Aus der Klinik und Poliklinik für Kinder- und Jugendpsychiatrie, Psychosomatik

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Using diffusion imaging to explore the anatomical nature of early course schizophrenia

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Für meinen Dad: Danke, dass du mich immer unterstützt und für mich da bist und gleichzeitig dazu ermutigst mutig und selbstständig zu träumen und zu leben.

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List of abbreviations

AD *Axial diffusivity*

CB *Cingulum bundle*
CT *Computer tomography*

FA *Fractional anisotropy*
FOV *Field of view*

HC *Healthy controls*

ILF *Inferior longitudinal fasciculus*
IQ *Intelligence quotient*

MD *Mean diffusivity*
MRI *Magnetic resonance imaging*

n *Number of participants*

RD *Radial diffusivity*
ROI *Region of interest*

SANS *Scale for the assessment of negative symptoms*
SAPS *Scale for the assessment of positive symptoms*
SCZ *Schizophrenia*
SLF *Superior longitudinal fasciculus*

TR *repetition time*
TE *echo time*

UF *Uncinate fasciculus*

Voxel *Volume of a pixel*
VBM *Voxel based morphometry*

Publication record of the presented work

The presented work is based on the following two papers:

1. **Seitz J,** Zuo JX, Lyall A, Makris N, Kikinis Z, Bouix S, Pasternak O, Fredman E, Duskin J, Goldstein JM, Petryshen TL, Mesholam-Gately R, Wojcik J, McCarley RW, Seidman LJ, Shenton M, Koerte I, Kubicki M: **Tractography analysis of 5 white matter bundles and their clinical and cognitive correlates in early-course schizophrenia** (Schizophrenia Bulletin, 2016)
    - Web of Science Core Collection impact factor 2016: 7.76
2. **Seitz J**, Rathi Y, Lyall A, Pasternak O, del Re EC, Niznikiewicz M, Nestor P, Seidman LJ, Petryshen T L, Mesholam-Gately RI, Wojcik J, McCarley RW, Shenton M, Koerte I, Kubicki M: **Alterations of gray matter microstructure in schizophrenia** (Brain Imaging and Behavior, 2017)
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Summary

Schizophrenia (SCZ) is a serious brain disorder that affects around 1% of the world population. Despite a long history of research in diagnosis and treatment of SCZ, we are still far from being able to explain the origin of the disease and the interindividual differences in the trajectory of the disease. The neurodevelopmental hypothesis states that SCZ is caused by early maturational abnormalities, which interact with later brain development.

Neuroimaging provides a noninvasive opportunity to study this theory *in vivo*. Traditionally, Magnetic resonance imaging (MRI) has been used to examine macrostructural gray matter features such as gray matter volume or cortical thickness and SCZ has been established as a *brain disorder* hereinafter. Diffusion tensor imaging (DTI) allows to investigate the microstructure of brain tissue. It measures the magnitude and direction of water molecule`s diffusion and is highly sensitive to alterations of gray and white matter organization. Gray matter contains the neurons and the white matter contains myelinated axons and provides long and middle range connectivity between cortical neurons. White matter alterations observed in SCZ therefore support the disconnection theory stating that SCZ is a brain disorder with disrupted integration of different brain systems.

Finally, while early imaging research focused on chronic states of SCZ a shift of the field towards studying early stages can be observed in more recent years. Understanding early course SCZ raises the hope to improve diagnosis and subsequently prevention and intervention.

In line with this research the aim of the presented studies is to characterize microstructural white and gray matter alterations in early course SCZ using diffusion MRI combined with advanced post-processing techniques, which are sufficiently sensitive to detect subtle brain conspicuities. Implications of and associations with neuropsychological and clinical symptoms and diagnosis of SCZ will be discussed subsequently.

Paper 1

The purpose of the first project is to characterize white matter organization in patients with early course SCZ. To my knowledge this is the first study investigating five main intra-hemispheric cortico-cortical white matter tracts using manual guided tractography in early course SCZ. The tracts were selected based on previous findings: uncinate fasciculus (UF), cingulum bundle (CB), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF) and arcuate fasciculus (AF). Diffusion parameters (fractional anisotropy [FA], trace, axial diffusivity [AD] and radial diffusivity [RD]) were computed for each tract and compared between patients with early course SCZ (number [n]=30) and healthy controls (HC) (n=30). The association of the diffusion parameters of the tracts with clinical

symptoms, memory performance, and processing speed was examined afterwards. A significant group effect, represented by reduced FA and increased RD and trace in the patients' group compared to HC was observed for the right AF (FA [F=5.94, df=1, p=.016]; RD [F=5.60, df=1, p=.020]), CB (FA [F=9.35, df=1, p=.003]; RD [F=11.55, df=1, p=.0010] and ILF (FA [F=14.77, df=1, p=.004]; RD [F=13.25, df=1, p<.0001]). The pattern of lower FA and higher RD is indicative for myelin abnormalities. Structural alterations were correlated with positive symptoms (ILF, AF), and cognitive performance (CB), which points to the clinical relevance of the observed white matter conspicuities.

Paper 2

In the past, DTI has mainly been used to study white mater, because technical challenges limited the use of DTI for the characterization of gray matter organization. However, as an extension of the classical disconnection theory one would not only expect dysconnectivity in white matter, but also a disruption of gray matter organization. The aim of this study therefore is to use novel DTI method-*heterogeneity*- to study the microstructural gray matter organization over the course of SCZ. In comparison to traditional diffusion indices, which focus on intra-voxel diffusion properties, heterogeneity captures the microstructural organization of a larger cortical area. After applying a *free water correction* to control for partial volume effects, T1 and diffusion images were registered to each other and the variability (=heterogeneity) of diffusion parameters within the four brain lobes defined by automatic parcellation method was calculated. Patients with chronic SCZ (n=27) did not show differences of cortical organization when compared to HC (n=22). However, patients with early course SCZ (n=19) showed increased heterogeneity in the frontal lobe when compared to HC (n=15) (F=10.68, df=1, p<.0030). This indicates a lower grade of cortical organization in patients than in HC. It is suggested that this can be explained by neurodevelopmental abnormalities, plausibly caused by abnormal synaptic reorganization and pruning during adolescence and early adulthood in SCZ.

Zusammenfassung

Schizophrenie ist eine schwerwiegende Erkrankung des Gehirns, die ungefähr 1% der Bevölkerung betrifft. Trotz jahrzehntelanger Erforschung der Ätiologie, Diagnose und Therapie der Erkrankung können wir bisher weder die Ursache noch inter-individuelle Unterschiede im Krankheitsverlauf suffizient erklären. Die *neurodevelopmental hypothesis* besagt, dass Schizophrenie eine Krankheit der Gehirnentwicklung ist, bei der frühe strukturelle Anomalitäten die weitere Reifung des Gehirns beeinträchtigen.

Magnetresonanztomographie ermöglicht es diese vermuteten Pathomechanismen *in vivo* und nicht invasiv zu untersuchen. In der Vergangenheit wurde vor allem der Kortex (kortikale Dicke und Volumen) mit Hilfe der Magnetresonanztomographie untersucht und aufgrund der gefundenen Unterschiede zu Kontrollgruppen wurde Schizophrenie daraufhin erstmals als *Gehirnerkrankung* charakterisiert. Diffusions-Tensor-Magnetresonanztomographie (engl. Diffusion tensor imgaging [DTI]) erlaubt es die Mikrostruktur des Gehirngewebes der grauen und weißen Substanz zu untersuchen. Das Signal basiert hierbei auf der Diffusion von Wassermolekülen im Gehirn. Während die graue Substanz auch aus Neuronen und ihren Verbindungen besteht, stellt die weiße Gehirnsubstanz die *Faserbündel des Gehirns* dar*,* welche verschiedene kortikale Areale miteinander verbinden. Veränderungen der weißen Substanz unterstützen die *disconnection theory,* welche Schizophrenie als Erkrankung beschreibt, bei der die Integration verschiedener kognitiver und psychologischer Domänen gestört ist.

Während sich die neuroradiologische Forschung lange Zeit auf die chronischen Stadien der Schizophrenie fokussiert hat, werden in letzter Zeit vermehrt frühe Krankheitsstadien erforscht. Hiervon erhofft man sich neue Erkenntnisse für Diagnostik, Prävention und Behandlung zu gewinnen.

Im Sinne dieser Strömung ist das Ziel der vorliegenden Arbeit, sowohl die mikrostrukturelle Organisation der weißen als auch der grauen Gehirnsubstanz in frühen Stadien der Schizophrenie zu untersuchen. Die hierfür verwendeten innovativen Methoden sind ausreichend sensitiv, um bereits geringfügige Veränderungen des Gehirns aufzuzeigen. Darüber hinaus wird der Zusammenhang mit neuropsychologischen und psychiatrischen Symptomen diskutiert, um die Bedeutung von strukturellen Auffälligkeiten zu erfassen.

1.Arbeit

Das erste Projekt dient der Charakterisierung der Organisation der weißen Substanz bei Patienten mit frühen Schizophrenie Stadien. Dabei ist dies meines Wissens nach die erste Arbeit, welche die fünf wichtigsten intra-hemisphärischen kortiko-kortikalen Faserbündel des Gehirns und ihre Veränderung in frühen Schizophreniestadien untersucht und hierfür manuelle Traktographie verwendet.

Basierend auf bisherigen Befunden wurden der Fasciculus uncinatus (engl. Uncinate fasciculus [UF]), das Cingulum (englisch cingulum bundle [CB]), der Fasciculus longitudinalis inferior (englisch inferior longitudinal fasciculus [ILF]), der Fasciculus longitudinalis superior (englisch superior longitudinal fasciculus [SLF]) und der Fasciculus arcuatus (englisch arcuate fasciculus [AF]) gewählt. Die wichtigsten Diffusionsparameter (englisch fractional anisotropy [FA], trace, axial diffusivity [AD] and radial diffusivity [RD]) wurden für diese Faserbündel ermittelt und zwischen Patienten (n=30) und gesunden Kontrollen (n=30) verglichen. Zusätzlich prüften wir den Zusammenhang zwischen strukturellen Anomalitäten und klinischen Symptomen, sowie Leistungen in den kognitiven Domänen „Arbeitsgedächtnis“ und „Verarbeitungsgeschwindigkeit“. Verglichen mit den Kontrollen zeigten Patienten mit Schizophrenie signifikant reduzierte FA, sowie gesteigerte RD Werte im Bereich des rechten AF (FA [F=5.94, df=1, p=.016]; RD [F=5.60, df=1, p=.020]), des rechten CB (FA [F=9.35, df=1, p=.003]; RD [F=11.55, df=1, p=.0010]) und des rechten ILF (FA [F=14.77, df=1, p=.004]; RD [F=13.25, df=1, p<.0001]). Dies wird wahrscheinlich durch pathologische Veränderungen der Myelinscheide verursacht. Die Assoziation der strukturellen Anomalitäten mit klinischen Symptomen (ILF, AF) und kognitiver Leistung (CB) untermauert die klinische Relevanz dieser Arbeit.

2. Arbeit

Bisher wurde DTI auf Grund von technischen Schwierigkeiten selten für die Untersuchung der grauen Substanz eingesetzt. Allerdings ist im Sinne einer erweiterten *disconnection theory* nicht nur eine Veränderung der Mikrostruktur der weißen Substanz, sondern auch der grauen Substanz zu erwarten. Deshalb ist es das Ziel dieser Arbeit mit Hilfe einer neuen DTI Methodik- *Heterogenität*- mikrostrukturelle Veränderungen der grauen Substanz bei Patienten mit Schizophrenie aufzudecken. Im Gegensatz zu klassischen Diffusionsparametern, bezieht sich Heterogeneität nicht auf das Diffusionsverhalten innerhalb eines Voxels, sondern erfasst die Organisation innerhalb eines größeren kortikalen Gebietes. Nach Korrektur für *freies Wasser* wurden T1 und Diffusions-gewichtete Bilder aufeinander registriert und die Variabilität von Diffusionsparametern (=Heterogenität) innerhalb eines Kortexareals ermittelt. Patienten mit chronischer Schizophrenie (n=27) unterschieden sich bezüglich ihrer kortikalen Organisation nicht von gleichaltrigen Kontrollen (n=22). Im Gegensatz dazu war die Heterogenität bei Patienten zu Beginn der Krankheit (n=19) im Bereich des frontalen Kortex größer als bei den Gesunden (n=15) (F=10.68, df=1, p<.0030). Dies deutet darauf hin, dass das Frontalhirn bei Patienten mit Schizophrenie weniger gut organisiert ist als bei Gesunden. Wobei diese Auffälligkeiten auf Grund des frühen Auftretens Entwicklungs- und nicht degenerativ bedingt sind. Sie könnten möglicherweise durch anormale synaptische Reorganisation verursacht werden, welche in post-mortem

Studien junger Patienten mit Schizophrenie beobachtet wurde. Zukünftige Forschung muss untersuchen, inwiefern sich Heterogenität als ein Biomarker für das Risiko an Schizophrenie zu erkranken eignet.

I. Introduction

I.1 Schizophrenia

I.1.1 Prevalence, Diagnosis and Treatment

Schizophrenia (SCZ) is a serious disorder with a lifetime prevalence of 0.3-1% [1-4] across different cultures and countries [5]. Both sexes are affected equally [6], while men show an earlier average age of onset [7]. SCZ can break out during the whole lifespan [8], with the first episode normally occurring between 16 and 30 years of age [9]. Only 5% of patients experience their first episode before the age of 15 [10]. A secondary peak of disease onset can be observed after the age of 45 especially for women [11]. SCZ has significant consequences for the quality of life of patients, caregivers, and society and is therefore listed as one of the top ten causes of loss of healthy life years by the World Health organization [12-14]. Morbidity and mortality are increased [15] and patients' life expectancy is reduced by 15-20 years [16, 17]. Patients with SCZ are more likely to commit suicide [18-20], are involved in more accidents [21], live more often in poor social circumstances [22, 23] and suffer from comorbidities [24-26] than healthy individuals. The prevalence of somatic diagnoses, such as cardiovascular [27], infectious [28] or neoplastic [3] disorders is substantially increased and patients with SCZ receive poorer treatment for the aforementioned illnesses which leads to inferior long term outcome. Additionally, axis one (e.g. depression or dependency) [29-31] and axis two (e.g. personality disorders) [32] psychiatric diseases are often seen as co-diagnosis in patients. Not only patients [33, 34], but also their relatives experience stigma and exclusion [35] which increases the strain for caregivers [36]. For society SCZ poses a burden by entailing high economical costs [37, 38] and presenting a risk of increased violent behavior of patients [39].

The first report of symptoms resembling what we know as SCZ today can be found in the *Ebers papyrus* from over 1500 years before Christ and SCZ like symptoms are described throughout whole mankind history [40]. However, it took until the late 19th century when Kraepelin was the first to distinguish psychosis, which he called *dementia praecox*, from other psychiatric disorders [41, 42]. Bleuler coined the phrase *schizophrenia* for Kraepelin`s *dementia praecox* shortly afterwards and introduced the concept of primary and secondary symptoms [43]. This was later adapted by Schneider and is to some extend still valid to date [44]. Diagnosis is based on either the DSM V (diagnostic and statistical manual of mental disorders) [45] or the ICD 10 (international classification of diseases) [46] (Table 1). In comparison to the predecessor DSM IV, a conceptual psychosis continuum was introduced in the DSM V and classical SCZ subtypes were eliminated [47]. Various approaches of grouping the different symptoms into dimensions have been suggested [48-50]. The most accepted models are either the three dimensional model (with psychotic, negative and disorganized symptoms) [51, 52] or the two dimensional model (with

positive and negative symptoms) [53]. Positive symptoms like hallucinations and delusions are often considered as core concept of SCZ. Both occur in the healthy population [54, 55]. However, they are more common, more extreme and more often unpleasant or frightening in patients with SCZ [56, 57]. Negative symptoms (presented by diminished basic emotional and behavioral processes) can arise even before disease onset and are more relevant for long-term outcome than positive symptoms [53, 58]. The variety of symptoms nevertheless challenges diagnostic possibilities and the boundaries towards other disorders or healthy behavior are often blurred [59-61]. Newer approaches aim on tackling these challenges by using advanced methodical approaches[62] such as multimodal (imaging) studies[63, 64] or machine learning [65-69].

Table 1: Diagnostic criteria for SCZ

	DSM V [45]	**ICD 10** [46]
Duration	6 month of disturbance 1 month of symptoms	1 month of disturbance/ symptoms
Symptoms	1. Delusions 2. Hallucinations 3. Disorganized speech 4. Grossly disorganized or catatonic behavior 5. Negative symptoms	1. Thought echo, insertion, withdrawal or broadcasting 2. Delusions (body, specific thoughts, action or sensation related), delusional perception 3. Hallucinatory voices 4. Persistent delusions of other kinds 5. Persistent hallucinations 6. Neologisms, breaks or interpolations in thought or speech 7. Catatonic behavior 8. Negative symptoms
	Two or more symptoms At least one of 1-3	At least one: 1-4 At least two: 5-8
Further criteria	Social/occupational dysfunction No schizoaffective or major mood disorder No substance/ general medical condition If global developmental delay or autistic disease: delusions or hallucinations	Social/occupational dysfunction No major mood disorder Not alcohol or drug related No organic brain disease

While there is no curative therapy available [70] psychopharmacological and non-psychopharmacological treatments can positively modulate the course and outcome of SCZ [71-73]. Therapy options can

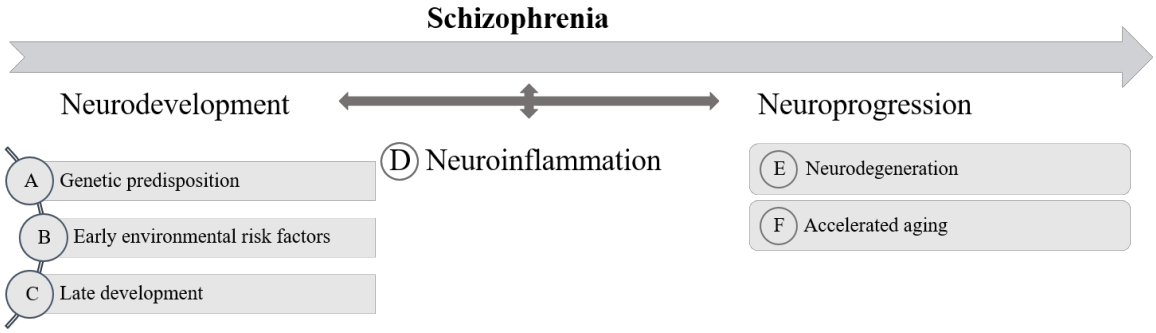
grossly be categorized into three different types: antipsychotic medication, non- antipsychotic medication and other biological, psychological and social interventions. Antipsychotics are the basis of treatment. Early medication studies showed the involvement of the dopamine system in SCZ [74, 75], whereby hyperactivity in subcortical and hypoactivity in prefrontal regions and an association with positive symptoms [76] has been suggested. The contribution of the glutamate [77-79], GABAergic [80, 81] and acetylcholinergic [82] transmitter systems has been shown subsequently. Antipsychotics can either be classified as first generation or conventional antipsychotics or as second generation or atypical antipsychotics [83]. Low potency first generation antipsychotics show more anticholinergic effects than high potency first generation antipsychotics which mainly work as dopamine antagonist. Second generation antipsychotics are more specific for serotonin and dopamine receptors [83-85]. Newer second generation antipsychotics additionally effect some receptors as agonists rather than as antagonists [86-88]. On average all first generation antipsychotics cause similar outcomes [89] and side effects are often extrapyramidal symptoms such as akathisia or parkinsonism [90, 91]. Second generation agents are in general associated with more metabolic and less motoric side effects than first generation antipsychotics. While the effectiveness between different agents is again comparable, Clozapine, is considered as superior for treatment refractory SCZ [92, 93]. It is recommended to start antipsychotic treatment as early as possible in an episode [94] and maintain therapy for at least five years – if not lifelong- to prevent psychotic relapse and manage functional impairments [95-101]. Newer developments suggest the advantage for medical adherence of long lasting injectable agents [102], however safety risks still need to be evaluated [103]. Additionally to antipsychotics other medications are promising for the treatment of symptoms - such as antidepressants for depressive [104, 105] and obsessive compulsive symptoms [106] , anti-inflammatory medication [107, 108] or agents which interact with the nicotine-cholinergic [109] or oxytonergic [110, 111] system. Newest studies also suggest histone-deacetylase 1 inhibition as potential medication [112]. Further biological therapy approaches include non-invasive brain stimulation (e.g. electroconvulsive therapy/transcranial magnetic stimulation [113-119]) or experimental efforts for interneuron transplants [120]. Psychosocial and cognitive behavioral therapy supports the recovery, increases the compliance and reinforces the stabilization [121-123], and is especially suitable for children and adolescents [124], patients with treatment resistant SCZ [125] or older patients [126]. Psychoeducation for affected people and their family members [127-129], nutritional approaches [130] and physical exercise [131-135] can support recovery, improve medication adherence, clinical symptoms, quality of life and functioning [136, 137].

I.1.2 Etiology

Given that SCZ is an incurable disorder with comparable high prevalence and early onset [138] an improvement of diagnostic skills seems essential [139]. Additionally, although there is a wide range of

therapeutic options, we are far from understanding individual responses and non- responses to treatment. Moreover, despite all treatment options described above the rate of recovery from SCZ is not increasing [140] - leading to a "pressing need for more effective treatments and delivery of services" [141]. Therefore it seems suitable to focus on underlying neurobiological pathologies rather than mere description of symptom clusters to study the heterogenous group of SCZ [142] and hopefully be able to use neurobiological findings for optimizing diagnosis and treatment. Several models and hypotheses about the etiology of SCZ have been proposed (Figure 1).

Figure 1: Etiology of SCZ



The early neurodevelopmental hypothesis suggests that genetic (Figure 1, A) and early environmental risk factors (Figure 1, B) cause early developmental abnormalities [143] leading to behavioral [144-148], intellectual [149, 150] , and morphological conspicuities [151] and finally to disease onset. Others emphasize the importance of late developmental events (Figure 1, C) like dysfunctional synaptic pruning during adolescence [152]. The two-hit hypothesis combines these two attempts by assuming that SCZ is the final state of a neurodevelopmental disorder where early neurodevelopmental abnormalities interact with later maturational abnormalities [153-155]. Recent clinical, epidemiological and experimental studies emphasize the importance of inflammatory conditions for SCZ (Figure 1, D) [156-158]. Neuroinflammation may be present at onset [159, 160] and prolonged inflammation may promote subsequent degeneration [161, 162]. The potentially degenerative effects (Figure 1, E) of SCZ are discussed controversially. Though Kraepelin [41] introduced SCZ as *dementia praecox*, post mortem studies do not find typical degenerative abnormalities like gliosis [163] or neurofibrillary tangles [164]. Nevertheless SCZ may be a syndrome of accelerated aging (Figure 1, F) were "a state of decreased cerebral reserve (…) causes persons with schizophrenia to be more vulnerable to the toxic effects of even *normal* accumulations of age-related neurodegenerative lesions" [165]. This idea is being supported by genetic [166, 167], metabolic [168, 169], immunological [170] and morphological[171 172] (especially for white matter[173, 174]) similarities between patients with SCZ and healthy aging people. Some authors even assume SCZ to be a whole-

body progeria syndrome [175, 176]. However other clinical, (neuro)psychological and morphological findings do not clearly support the idea of accelerated aging [177-190].

Taken together patients with SCZ show an atypical maturational trajectory during their whole life span, most certainly caused by early neurodevelopmental abnormalities and influenced by genetic susceptibility, environmental risk factors, neuroinflammation and the state of being chronically ill (for an overview on factors associated with SCZ please see Table 2).

Table 2: Factors potentially influencing SCZ

Genetic susceptibility	
Heritability	Approximatly 80% contribution [191] Children of parents with SCZ have a ten times higher risk to get SCZ, even if they were adopted short after birth [192-194]
Possible genes	No single gene has a large effect, several common genetic variants with small effects (microdeletions, microduplication, single- nucleotide polymorphisms) [195, 196 197-201] Role of micro RNA alterations[202], epigenetics [203],
Gender	Female gender associated with better outcome, less deficits syndroms [204]
Association with environmental factors	
Early environmental factors	Paternal Age [205] Maternal illness, infections and inflammation during pregnancy [206-208] Obstetric complications (e.g.hypoxia) [209, 210] Winter birth in northern hemisphere [211] Urban birth and living [212, 213]
Later environmental factors [214, 215]	Migration [216, 217] Ethnic minority status [218] Role of toxoplasmosis [219] Negative family emotional environment, single status [220] Parental separation or death [221] Trauma [221], sexual abuse [194, 222] Low intelligence, psychopathology, cognitive or motor dysfunction during childhood [206, 223] Cannabis and other substance abuse [224] Poor education [225], Unemployment [226]

I.2 Neuroimaging

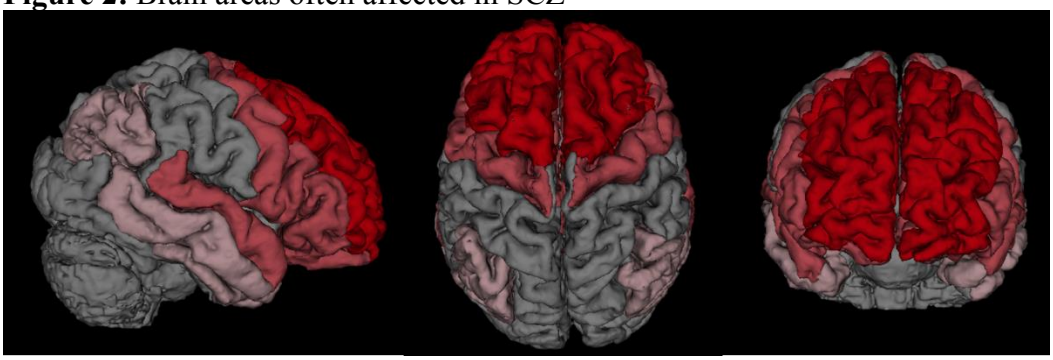
Though Bleuler and Kraepelin already assumed that SCZ is a *brain disorder* [227], early post mortem studies did not find significant alterations in brain tissue [228] and therefore researchers dropped the idea of a *brain disorder* until the first computed tomography (CT) study showed enlarged ventricles in patients with SCZ [229]. Neuroimaging provided a new approach, because it allowed to study the brain

in vivo, it is non-invasive, and provides three-dimensional, high contrast picture of the entire brain at once.

I.2.2 Classical MRI

The discovery of nuclear magnetic resonance in the 1970s by Mansfield and Lauterbur and the following development of magnet resonance imaging (MRI) supplied an even more powerful tool than CT for brain research. MRI is more sensitive for soft tissue contrast than CT, does not require ionizing radiation, and no adverse effects and only few contra-indications are known. Using MRI several brain abnormalities in SCZ have been detected, with enlarged ventricles, cavum septum pellucidum abnormalities and loss of cortical substance being the most prominent ones [for review see 230]. Loss of cortical volume and thickness is most pronounced in prefrontal areas (Figure 2, areas in dark red) already occurring before onset and being influenced by genetic load [231-235]. During the first episode (=period of first onset of disease) additional cortical decrease can be observed in the inferior frontal regions, cingulate, superior temporal gyrus, insula and precentral gyrus [179, 180] (Figure 2, middle red). With progression of disease changes get more noticeable in the temporal and parietal lobes (Figure 2, light red) [230]. However, one needs to notice that findings are not unambigous[236, 237] and that many questions remain unresolved: e.g. what are the exact locations, extend and progression of structural alterations [173, 174]? While MRI was essential to establish the concept of SCZ as *brain disorder,* available measurements are quite nonspecific and unprecise and therfore potentially not suitable to identfy early subtle changes [238] and answer the aformentioned questions. (Figure 2 is adapted from figure created by the author in[239])

Figure 2: Brain areas often affected in SCZ



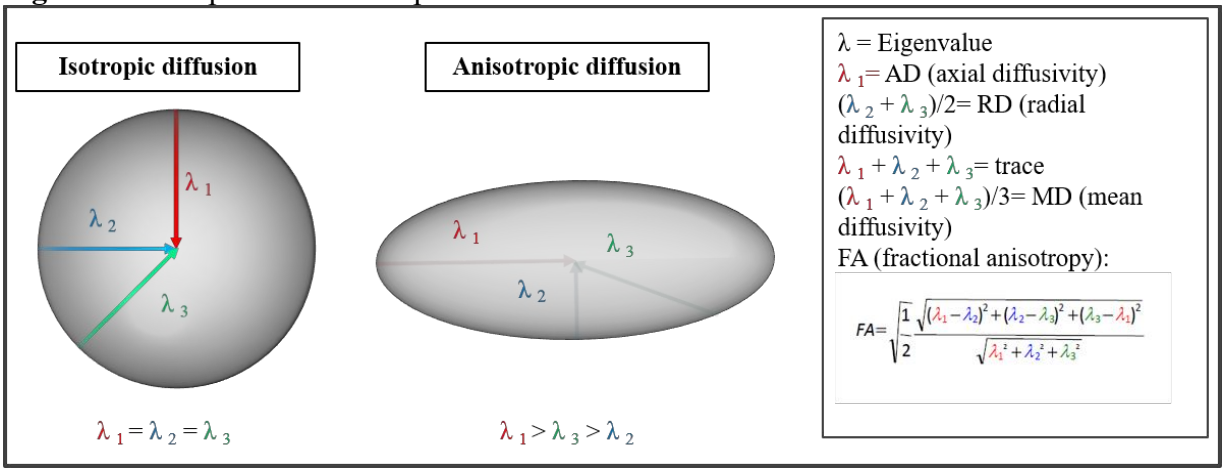
I.2.3 DTI

A method more sensitive to changes in brain microstructure than conventional MRI is *diffusion tensor imaging (DTI)*. DTI was introduced by Basser and colleagues and it provides a way of indirectly examining tissue microstructure. Brain water molecules move (diffusely) in the tissue following

Brownian motion rules. DTI measures the magnitude and main directionality of diffusion [240-242]. Therefore a diffusion tensor (a tree-dimensional vector) is estimated from the data for each voxel of the brain for three perpendicular axes [243]. The magnitude of diffusion along each axis is represented by an "eigenvalue" and the directionality by an "eigenvector". The diffusion is called isotropic, or spherical if it is more or less the same along all axes (Figure 3). Isotropic diffusion can be found in the cerebrospinal fluid. In white matter diffusion is affected and restricted by the organization of tissue in fiber bundles. Water molecules most likely diffuse along the main direction of the fibers. This is called anisotropic or ellipsoidal diffusion.

From the diffusion tensor, several diffusion indices like fractional anisotropy (FA), mean diffusivity (MD), trace, axial diffusivity (AD), or radial diffusivity (RD)[244] can be computed to quantify diffusion behavior. FA represents the normalized variance of the diffusivity along all three axes estimating how anisotropic the diffusion is in an examined voxel. Values range between 0 and 1, with values close to 0 indicating isotropic diffusion and values close to 1 indicating anisotropic diffusion [245]. FA is often used as an index for overall white matter organization. Trace and MD are indices for the overall magnitude of diffusion in a voxel. Trace is calculated by summing up the eigenvalues of all three directions, while MD represents the averaged diffusivity over all three directions. AD and RD can give further insights into possible underlying neuropathology. It is assumed that AD ("the magnitude of diffusion parallel to the fiber axis"[246]) represents the axon integrity, while RD (the magnitude of the diffusion perpendicular to the main diffusion direction) provides an index for myelin integrity[247]. Using these indices, microstructure of a region-of-interest can be quantitatively described. An enlarged extracellular space for example will lead to enhanced mean diffusivity of water molecules (and therefore larger MD and trace) and less restriction of diffusion direction (and therefore lower FA). However one needs to keep in mind that these measurements are indirect rather than direct indices for microstructural characteristics [159, 248, 249].

Figure 3: Isotropic and anisotropic diffusion figure adapted from 250



FA, MD/trace, RD and AD brain maps can be analyzed in different ways. In the context of this work the main analysis approaches- *voxel based*, *tractography* and *regions-of-interest approaches* are introduced hereafter.

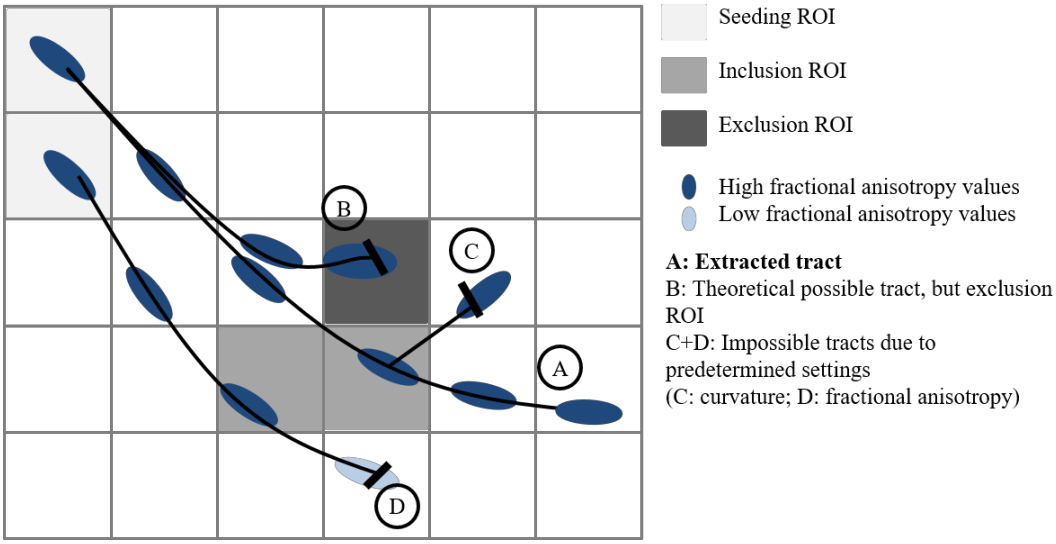
Voxel based morphometry (VBM)

VBM attempts to compare whole brains- voxel by voxel using parametric mapping. All datasets are therefore registered to a template and brain images are smoothed afterwards. Diffusion parameters are then compared at each voxel. TBSS is an optimized, white matter specific VBM analysis, which uses the registered datasets to create a *mean white matter skeleton* (skeletonized average of all FA values at one voxel). All individual FA maps can be projected onto that skeleton afterwards and voxels which are part of the skeleton are used for individual statistics. Using TBSS minimizes the need for data smoothing and therefore leads to less partial volume effects and greater statistical power than classical VBM [251]. However since tract extraction is performed automatically and does not use prior anatomical information, this method is susceptible to misalignment/registration errors [252]. Further, given that TBSS is based on the maximal value projection, it is less suitable to study subtle changes or pathologies which show high- inter-individual variability.

Tractography

Tractography provides the opportunity to delineates tracts and analyze diffusion properties (please see Figure 4 [figure adapted from253]). In tractography fiber bundles are usually calculated from a seeding region. Using manual guided streamline tractography [254] seeding regions are either drawn manually on diffusion images guided by anatomical knowledge [255] or derived from automated segmentation techniques. Additional inclusion and exclusion regions can be determined. The tractography than follows two criteria: the FA value of each voxel and the curvature of the fibers between voxels. Fibers terminate if either the radius of curvature gets below a certain threshold (Figure 4-C) or if FA undercuts a certain value (Figure 4-D). Diffusion parameters can be extracted for the identified fiber tracts afterwards and if necessary averaged over each tract or for subsections along the tract for each subject and used for statistics. Manual tractography is the anatomically most accurate way to determine tracts based on diffusion MRI, however it requires high resolution diffusion MRI data, computational power, profound anatomical knowledge and is time consuming.

Figure 4: Schematic representation of streamline tractography figure adapted from 253



Region of interest approaches

ROI analyses are analyses were brain areas of interest are selected (similar to seeding and inclusion ROIs in tractography) and diffusion values are extracted and averaged for each subject over the selected regions. Brain regions can be identified manually, semi-automatically or automatically by segmentation software (e.g., FreeSurfer). Comparable to tractography and in contrast to VBM manually traced ROI analyses are anatomically more accurate and better suitable for data sets with high inter-individual variability. ROI analyses may not include whole tracts but may include a region with several tracts [256].

As opposed to TBSS or tractography approaches, which are specific to white matter, ROI analysis can also be used to study gray matter. As aforementioned, conventional macrostructural gray matter MRI studies may not be sensitive enough to study subtle brain alterations, whereas DTI may capture those [257]. However, very few studies using DTI to examine gray matter in SCZ have been conducted [258-262]. This is due to several reasons: Even when using high resolution data, DTI is especially prone to partial volume effects. Additionally, gray matter is organized in a very different way with cell bodies and processes determining water diffusion rather than bundles of axons. This leads to no preferred direction of diffusion in a voxel of gray matter and rather isotropic diffusion properties. Finally, diffusion properties vary significantly between voxels in gray matter [263], which makes them insensitive for detecting subtle group differences. If one would still like to use DTI to study gray matter microstructure, one needs to look at parameters that do not cover intra-voxel diffusion parameters, but overall inter-voxel diffusion properties to study overall cortical organization [264-266].

Diffusion findings in patients with SCZ

Using the above described methods several studies suggest that SCZ is a disorder were white matter integrity is disrupted[267, 268]. This is in line with genetic, neuropathological, functional MRI and electro/magnetoencephalography studies [269-272.] Most of those studies have focused on chronic disease state. In chronic SCZ FA is reduced all over the white matter [273,] while this decrease is particularly pronounced in frontal, temporal, and cingulate areas [274]. It seems that the disruption of the corpus callosum, fronto-temporal, limbic and cerebello-thalamo-cortical fibers [275-280] is characteristic for chronic SCZ. The magnitude of white matter alterations is thereby associated with clinical symptoms [281-283], cognitive impairments [284] and global functioning [285]. Findings are influenced by many factors such as sex [286, 287 288], medication [289-291], age [292-295] and potentially illness duration [296-298]. Recently a shift of the research field towards studying earlier stages of disease can be observed, driven by the hope to understand early abnormalities that are not confounded by illness chronicity or medication, and subsequently improve early diagnosis, prevention and intervention. Studies find white matter alterations in healthy individuals with family risk for SCZ, which are similar but less pronounced than findings in patients [299]. Additionally, wide-spread white matter changes [300-302] can be observed in patients with risk for SCZ based on clinical presentation (e.g., individuals with psychotic experiences) and a progression of brain abnormalities is associated with and predictive for a progression of symptoms [303-306]. These findings indicate that connectivity abnormalities, influenced by neurodevelopmental factors, are not primarily a consequence of psychosis, but may represent vulnerability to develop psychosis. However, alterations before onset may not be specific for SCZ or psychotic risk [307], but rather reflect a *general state of vulnerability of the brain*. It is therefore informative to also study patients with early course/first episode SCZ. Those individuals already show SCZ specific symptoms but have not been suffering from a chronic disorder or received long-term medication. Reduced FA has been reported in early course/first episode SCZ in several areas including the corpus callosum, cerebellum, brainstem, internal capsule, cingulum bundle, corona radiata, superior longitudinal fasciculus, inferior longitudinal fasciculus, fornix, uncinate fasciculus and anterior commissure [308-317] and again an association with symptom severity has been observed [318-320].

I.3 Motivation for this work

The aim of this study is to identify conspicuities of microstructural brain organization in early course SCZ. Given the inconsistency of findings in the literature [e.g.236, 237] and the fact that brain alterations are suspected to be less pronounced in patients with early course SCZ [238] anatomically precise methods are chosen. As described above, the most anatomically accurate way of studying white matter

is tractography. Nevertheless, given the time-consuming nature of manual tractography, most early course SCZ studies have either not used it and those few studies that did perform manual tractography only looked at a small number of tracts [321-323], even though it is widely accepted that structural abnormalities in SCZ are rather widespread than regional [275-277]. For technical reasons [324] even fewer studies used DTI to examine gray matter changes in SCZ [258-262] , and most importantly, there is no study in early course SCZ. Given the differential organization of gray matter when compared to white matter, it is important to capture cortical organization on a bigger scale. Promising methods to study gray matter microstructure are therefore based on inter-, rather than intra-voxel organization. A promising *inter-voxel method* is *heterogeneity* [325], which reflects the variability of diffusion properties within a given cortical area.

The presented work intends on combing the aforementioned methods in a unique way to study brain microstructure in both white and gray matter organization in patients with early course SCZ. Additionally, driven by the hope that better understanding of neurobiological features will in the long-term improve diagnosis, prevention and intervention [326], the work also focuses on associations of structural abnormalities with clinical symptoms, cognitive impairments and diagnosis categories. To reach these aims, two studies were conducted:

1. The first study investigates the most important cortical intra-hemispheric white matter bundles: uncinate fasciculus (UF), cingulum bundle (CB) , inferior longitudinal fasciculus (ILF) , superior longitudinal fasciculus (SLF) and arcuate fasciculus (AF) using manual tractography. We assumed that diffusion measures of these tracts would be most likely associated with core cognitive domains disturbed in early course SCZ (working memory and processing speed), as well as early occurring neuropsychological symptoms.
2. The second aim was to gain *in vivo* insights into the microstructural organization of gray matter in patients with SCZ in comparison to HC. We therefore used *heterogeneity*, which we expected to be more sensitive to gray matter microstructural pathology than traditional measures.

II Paper 1

II.1 Background

To our knowledge this is the first paper examining the five main intra-hemispheric fiber associations at once in early course SCZ using manually guided tractography. The UF - associated with acoustic memory, visual information and emotional response [327]- is often altered in patients with SCZ who show negative symptoms and poor outcome [328-331]. The CB – "involved in emotional expression, attention, motivation and working memory" [327]- is most likely associated with the impairment in executive functions in SCZ [282, 331-335]. The ILF – "implicated in visual representation, facial recognition, and emotional perception" [327]- has often been reported to be disrupted in SCZ, where this seems to be associated with positive symptoms [336-338]. Though being two distinct structures, many studies so far examined the SLF and the AF together and abnormalities in SCZ have often been reported [338, 339]. However these tracts are separate neuroanatomical structures [340], where the SLF is related to spatial attention and memory [327] and the AF is linked with language and spatial information [327]. Additionally, an association of the AF with positive symptoms in SCZ has been found [341, 342].

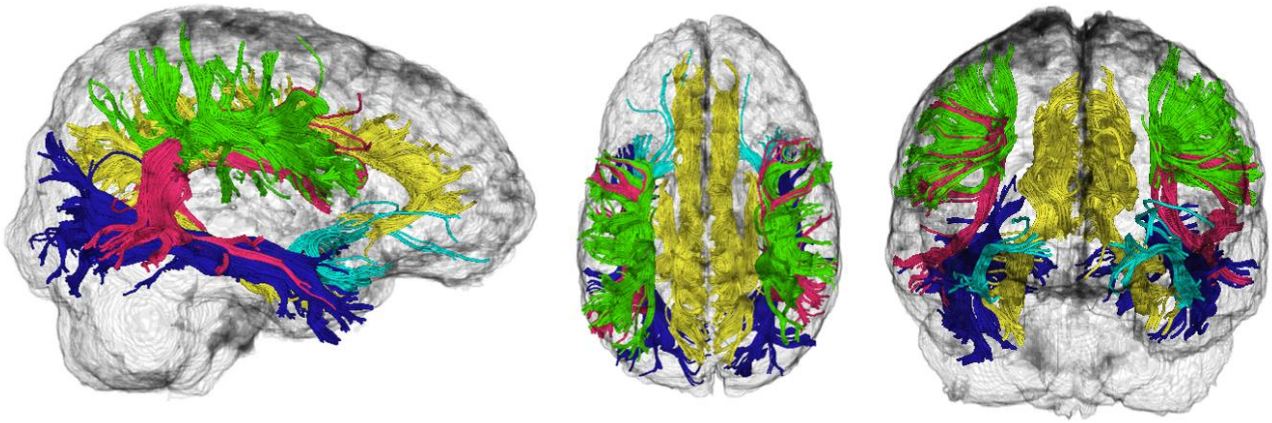
While our first aim is to detect structural alterations of these tracts, the second aim of the presented study is to investigate the association of potential structural abnormalities with symptom severity and neuropsychological measurements. Symptom severity is measured by the Scale for the assessment of negative symptoms (SANS) [343] and the Scale for the assessment of positive symptoms (SAPS [344]). For cognitive measures, we are focusing on working memory and processing speed. These are basic cognitive processes which are needed for several other cognitive operations [345-347] and influence one and another [348, 349]. They are "associated with white matter integrity and development" [345, 350-352] and impairments in SCZ have been reported [353-355]. Impairments furthermore seem to be typical for SCZ [356, 357] and specific for early disease stages [358, 359], or even SCZ risk [355, 360]. Additionally, both measurements are clinical relevant, because performance in these domains is related to later poorer functional outcome in patients with SCZ [361-363].

II.2 Methods

Diffusion MRI was acquired in 30 patients with early course SCZ and 30 HC on a 3 Tesla whole body scanner (General Electric Medical Systems, Milwaukee, WI) using a high spatial resolution twice refocused echoplanar sequence (Repetition time [TR]=17 s, echo time [TE]=80 ms, flip angle 90°, field of view [FOV] 240 x 240 mm, 85 slices, 1.7 mm x 1.7 mm in-plane, 1.7 mm slice thickness, 51

gradient directions with b= 900 s/mm^2, and eight baseline scans with b= 0). Patients and HC were age, gender, handedness, parental socioeconomic status and premorbid IQ matched. To account for a scanner update, this was included as a covariate in analyses. Trained neuropsychological testers conducted the clinical and neuropsychological tests. Diffusion tensors were estimated from the images using a weighted least square approach and manually guided tractography was performed. We extracted the five main intra-hemispheric association bundles: UF, CB, ILF, SLF and AF (Figure 5) using manual guided tractography. Diffusion parameters (FA, trace, RD and AD) were extracted for each tract with MATLAB [364]. Group differences were examined using a MANCOVA- with dependent variables FA/trace/AD/RD of the five fiber bundles, independent variables group, hemisphere, and group x hemisphere and covariates gender, age, handedness and scanner software upgrade. Additionally, post hoc ANCOVAs and t-tests were conducted. A discriminant analysis was performed to see if FA of tracts predicts group affiliation (HC versus patients with SCZ) and correlation analysis (Spearman R) between DTI measure sand clinical test results were calculated. All statistical tests, except for the post hoc tests, are Bonferroni corrected.

Figure 5: ILF (dark blue), SLF (green), AF (red), UF (light blue) and CB (yellow) as modeled by manual tractography adapted from Paper 1, 352.



II.3 Results

MANCOVAs showed significant group and hemisphere effects for FA, trace and RD and significant hemisphere effects for AD. Post hoc ANCOVAs and t-tests were significant for the right AF, right CB and right ILF (Table 3, adapted from Paper 1 [352]). Using the given white matter properties 80% of the population could be classified right, whereby the right ILF, right AF and right CB were most important for predicting group affiliation. FA and RD of the right ILF and AF showed an association with positive symptoms, while CB white matter properties were correlated with memory performance and processing speed.

II.4 Conclusion

The study presented here aimed to investigate five major association bundles in early course SCZ using high resolution diffusion data and manual guided tractography. An alteration of white matter- of the CB, ILF and AF was found. The pattern of diminished FA values and elevated trace and RD is indicative for white matter disruption most likely caused by myelin abnormalities [247]. Diffusion measurements of the CB were associated with speed of processing and memory. Furthermore, an association of the white matter of the ILF and AF with positive symptoms was found. This clearly demonstrates the clinical relevance of the presented structural abnormalities.

In contrary to other studies, no alterations of the UF [328-331] and the SLF [338, 339] were observed. Alterations of the UF in SCZ are being discussed controversially [365, 366] and are most likely associated with deficit SCZ and negative symptoms [285, 331] and therefore chronical disease states. The lack of SLF findings can be explained by the fact that unlike other studies, the SLF and AF were considered as two separate tracts in this study. The separation of the SLF and AF is only possible when using tractography. We found differences for the AF, which proves how essential it is to investigate these two tracts separately.

Interestingly almost all group differences were observed for the right hemisphere only, which is in accordance with other studies in individuals at risk [367], or in early course SCZ [312, 368-370] suggesting that these abnormalities might be dominant in early course SCZ.

Taken together we observed alterations of the white matter of three major tracts in the right hemisphere in early course SCZ indicating that white matter changes take place very early during disease. Manual guided tractography seems to be a valid method to investigate these early changes. The association with clinical impairments and the high ability to predict group affiliation based on diffusion properties shows that these early changes are clinically relevant. Future studies should include potential confounding variables like duration of illness, medication or kind of symptoms [321, 322, 328, 371-375]. Additionally, methods need to be improved further (e.g. use of two tensor tractography [376]) so that the presented findings may be used as prognostic factors in the future.

Table 3: Significant differences between HC and patients with SCZ adapted from Paper 1, 352

		FA	Trace	AD	RD
MANOVA	Hemisphere	(F=44.46, df=5, p<.0001)	(F=12.99, df=5, p<.0001)	(F=27.23, df=5, p<.0001)	(F=16.30, df=5, p<.0001)
	Group	(F=5.27, df=5, p<.0001)	(F=3.33, df=5, p=.008)		(F=4.92, df=5, p<.0001)
Post hoc ANOVA (Group)		AF (F=5.94, df=1, p=.016)			AF (F=5.60, df=1, p=.020)
		CB (F=9.35, df=1, p=.003)	CB (F=8.15, df=1, p=.005)		CB (F=11.55, df=1, p=.0010)
		ILF (F=14.77, df=1, p=.004)	ILF (F=11.17, df=1, p=.001)		ILF (F=13.25, df=1, p<.0001)
Post hoc two-tailed t-tests		Right AF (t=2.87, df=55,p=.0061)			Right AF (t=2.46, df=55, p=.018)
		Right CB (t=2.29, df=58, p=.027)	Right CB (t=2.02,df=58, p=.048)		Right CB (t=2.49, df=58, p=.017)
		Right ILF (t=3.28, df=58, p=.0020)	Right ILF (t=2.54, df=58, p=.014)		Right ILF (t=3.40, df=58, p=.001)
			Left ILF (t=2.13, df=58, p=.038)		Left ILF (t=2.27, df=58, p=.028)

III Paper 2

III.1 Background

The study presented here suggests the use of a novel DTI measurement- heterogeneity [325]- to investigate cortical organization on a microstructural level. Heterogeneity is a way to statistically determine the variability of diffusion parameters in a brain region rather than looking at single values within a voxel. The heterogeneity of FA for example, represents the variability of FA values within a region (e.g. a cortical lobe). Heterogeneity is therefore able to overcome difficulties of using DTI in gray matter (please see Introduction I.2.3 DTI - Region of interest approaches). First it considers the variation of diffusion properties between voxels [263] which makes it more sensitive for detecting group differences. Secondly, it copes with the special nature of gray matter organization: rather than focusing on the tensor shape in a voxel which is isotropic in gray matter, it captures cortical organization on a bigger scale. Last, the method introduced here corrects for partial volume effects (please see method description below).

The healthy cortex is characterized by low heterogeneity, indicating a relatively consistent organization of tissue within a region. One would predict that pathological reorganization processes of the brain would in some areas disrupt this consistent organization leading to higher variability and therefore higher heterogeneity of diffusion properties. Given that SCZ is proposed to be a disorder where the brain is less connected/ less organized the aim of this study was to investigate this potential disorganization in patients with SCZ. In addition studying early course SCZ (using a subset of the cohort of paper 1), we also include a comparison group of patients with chronic SCZ.

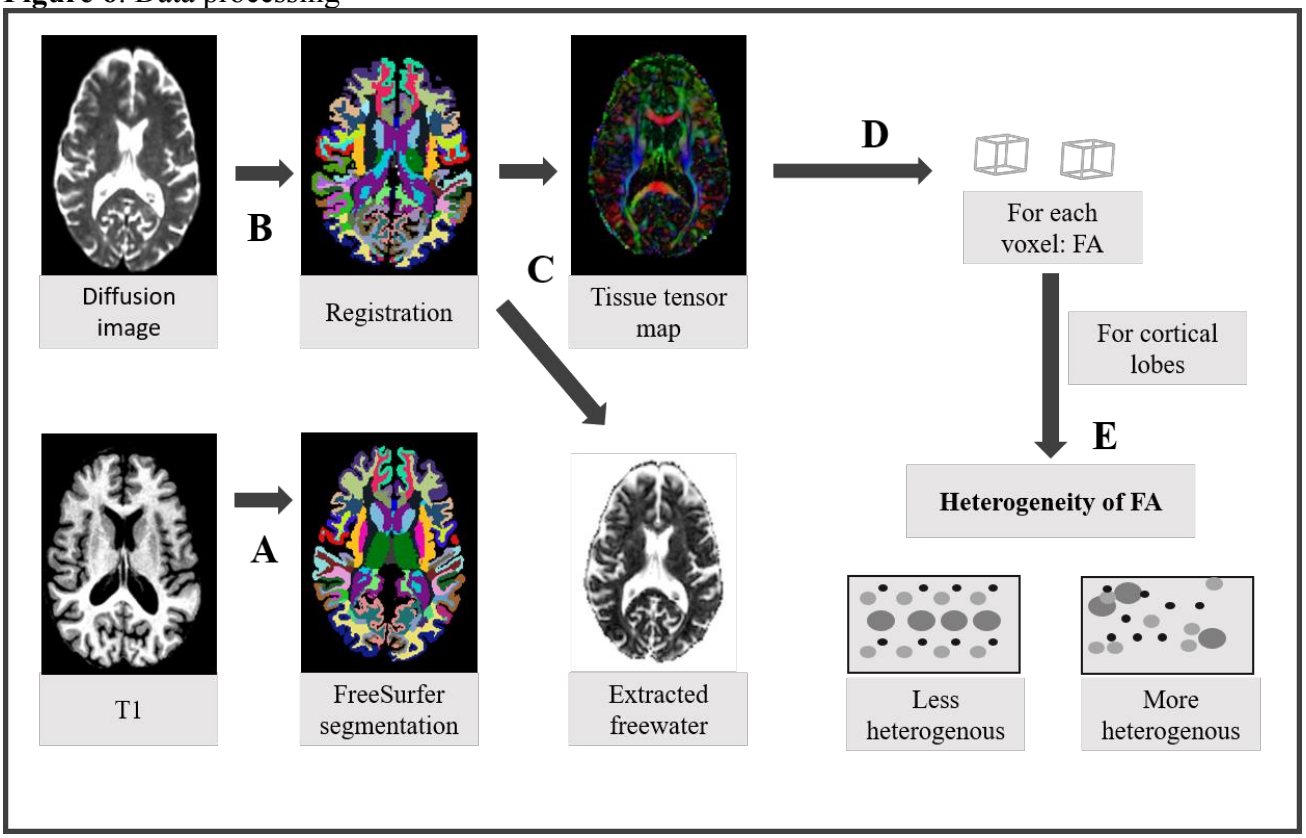
III.2 Methods

Fourty-six patients with SCZ and 37 HC matched on age, sex, parental socioeconomic status, and estimated premorbid IQ matched HC were scanned on a 3 Tesla whole body General Electric MRI scanner (GE Medical Systems, Milwaukee). A high-resolution 3D T1 (IR-FSPGR, TR 7.8 ms, TE 3 ms, TI 600 ms, flip angle 10°, FOV matrix size 256x256, 176 slices, 1mm slice thickness) and a high spatial resolution twice refocused echoplanar DTI sequence (TR=17 s, TE=80 ms, flip angle 90°, FOV 240 x 240 mm, 85 slices, 1.7 mm x 1.7 mm in-plane, 1.7 mm slice thickness, 51 gradient directions with b= 900 s/mm^2, and eight baseline scans with b= 0) were acquired. As described above a scanner update took place during the study and was therefore included as covariate. The sample consisted of two subsamples: patients with early course SCZ (19 patients, 15 HC) and patients with chronic SCZ (27 patients, 22 HC). For image processing please see also Figure 6 (adapted from paper 2 [377]). T1

images were visually inspected, realigned and parcellated using FreeSurfer (Figure 6 A). Diffusion images were corrected for motion, rotation and distortion and registered to the FreeSurfer parcellations (Figure 6 B). We applied a *freewater correction* [378, 379] to the diffusion images. This is, additionally to high spatial resolution, a way to control for partial volume effects. The diffusion signal is therefore separated into two signals- one from the free water compartment (were the diffusion of water molecules is not restricted at all, and follows the self-diffusion coefficient of $3x10^{-3}mm^2/s$ [380, 381]) and one from the tissue compartment (Figure 6 C). After removing the freewater component from the overall signal one can model the diffusion tensors as previously described [382]. After obtaining diffusion properties for each voxel (Figure 6 D), heterogeneity can be calculated for a predefined brain region (Figure 6 E).

We calculated heterogeneity for the four cortical lobes (frontal, parietal, temporal and occipital lobe) and analyzed group differences between patients and HC for early course and chronic SCZ separated using MANCOVAs. Independent variable was group affiliation (patient versus HC), dependent variables were FA, MD, volume, heterogeneity of FA of the cortical lobes. Age, gender, and scanner update were included as covariates. Afterwards post hoc ANCOVS and receiver operating characteristic curves were conducted. Areas under the receiver operating characteristic curves were used to quantify of how well dependent variables predicted group affiliation. All analyses are Bonferroni corrected for multiple testing.

Figure 6: Data processing adapted from paper 2, 377



III.3 Results

For patients with chronic SCZ none of the MANCOVAs showed significant group differences. For patients with early course SCZ the MANCOVA for heterogeneity of FA ($F=5.50$, $df1=4$, $df2=26$, $p<.0020$), but not for FA ($F=2.83$, $df1=4$, $df2=28$, $p<.048$), MD ($F=3.02$, $df1=4$, $df2=26$, $p<.036$) or volume ($F=2.04$, $df1=4$, $df2=26$, $p<12$) showed significant group differences. Subsequently, we conducted ANCOVAs for the four cortical lobes for heterogeneity of FA. Heterogeneity of the frontal lobe showed significant group differences between patients with early course SCZ and HC ($F=10.68$, $df=1$, $p<.0030$). Additionally, heterogeneity of the frontal lobe appeared as excellent group discriminator ($AUC=0.82$, $p<.023$).

III.4 Conclusion

The presented work used a novel DTI measure to investigate age dependent cellular microstructural gray matter changes in SCZ. We saw group differences for heterogeneity of FA in the frontal lobe. Higher heterogeneity in the frontal lobe in patients is indicative for late neurodevelopmental abnormalities. Post mortem studies show that the synaptic reorganization in frontal regions is ongoing beyond adolescence [383-386] and that disruptions of these late neurodevelopmental processes are associated

with SCZ [154, 387]. Heterogeneity may reflect these neurodevelopmental abnormalities of the maturational trajectory of the brain and may therefore serve as a potential biomarker for early disease stages and SCZ risk.

The absence of group differences in the chronic cohort compared to HC suggests no progressive neurodegeneration of microstructural gray matter organization. Consequently, progressive neurodegeneration in SCZ, if existing, may rather be related to extracellular gray matter (e.g. enlarged ventricles) or to white matter pathologies than to microstructural cellular gray matter alterations.

To establish heterogeneity as a biomarker for SCZ risk further studies are needed, which need to include patients at risk to develop SCZ as well as patients with prodromal SCZ and unmedicated SCZ to determine if heterogeneity is altered before disease onset- Finally, heterogeneity could also be used to study the influence of microstructural cellular gray matter and extracellular gray matter changes in other brain disorders like Alzheimer disease or tumors [388, 389] to get deeper insights into underlying pathologies.

IV Original articles

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Tractography Analysis of 5 White Matter Bundles and Their Clinical and Cognitive Correlates in Early-Course Schizophrenia

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Purpose: **Tractography is the most anatomically accurate method for delineating white matter tracts in the brain, yet few studies have examined multiple tracts using tractography in patients with schizophrenia (SCZ). We analyze 5 white matter connections important in the pathophysiology of SCZ: uncinate fasciculus, cingulum bundle (CB), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus, and arcuate fasciculus (AF). Additionally, we investigate the relationship between diffusion tensor imaging (DTI) markers and neuropsychological measures.** ***Methods:*** **High-resolution DTI data were acquired on a 3 Tesla scanner in 30 patients with early-course SCZ and 30 healthy controls (HC) from the Boston Center for Intervention Development and Applied Research study. After manually guided tracts delineation, fractional anisotropy (FA), trace, radial diffusivity (RD), and axial diffusivity (AD) were calculated and averaged along each tract. The association of DTI measures with the Scales for the Assessment of Negative and Positive Symptoms and neuropsychological measures was evaluated.** ***Results:*** **Compared to HC, patients exhibited reduced FA and increased trace and RD in the right AF, CB, and ILF. A discriminant analysis showed the possible use of FA of these tracts for better future group membership classifications. FA and RD of the right ILF and AF were associated with positive symptoms while FA and RD of the right CB were associated with memory performance and processing speed.** ***Conclusion:*** **We observed white matter alterations in the right CB, ILF, and AF, possibly caused by myelin disruptions. The structural abnormalities interact with cognitive performance, and are linked to clinical symptoms.**

Key words: diffusion MRI/positive symptoms/cognitive impairments/lateralization/uncinate fasciculus/cingulum bundle/inferior longitudinal fasciculus/superior longitudinal fasciculus/arcuate fasciculus

Introduction

Although the etiology of schizophrenia (SCZ) remains unknown, pathophysiological evidence from various studies supports the disconnection hypothesis, in which SCZ is proposed to be a syndrome of abnormal integration of functional brain systems eg, Friston[1] and Schmitt et al.[2] It is further suggested that structural changes in the cerebral white matter underlie connectivity abnormalities that result in clinical symptoms and cognitive impairments. Diffusion tensor imaging (DTI) provides a

noninvasive window to investigate tissue microstructure in vivo and makes it possible to study white matter and its microstructural alterations.[3,4] Previous DTI studies of patients with SCZ have reported disruptions in cerebello-thalamo-cortical and fronto-temporal networks.[5–7] Most of these SCZ DTI studies have used fractional anisotropy (FA), an index of white matter organization, and trace, an index of the magnitude of water diffusion, to quantify white matter abnormalities in vivo.

DTI quantitative measurements can be extracted from the diffusion data in several ways. Studies using voxel-based morphometry (VBM) register the brain of every subject to a template, and diffusion measurements are extracted from the corresponding voxels across the entire sample. Though this allows for the study of the entire brain, the required data smoothing and registration steps are problematic.[8] Registration is always performed automatically, without prior anatomical knowledge, and thus misregistration errors are common. To account for such errors, studies use a great variety of smoothing filter sizes. This not only makes results difficult to compare, but also, after smoothing, many voxels do not show a Gaussianity of residuals, a main assumption for parametric statistical approaches, which are used commonly in such analyses. Another approach to data analysis is to use Tract-Based Spatial Statistics (TBSS), which utilizes a white matter skeleton template and results in a lower risk of partial volume effects and greater statistical power.[9] Nonetheless, automatic registration can still lead to misalignment, as it is solely based on FA maps that do not include orientation information.[10] Furthermore, TBSS represents the maximal value projections, which may not be related to potential, subtle structural abnormalities. Finally, DTI measurements can be extracted and compared through manually drawn regions of interest (ROIs). However, implementation of ROI methods can be problematic because a single ROI can include several tracts.[11]

Currently, tractography is the only in vivo method that makes it possible to investigate entire white matter tracts. With this method, unlike with other available tools, microstructural properties of white matter can be measured and averaged over the entire anatomical structure (tracts), which provides greater anatomical specificity and allows for investigating anatomo-functional relationships. To date, however, most SCZ studies have not used tractography because it requires high-resolution data, is computationally expensive, and requires a high degree of anatomical knowledge. Thus, despite many reports of widespread structural white matter abnormalities in patients with SCZ, very few studies[12–14] have combined tractography of several tracts, and, to our knowledge, none have investigated early-course SCZ.

Investigating early-course SCZ is important for understanding the pathophysiology of SCZ, because it minimizes the influence of medication and illness chronicity. Additionally, any potential intervention strategies are likely to have the most impact early in the course of illness, before further alterations occur in the brain.[15] In this study, we investigate 5 major association tracts using tractography in early-course SCZ. We measure FA, trace, radial diffusivity (RD), and axial diffusivity (AD). While FA and trace are often used to describe microstructural white matter pathologies in general, RD and AD can provide greater insights into the potential underlying neuropathology. AD describes the diffusion along the main diffusion direction, while RD captures the diffusion perpendicular to it. AD changes have been reported to be associated with axonal alterations, while RD changes have been reported to be associated with myelin damage.[16] The 5 selected bilateral tracts have all been proposed to play an important role in the pathophysiology of SCZ, including the uncinate fasciculus (UF), cingulum bundle (CB), inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF), and the arcuate fasciculus (AF).[17–19] We focused our analysis only on the main cortico-cortical long association intra-hemispheric fiber tracts, as we believe that these discrete tracts are associated with cognitive domains that are disrupted in SCZ. In this selection process, we excluded other tracts such as the corpus callosum, which have also been associated with SCZ pathophysiology.[20,21]

More specifically, the UF interconnects regions that support acoustic memory, visual information, and emotional response.[22] Studies have reported a reduction in FA in patients with SCZ as well as an association with poorer outcome and negative symptoms (although somewhat inconsistently).[17,18,23,24] The CB is involved in emotional expression, attention, motivation, and working memory processing.[22] Studies have shown DTI abnormalities in CB in first episode and chronic SCZ, but the results are not consistent. Further, an association of DTI measures in CB with executive functioning has been reported.[18,25–29] The ILF is implicated in visual representation, facial recognition, and emotional perception.[22] DTI abnormalities of the ILF appear at different stages of the disease and are likely related to positive symptoms.[19,30,31] White matter abnormalities in SCZ have also frequently been reported in the SLF.[19,32] The SLF is important for spatial attention and memory.[22] Interestingly, most studies investigated the AF and the SLF together, although they are distinct anatomical structures.[30,31] The AF connects cortical areas that are involved in spatial information and language processing and studies have shown that the AF is impaired in SCZ and is associated more with positive symptoms.[33,34]

The inconsistency of the aforementioned reported findings may be due to differences in data acquisition and population characteristics. However, it is also likely that such discrepancies might be the result of differences in study design (eg, the use of VBM methods like TBSS or analysis based on a single ROI which can, as previously noted, lead to imprecise results). Accordingly, using

high-resolution DTI data and tractography to study 5 tracts in a group of patients with early-course SCZ and HC, we matched groups on age, gender, and handedness. We predicted decreased FA and increased trace, RD, and AD in patients with early-course SCZ compared to HC.

We also explored associations between DTI indices and symptom severity and cognitive impairments. Here, we were especially interested in the association of white matter alterations with working memory and processing speed. Both are basic cognitive processes that are critical for a number of higher level operations[35] such as learning, reasoning,[36] encoding and retrieval, and decision making.[37] Furthermore working memory and processing speed influence each other.[38,39] On the one hand both have been reported to be clearly associated with white matter integrity and development.[35,40,41] On the other hand many studies show that these functions are impaired in SCZ.[42–44] In fact, memory and processing speed are the most prominent neurocognitive impairments in early-course SCZ[45,46] and they seem to be more specific to SCZ than other cognitive measurements.[47,48] Additionally, such impairments have also been shown to be common in ultra high-risk individuals who go on to develop psychosis.[44,49] Finally, these cognitive impairments have been associated with poorer functional outcome.[50–52]

Methods

Participants

Thirty individuals with early-course SCZ (1–39 months after disease onset) and 30 HCs were selected based on DTI data availability from the larger sample recruited via the Boston Center for Intervention Development and Applied Research study (CIDAR) study (www.bostoncidar.org, accessed November 19, 2015) (see table 1 and supplementary material 1). SCZ participants were recruited from local hospitals and outpatient clinics, referrals from clinicians, advertisements, and outreach presentations. HCs were recruited from the general community via advertisements.

Clinical diagnoses were based on interviews with the Structured Clinical Interview for the DSM-IV-TR, Research Version (SCID)[53] for ages >18, or the KID–SCID[54] for subjects 13–17 years of age, as well as information from available medical records. All SCZ participants met DSM-IV-TR criteria for SCZ, schizoaffective disorder or schizophreniform disorder. HCs were drawn from the same geographic base as the SCZ group with comparable age, gender, race and ethnicity, handedness, and parental socioeconomic status, and were screened for Axis I disorders using the SCID for DSM-IV-TR, Nonpatient Edition.[55] No HCs met criteria for any current major DSM-IV-TR Axis I disorders, or any history of psychosis, Major Depression (recurrent), Bipolar disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder, or developmental disorders. HCs were also excluded for any history of psychiatric hospitalizations, prodromal symptoms, schizotypal, or other Cluster A personality disorders, first-degree relatives with psychosis, or any current or past use of antipsychotics (other past psychotropic medication use was acceptable, but the individual must have been off medicine for at least 6 months before participating in the study, except for p.r.n. medications such as sleeping or anxiolytic medications). Exclusion criteria for all participants were: sensory-motor handicaps, neurological disorders, medical illnesses that significantly impair neurocognitive function, diagnosis of mental retardation, education <5th grade if <18 years or <9th grade if ≥18, not fluent in English, DSM-IV-TR substance abuse in the past month,

Table 1. Sample Characteristics of Early-Course SCZ and HC Groups

			Statistical Test		
	SCZ (n = 30)	HC (n = 30)	t	Df	P
Age (y)	21.76 ± 4.73[a]	21.88 ± 3.38[a]	0.12	58	.91
Age range (y)	13.92–31.67	14.67–29.25	—	—	—
Gender (male/female)	20/10	18/12	Fisher`s exact test: P = .79		
Race (Caucasian/not Caucasian)	21/9	16/14	Fisher`s exact test: P = .29		
Ethnicity (Hispanic or Latino/not Hispanic or Latino)	6/24	4/26	Fisher`s exact test: P = .73		
Handedness (right/nonright)	24/5	27/3	Fisher`s exact test: P = .47		
Parental socioeconomic status[b]	2.10 ± 1.09[a]	1.83 ± 0.91[a]	−1.03	58	.32
WRAT-4 Reading subtest[87]	105.10 ± 2.45[a]	103.30 ± 2.56[a]	−0.039	57	.97
Education (y)	13.20 ± 2.95[a]	14.17 ± 2.39[a]	1.39	58	.17
WASI	109.3 ± 13.94	115.7 ± 14.16	1.73	56	.0090

Note: SCZ, Schizophrenia; HC, Healthy Controls; WRAT-4, Wide Range Achievement Test-4 (premorbid IQ estimate based on Reading subtest); WASI, Wechsler Abbreviated Scale of Intelligence (current IQ estimate based on Vocabulary and Block Design subtests).
[a]Mean ± SD.
[b]Hollingshead score (1–5 scale, 1 highest).

DSM-IV-TR substance dependence, excluding nicotine, in the past 3 months, current suicidality, a history of ECT within the past 5 years for patients and a history of ECT ever for controls, or study participation by another family member. Prior to MRI scanning, all subjects were screened for foreign metal in their body, pacemakers, pregnancy, claustrophobia, or any other health risks.

This study was approved by the local institutional review board committees at Beth Israel Deaconess Medical Center, the Veterans Affairs Boston Healthcare System (Brockton campus), Harvard Medical School, Brigham and Women's Hospital, and Massachusetts General Hospital. Each subject, or legal guardian for those under 18 years, provided written informed consent before participating.

Image Acquisition

Images were acquired on a 3T whole body scanner (General Electric Medical Systems). Diffusion weighted images were acquired with a high-spatial resolution twice refocused echo-planar imaging sequence (TR = 17 s, TE = 80 ms, flip angle 90°, FOV 240 × 240 mm, 85 slices, 1.7 × 1.7 mm in-plane, 1.7 mm slice thickness, 51 gradient directions with b = 900 s/mm^2, and 8 baseline scans with b = 0). One scanner software upgrade took place during the course of the study. We, therefore, included a covariate for scanner upgrade in our statistical analysis.

Image Processing

Diffusion images were visually inspected, and then corrected for head motion and eddy current distortion by performing an affine registration of each gradient weighted image to the baseline using FLIRT (FSL, Oxford; http://fsl.fmrib.ox.ac.uk/fsl[56], accessed November 19, 2015). A custom in-house script reoriented the corresponding gradient direction based on the computed affine transformation. The resulting corrected images were then input into 3D slicer (www.slicer.org, accessed November 19, 2015), which was used for weighted least square tensor estimation followed by human expert supervised streamline tractography (neuroanatomist NM). ROIs for defining the tracts were drawn, blind to diagnosis, guided by color orientation maps.[57] For further information about the tractography see figures 1 and 2. FA, trace, RD, and AD were calculated and averaged over each tract using MATLAB.[58] To determine reliability, 2 additional raters performed tractography in 10 randomly selected subjects. The intraclass correlation coefficients for FA were greater than 0.79.

Clinical Symptoms and Cognitive Tests

Trained and skilled interviewers and neuropsychological testers conducted all clinical and cognitive assessments. The Scale for the Assessment of Negative Symptoms (SANS)[59] and the Scale for the Assessment of Positive Symptoms (SAPS)[60] were used to measure symptom severity. In addition to the total scores, SANS and SAPS global ratings were examined (supplementary material 1). The symbol coding test and the Trail Making Test-Part A were used for processing speed, and the Spatial Span and Letter-Number Span tests were used for assessing working memory (table 2), following standardized guidelines for examining these functions in SCZ.[35,36] All tests were part of an extensive neuropsychological test battery for all studies that were part of the Boston CIDAR studies.

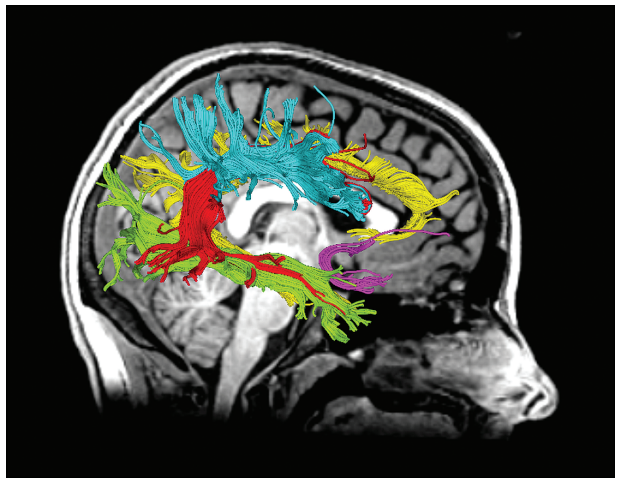

Fig. 1. Tractography of 5 white matter tracts. Sagittal view of the tractography of the inferior longitudinal fasciculus (green), the superior longitudinal fasciculus (blue), arcuate fasciculus (red), uncinate fasciculus (pink), and the cingulum bundle (yellow).

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences version 22.0[61] and Prism.[62]

Group Differences. We conducted 4 separate MANCOVAs (1 for each FA, trace, RD, and AD). Dependent variables were, respectively, diffusion parameters of the 5 tracts (eg, dependent variables in the FA MANCOVA were FA of AF, FA of UF, FA of SLF, FA of CB, and FA of ILF). For each test, independent variables of interest were group, hemisphere, and group × hemisphere, with scanner upgrade, gender, age, and handedness as covariates. In case of a significant group effect (P < .013- Bonferroni corrected for 4 tests) post hoc ANCOVAs were calculated for each tract separately. Dependent variables were FA/trace/RD/AD, and independent variables and covariates were the same as in the MANCOVA. In case of a significant group effect for a tract in the ANCOVA, 2-tailed t tests were performed to investigate whether group differences appeared for both or for 1 hemisphere only.

Since we wanted to explore the clinical relevance of white matter abnormalities found with DTI and their potential use for future group membership, we conducted

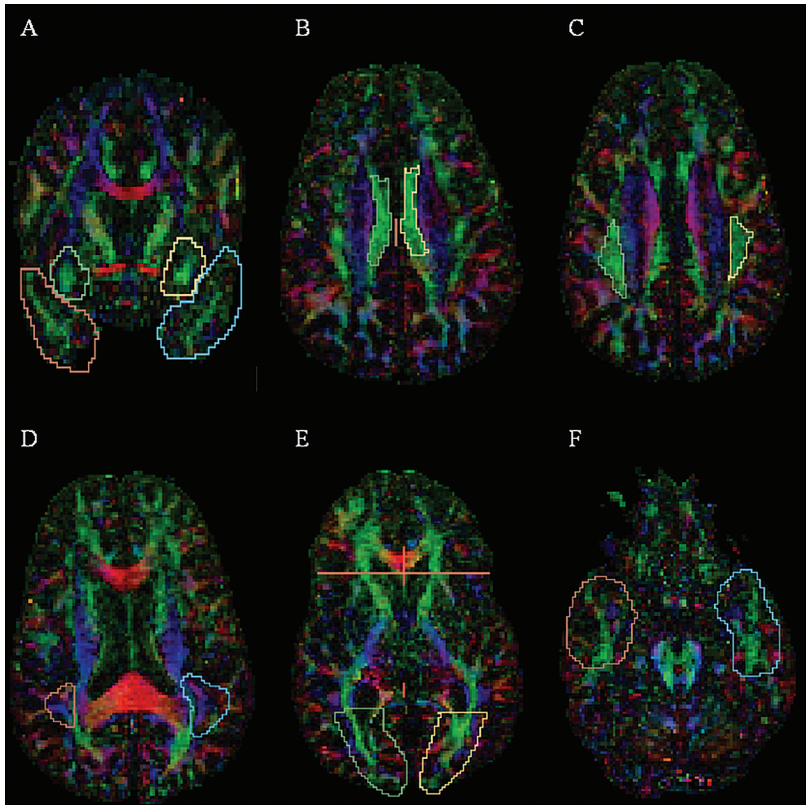

Fig. 2. Region of interest (ROI) placement. ROIs were drawn blind to diagnosis, guided by color orientation maps,[54] and supervised by a neuroanatomist (NM). **Uncinate fasciculus:** 1 seeding (green and yellow) and 1 inclusion (red and blue) ROI 2 slices anterior to the coronal slice where the temporal stem no longer connects to the rest of the temporal lobe (A). **Cingulum bundle**: 1 seeding ROI on approximately 20 axial slices (B). **Superior longitudinal fasciculus (SLF) and arcuate fasciculus (AF):** Initial seeding ROI on 5 axial slices lateral to the corona radiata for both tracts (C), additional ROI where the AF curves down into the temporal lobe (exclusion ROI for the SLF and inclusion ROI for the AF) (D). **Inferior longitudinal fasciculus**: Occipital ROI on 12 axial slices (E) and temporal ROI on 5 axial slices in the anterior portion of the temporal lobe (F).

a discriminant analyses. Discriminant analyses are used to predict memberships to mutually exclusive groups (the grouping variable here was: HC vs patients with SCZ). The independent variables were the FA values of the 10 tracts. Since we were interested in the possible impact of all these variables, we entered them all together, rather than choosing a stepwise approach.

Clinical Symptoms and Cognitive Tests. We conducted Spearman's correlation analyses between overall SANS and SAPS scores, SANS/SAPS subscales, and cognitive measures, with DTI measurements. We chose Spearman`s rank-order correlations, instead of Pearson product moment correlation, because clinical symptom and cognitive test results are ordinal, rather than continuous variables and, therefore, not necessarily normally distributed. This analysis was followed by the Fisher`s Exact Score to test whether these correlation coefficients significantly differ from 0.

Additional Analyses. We used correlation analysis to investigate associations between DTI measurements and duration of illness, medication dose, and age of onset. We used Spearman's R to examine the correlations, because not all measurements were normally distributed (as shown by Shapiro-Wilk tests).

Results

Group Differences

The MANCOVA for FA (table 2 and figure 3) revealed significant main effects for group and hemisphere but no significant group × hemisphere interaction. Post hoc ANCOVAS for each of the 5 tracts separately showed significant group effects for AF, CB, and ILF. Post hoc 2 tailed *t* tests for the left and right AF, CB, and ILF showed significant group differences for the right hemisphere only. The MANCOVA for trace showed significant main effects for group and hemisphere, but no group × hemisphere interaction. Post hoc ANCOVAS revealed significant group effects for CB and ILF. Post hoc 2-tailed *t* tests for left and right CB and ILF revealed significant group differences for right CB, right ILF, and left ILF. The same MANCOVA for AD revealed a significant main effect for hemisphere only. The MANCOVA for RD showed significant group and hemisphere main

Table 2. Significant Group Differences Between Patients With SCZ and HC

		FA	Trace	AD	RD
MANCOVA	Hemisphere	($F = 44.46$, $df = 5$, $P < .0001$)	($F = 12.99$, $df = 5$, $P < .0001$)	($F = 27.23$, $df = 5$, $P < .0001$)	($F = 16.30$, $df = 5$, $P < .0001$)
	Group	($F = 5.27$, $df = 5$, $P < .0001$)	($F = 3.33$, $df = 5$, $P = .008$)	—	($F = 4.92$, $df = 5$, $P < .0001$)
Post hoc ANCOVA	Group	AF ($F = 5.94$, $df = 1$, $P = .016$) CB ($F = 9.35$, $df = 1$, $P = .003$) ILF ($F = 14.77$, $df = 1$, $P = .004$)	CB ($F = 8.15$, $df = 1$, $P = .005$) ILF ($F = 11.17$, $df = 1$, $P = .001$)	—	AF ($F = 5.60$, $df = 1$, $P = .020$) CB ($F = 11.55$, $df = 1$, $P = .0010$) ILF ($F = 13.25$, $df = 1$, $P < .0001$)
Post hoc 2-tailed *t* tests	Group	Right AF ($t = 2.87$, $df = 55$, $P = .0061$) Right CB ($t = 2.29$, $df = 58$, $P = .027$) Right ILF ($t = -3.28$, $df = 58$, $P = .0020$)	Right CB ($t = 2.02$, $df = 58$, $P = .048$) Right ILF ($t = 2.54$, $df = 58$, $P = .014$) Left ILF ($t = 2.13$, $df = 58$, $P = .038$)	—	Right AF ($t = 2.46$, $df = 55$, $P = .018$) Right CB ($t = 2.49$, $df = 58$, $P = .017$) Right ILF ($t = 3.40$, $df = 58$, $P = .001$) Left ILF ($t = 2.27$, $df = 58$, $P = .028$)

Note: FA, Fractional Anisotropy; AD, Axial Diffusivity; RD, Radial Diffusivity; AF, Arcuate Fasciculus; CB, Cingulum Bundle; ILF, Inferior Longitudinal Fasciculus.

effects, but no group × hemisphere interaction. Post hoc ANCOVAs showed significant group effects for AF, CB, and ILF. Post hoc 2-tailed *t* tests were significant for right AF, right CB, right ILF, and left ILF.

In the discriminant analysis, 80% of the population could be classified correctly into groups (Wilks Lambda = .63, $X^2 = 22.45$, $df = 10$, $P = .013$). The proportion of variance explained by FA was 60% (indicated by an eigenvalue of .60). The right ILF (correlation with discriminant function = .53), right AF (correlation with discriminant function = .45) and right CB (correlation with discriminant function = .31) contributed the most for predicting group affiliation. These findings suggest that the FA values of the 3 tracts which showed white matter alterations have the most potential for future group membership classifications.

Clinical Symptoms and Cognitive Tests

The *P* values for all reported results were significant by Bonferroni correction (supplementary material 2).

The correlation of FA of the right ILF, AF, and CB with the SANS and SAPS total scores revealed a significant correlation between right ILF and SAPS score ($\rho = -0.37$, $P = .0034$). Further correlation analyses of the right ILF with the 4 SAPS subscales showed significant correlations with hallucinations ($\rho = -0.36$, $P = .0050$) and delusions ($\rho = -0.37$, $P = .0039$). Correlations between RD of the right ILF, AF, CB, and the left ILF with the SAPS and SANS total scores revealed a significant correlation between right AF ($\rho = 0.37$, $P = .0046$) and right ILF with total SAPS score ($\rho = 0.40$, $P = .0016$). Further correlation analysis of these 2 tracts with the 4 SAPS subscales revealed a significant correlation for the right ILF with delusions ($\rho = 0.41$, $P = .0011$).

When correlating the FA of the right AF, CB, and ILF with the results from the neuropsychological tests, we found statistically significant correlations between right CB with symbol coding ($\rho = 0.37$, $P < .0039$) and letter number sequencing test ($\rho = 0.38$, $P < .0034$). The same correlation analysis for RD of the right AF, CB, ILF, and left ILF showed a significant correlation between the right CB and the symbol coding test ($\rho = -0.38$, $P < .0031$).

Additional Analysis

The correlation analysis of DTI measurements with age of onset, duration of illness, and chlorpromazine equivalent medication dose did not reveal any significant results (supplementary material 3).

Discussion

Using tractography, we reported white matter abnormalities in 3 out of 5 major fiber bundles (ILF, AF, and CB, but not SLF and UF) in early-course SCZ compared to a well-matched HC group. To the best of our knowledge, this is the first comprehensive study of white matter association fiber tracts to investigate connectivity disturbances in early-course SCZ. More specifically, we observed FA reduction with trace and RD increase for the CB, ILF, and AF tracts in the right hemisphere. A reduction of FA is thought to indicate alterations in white matter organization. A significant increase of RD with no concomitant changes in AD suggests that the alterations could be related to disrupted myelin.[16]

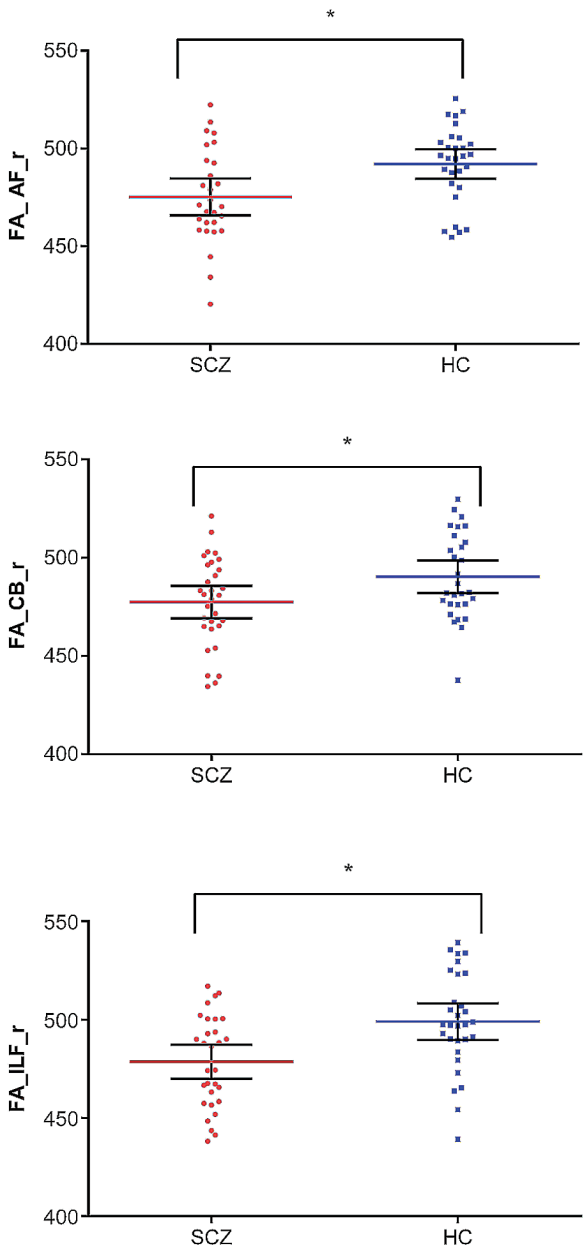

Fig. 3. Group differences of FA between patients with schizophrenia (SCZ) and healthy control individuals (HC). Group differences of fractional anisotropy of the right arcuate fasciculus (FA_AF_r), the right cingulum bundle (FA_CB_r) and the right inferior longitudinal fasciculus (FA_ILF_r) between patients with SCZ and HC.

While one needs to be careful in interpreting diffusion measures[63] as other anatomical (micro and macro structural) changes could also explain the abnormalities in these indices,[64,65] the associations of these measures with clinical and cognitive performance increase the likelihood that the observed patterns of abnormalities portray structural deficiencies that affect the function of the fibers.

We found that diffusion measures of the right CB were associated with performance on 2 cognitive tests (symbol coding and letter-number sequencing test). Structural abnormalities of the CB[25] and an association with executive functioning[28,29,66] have been reported previously. Our study adds to the current literature by showing an association between white matter alterations of the CB and processing speed and working memory, arguably measures of executive functioning. Finally, reduced FA in the right ILF and increased trace and RD in the right and left ILF were correlated with positive symptom scores.

Contrary to previous studies, we did not find any group differences for the SLF.[67,68] With respect to SLF, as mentioned previously, since SLF and AF are 2 separate anatomical structures that are involved in different cognitive processes, we analyzed them separately, whereas most previous studies have combined them for further analyses. Diffusion measures of the AF were associated with positive symptoms, which may reflect an impairment of the language processing system leading to hallucinations and delusions. This finding demonstrates the importance of separating SLF and AF in future studies.

We did not report any group differences for the UF. Results of previously published studies investigating UF are also inconsistent.[24,69] Some studies suggest that abnormalities in this structure are very subtle,[69,70] and might be associated with poor outcome and negative symptoms.[18,71] Of further note, poorer outcome and negative symptoms are more frequently found in chronic than in early-course SCZ. UF may thus be more relevant to progressive changes observed at later stages of the disease in those who evince a chronic course of illness.

It is important to note that almost all group differences in our study are observed in the right hemisphere. A similar lateralization pattern has been found in studies of individuals at clinical high risk of psychosis for white[72] and gray matter[73] and in patients with a first episode of SCZ,[74–76] but not in chronic SCZ. It has also been demonstrated that the right AF is particularly associated with positive symptom scores.[34,77] This may further suggest that white matter changes in the right hemisphere are typical for early stages of disease, suggesting a lack of lateralization or a particular (probably neurodevelopmental) early pathological disruption of white matter maturation in the right hemisphere. However, it has to be noted that most of our subjects are right-handed. Therefore, even though we controlled for the effect of handedness in our analysis, future studies need to investigate further the influence of handedness on white matter tracts in early-course SCZ.

Limitations and Future Directions

White matter changes in SCZ can be influenced by many potential factors, such as gender, age,[12,13] duration of illness,[17,19,78] age of onset,[79] type and severity of symptoms,[80,81] and medication.[67,82] Since every cohort varies on these parameters, it is difficult to generalize the results.

However, in our study, we controlled for gender and age, and our analyses showed that neither duration of illness, medication dosage, nor age of onset affected the DTI measures.

By choosing 5 white matter tracts, we excluded other tracts such as the corpus callosum,[20,21] the fornix,[83] or the internal capsule,[84] which have been reported to play a role in the pathophysiology of SCZ. Future studies should include even more tracts to have a more complete picture of the underlying white matter architecture.

Finally, tractography, although currently the most accurate method for investigating white matter in vivo, has its own limitations.[85] For example, averaging all voxels over the tract leads to loss of local information. Additionally, single tensor tractography is limited in the case of crossing fibers, suggesting that future studies need to attend to more precise methods for modeling water diffusion within a voxel, such as multi-tensor tractography.[86]

Conclusion

This is, to our knowledge, one of the first comprehensive investigations of white matter anatomy, pathology, and function in early-course SCZ. In this study, we used manually guided diffusion tractography, the most anatomically accurate way to investigate white matter structures, to examine 5 major association white matter tracts in a population of early-course SCZ, compared to HC. Changes observed were anatomically specific to ILF, CB, and AF, and only to the right side. We demonstrated further functional specificity of observed pathology, ie, CB was associated with processing speed and working memory, and ILF was associated with delusions. We, therefore, believe that our study will add to establishing DTI as a viable tool for investigating the nature of early pathology in SCZ, and as such may lead to imaging biomarkers that might prove useful for the detection of white matter disconnectivity, treatment monitoring, and outcome prediction in SCZ.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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ORIGINAL RESEARCH

Alteration of gray matter microstructure in schizophrenia

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Abstract Neuroimaging studies demonstrate gray matter (GM) macrostructural abnormalities in patients with schizophrenia (SCZ). While ex-vivo and genetic studies suggest cellular pathology associated with abnormal neurodevelopmental processes in SCZ, few in-vivo measures have been proposed to target microstructural GM organization. Here, we use diffusion heterogeneity- to study GM microstructure in SCZ. Structural and diffusion magnetic resonance imaging (MRI) were acquired on a 3 Tesla scanner in 46 patients with SCZ and 37 matched healthy controls (HC). After correction for free water, diffusion heterogeneity as well as commonly used diffusion measures FA and MD and volume were calculated for the four cortical lobes on each hemisphere, and compared between groups. Patients with early course SCZ exhibited higher diffusion heterogeneity in the GM of the frontal lobes compared to controls. Diffusion heterogeneity of the frontal lobe showed excellent discrimination between patients and HC, while none of the commonly used diffusion measures such as FA or MD did. Higher diffusion heterogeneity in the frontal lobes in early SCZ may be due to abnormal brain maturation (migration, pruning) before and during adolescence and early adulthood. Further studies are needed to investigate the role of heterogeneity as potential biomarker for SCZ risk.

Keywords Diffusion MRI · Heterogeneity · Schizophrenia · Neurodevelopment · Gray matter

Introduction

Schizophrenia (SCZ) is a severe psychiatric disorder with significant consequences for affected patients and society as a whole (Ratnasingham et al. 2013; Rössler et al. 2005; Whiteford et al. 2013). Patients with SCZ exhibit several

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macrostructural brain abnormalities including reduced regional gray matter volume and enlarged ventricles. The biological interpretations of these macrostructural gray matter alterations include abnormal cortical development, differential maturational trajectories (e.g., Nesvag et al. 2014; Vogeley et al. 2000), and possible neurodegeneration and/or accelerated aging (Assunção Leme et al. 2013; Egashira et al. 2014; Hulshoff Pol and Kahn 2008). However, since volumetric measures are biologically non-specific and appear to be present at all stages of the illness, associations of macrostructural changes with potential microstructural pathology have been difficult.

Diffusion tensor imaging (DTI) (Basser et al. 1996; Pierpaoli and Basser 1996) has been demonstrated to be sensitive to microstructural alterations (Spoletini et al. 2011). DTI provides information about the motion of water within brain tissue by quantifying the extent and directional preference of diffusion. Brain alterations at the cellular level lead to local modifications in water diffusion and can be detected by DTI even when macrostructural features do not change (Fjell et al. 2008). So far, DTI has primarily been used to evaluate white matter microstructure in SCZ (Kubicki et al. 2007) and very few SCZ gray matter DTI studies have been conducted (Anderson et al. 2013; Lee et al. 2009; Moriya et al. 2010; Park et al. 2014; Shin et al. 2006).

The most common DTI metrics that are being used to evaluate microstructural brain alterations include fractional anisotropy (FA), an index of white matter axonal organization and coherence, and mean diffusivity (MD), an index of overall white matter water content. While diffusion anisotropy and orientation of the fibers are of interest in white matter, both these measures are less relevant in gray matter. This is because the diffusion properties of gray matter reflect the diffusion of water hindered by cell bodies and their processes, while in white matter diffusion properties reflect the diffusion of water within bundles of long myelinated axons. Therefore, in gray matter there is no preferred direction of orientation that can be measured with current clinical imaging data resolution. It has been shown that the diffusion measures within the same anatomical structure in gray matter vary from voxel to voxel much more than in white matter (Vollmar et al. 2010). This variance in diffusion properties does not only indicate that FA or MD of a single voxel may not be appropriate to study gray matter, but it also makes these metrics insensitive to the detection of subtle abnormalities in the cortex, even when averaged over larger regions.

Therefore, we suggest that in addition to existing diffusion indices, it is important to consider DTI measurements that would capture cortical organization on a bigger scale, i.e. inter, rather than intra-voxel. For example, Kalus et al. (Kalus et al. 2004; Kalus et al. 2005a, 2005b) conducted inter-voxel coherence studies and they report reduced coherence in the amygdala and parahippocampical regions in patients with SCZ, which they interpreted to represent differences in local contents of ordered fiber systems.

Another possible measurement capturing inter-voxel organization was recently introduced by Rathi et al. (2014). It is derived from the diffusion signal and called "heterogeneity". Heterogeneity is a statistical measure that determines the variability of microstructural gray matter tissue properties reflected by diffusion measures within a given region of interest (ROI); e.g. the heterogeneity of FA (HFA) reflects the variability of FA within a defined ROI. It can therefore separate the effect of anisotropic diffusion from that of overall measure of diffusivity (MD). In contrast to other inter-voxel coherence measurements, one does not need to choose reference voxels and it can therefore be computed in arbitrarily large or small regions (e.g. for the four cortical lobes).

Normal, healthy cortex is characterized by relatively low heterogeneity of diffusion properties in gray matter, suggesting a consistent uniform tissue organization (Rathi et al. 2014). However, less consistent cellular organization within gray matter tissue, a result of possible age-dependent reorganization or faulty neurodevelopment of the cortex, would lead to more heterogeneous diffusion behavior that would be reflected as greater variability of diffusion properties, resulting in increased heterogeneity. Thus, heterogeneity has the potential to serve as a more sensitive indicator of microstructural organization of gray matter tissue than FA or MD to detect alterations in tissue organization, even in the absence of macrostructural volumetric changes.

The aim of the current study is to use heterogeneity to investigate gray matter microstructural organization in patients with SCZ. For reasons mentioned above, we expect our method to be more sensitive for differentiating between groups than traditional diffusion measurements, such as FA, MD or macrostructural gray matter measurements, i.e., cortical volume (Arnold 2001). We will apply heterogeneity in a cross-sectional study design to patients at different disease stages (early course and chronic) and well matched healthy control (HC) group. We predict higher gray matter heterogeneity in patients with SCZ (but not necessarily higher FA or MD), which we propose would reflect abnormal cortical organization in SCZ (Teffer and Semendeferi 2012).

Methods

Participants

Forty-six patients (9 females, 37 males) with SCZ and thirty-seven healthy control subjects (HC) (10 females, 27 males) were recruited from the Boston area. The entire sample was separated into two sub groups- patients within three years of onset of SCZ (19 patients, 15 HC) and patients with chronic SCZ (27 patients, 22 HC) (Supplement 1). Patients with early

course SCZ were recruited via the Boston Center for Intervention Development and Applied Research (CIDAR study) from outpatient clinics. Patients with a more chronic illness were recruited from the Veteran Affairs Boston Healthcare System, Brockton Division, MA.

Groups were matched on age, sex, handedness, parental socioeconomic status, and estimated premorbid intelligence (Reading scale of Wide Range Achievement Test- 3 (Wilkinson 1993)) (Table 1). Diagnoses were based on a diagnostic interview using the Structured Clinical Interview for the DSM-IV-TR, Research Version (SCID) (First et al. 2002b) for ages >18, or the KID–SCID (Hien et al. 1994) (for subjects 15–17 years of age). Patients were excluded if they had a history of electroconvulsive therapy within the past 5 years. Control subjects were screened for the presence of an Axis I disorder using the Structured Clinical Interview for DSM-IV-TR, Non-patient Edition (First et al. 2002a) and were excluded if they: 1) currently met criteria for any psychosis, major depressive disorder, dysthymic disorder, bipolar disorder, obsessive compulsive disorder, post-traumatic stress disorder, dissociative disorders, anorexia nervosa, bulimia nervosa, or developmental disorders; 2) had a history of any psychosis, major depression (recurrent), bipolar disorder, obsessive compulsive disorder, post-traumatic stress disorder, developmental disorder, or psychiatry hospitalization; 3) had current or past use of antipsychotics for any psychiatric condition (other past psychotropic medication use acceptable, but must been off medicine for at least 6 months before participating in the study, except for as the circumstances required medications such as sleeping medications or anxiolytic agents, or beta-blockers for performance anxiety, tremors, etc.); 4) had any history of ECT; 5) had evidence of any prodromal symptoms, or schizotypal or other Cluster A personality disorders; or 6) reported having a first-degree relative with psychosis.

For all subjects, exclusion criteria included sensory-motor handicaps (e.g. severe visual or auditory problems), neurological disorders, medical illnesses that significantly affect neurological functioning, diagnosis of mental retardation, education of less than 9th grade (or less than 5th grade for subjects under 18), non-fluency in English (exposure to English by age 6), substance abuse in the past month as defined by the DSM-IV-TR, substance dependence (excluding nicotine) in the past 3 months as defined by the DSM-IV-TR, and current suicidality. For their safety during the MRI scanning, all subjects were screened for foreign metal in their body, pacemakers, pregnancy, claustrophobia, or any other circumstance that might pose a health risk.

The Institutional Review Boards of the Veteran Affairs Boston Healthcare System, Brigham and Women's Hospital, Beth Israel Deaconess Medical Center, Massachusetts Department of Mental Health Central Office Research Review Committee, and Harvard Medical School approved the study. Each subject or legal guardian for those under

Table 1 Demographic characteristics for patients with schizophrenia (SCZ) and healthy control individuals (HC)

	SCZ	HC	Two sample t-test or Pearson-Chi-square test (2-tailed)		
			t	df	p
Number	**46**	**37**	-	-	-
Age (years)	36.07 ± 14.01[a]	36.27 ± 12.51[a]	-.070	81	.95
(Age range)	**(15.63–56.92)**	**(17.37–53.42)**	-	-	-
Gender	9f, 36 m	10f, 27 m	.65	1	.42
Handedness	40 left, 3 both, 3 right	36 right 1 both	3.27	2	.20
Parental socioeconomic status (PSES)[b]	2.52 ± 0.19[a]	2.25 ± 0.16[a]	1.07	80	.29
premorbid IQ (Wilkinson 1993)	105.80 ± 2.69[a]	104.50 ± 2.93[a]	0.31	61	.76
Scanner update (before/after)	25/21	17/20	.58	1	.45
Age of onset	24.26 ± 7.59[a]	-	-	-	-
Duration of illness SCZ	9.15 ± 10.25[a]	-	-	-	-
Medication dose SCZ (chlorpromazine equivalent dose)[c]	603.40 ± 913.90[a]	-	-	-	-
SANS [d]	32.05 ± 16.95[a]	-	-	-	-
SAPS [e]	27.18 ± 24.03[a]	-	-	-	-

[a] Mean ± Standard deviation ; [b] Higher numbers represent lower PSES; [c] Following the international consensus study of antipsychotic dosing (Gardner et al. 2010);

[d] SANS = The Scale for the Assessment of Negative Symptoms (N. C. Andreasen 1984a); [e] SAPS = Scale for the Assessment of Positive Symptoms (N. C. Andreasen 1984b)

Clinical and demographical information was not available for all subjects participating in this study

18 years provided understanding and written informed consent before participating.

Image acquisition

All images were acquired on a 3 Tesla whole body General Electric MRI scanner (GE Medical Systems, Milwaukee). The MRI sequences included a high-resolution 3D T1 (IR-FSPGR, TR 7.8 ms, TE 3 ms, TI 600 ms, flip angle 10°, FOV matrix size 256 × 256, 176 slices, 1 mm slice thickness). Diffusion weighted images (DWIs) were acquired using a twice refocused echo-planar imaging sequence (TR 17 s, TE 80 ms, flip angle 90°, FOV matrix size 144 × 144, 85 slices, 1.7 mm slice thickness, 51 gradient directions with b = 900 s/mm^2 and eight baseline scans with b = 0 stacked at the beginning of the image sequence). During the course of the study a scanner software upgrade took place. Even so HC and patients with SCZ did not differ in how many subjects were scanned before and after scanner upgrade (see Table 1), we included scanner update as a covariate in our analysis.

Image processing

For further information on the imaging process, see Fig. 1. The data was visually inspected for artifacts and signal drops. We checked our data for outliers (3 x interquartile range) and found that no subjects needed to be excluded from the analyses.

The T1 images were manually realigned and parcellated into 176 Gy matter, white matter, and cerebrospinal fluid regions using the FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/). The DWIs were corrected for motion by means of affine registration with a reference b0 volume (FLIRT, FSL, Oxford; http://fsl.fmrib.ox.ac.uk/fsl (Jenkinson et al. 2002)). Diffusion gradients were compensated for rotation and distortion. Next, the T1 FreeSurfer segmentations were registered to the DWIs using non-linear registration (FNIRT, FSL, Oxford; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT).

To minimize the impact of partial volume effects on diffusion measures, we applied a free water correction to the DWI data (Pasternak et al. 2009). The removal of the free water influence is especially important for gray matter studies, because it allows for more accurate diffusion parameters that are less influenced by the partial volume effects of extracellular components, and are thus more specific to cellular gray matter structure (Koo et al. 2009). For the free water correction, the diffusion signal is separated into two compartments - the free water and the tissue compartment. It is assumed that the diffusion of water molecules in the free water compartment is not restricted by any surroundings and can therefore be described as Gaussian distributed Brownian motion (Beaulieu 2002) with a self-diffusion coefficient of approximately 3×10^{-3} mm^2/s (Holz et al. 2000; Mills 1973). The diffusion in this environment is much greater than in cortical tissue where cellular processes lead to a hindered displacement profile (Assaf and Basser 2005; Helenius et al. 2002). This free water contribution can be removed from the overall diffusion signal, which is then described by the classical diffusion tensor model (Basser and Pierpaoli 1996). The diffusion parameters, such as FA and MD, are then calculated for this remaining tissue compartment for each voxel. These free water corrected diffusion measures will most likely capture tissue processes or extracellular processes in proximity to cellular membranes and therefore also specific to tissue changes (Pasternak et al. 2009).

Afterward free water correction, heterogeneity was calculated. Heterogeneity is mathematically defined as:

$$H(m) = \frac{1}{N^2}\sum_{i=1}^{N}\sum_{j=1}^{N}\left|\left|\mathrm{m_i}-\mathrm{m_j}\right|\right|$$

where N is the number of voxels in the ROI, and m is the value of a given diffusion measurement (such as FA) in a voxel, indexed by $_i$ or $_j$. Heterogeneity describes the statistical variability of a diffusion measurement in an ROI, while being more robust than classical variability measures like variance (Rathi et al. 2014).

The heterogeneity of FA (HFA) for four cortical lobes (frontal, parietal, temporal, occipital) was calculated using Matlab (TheMathWorks). The four cortical lobes were delineated by combining all corresponding FreeSurfer ROIs (e.g. the frontal lobe was generated by combining the 12 right frontal gray matter FreeSurfer ROIs with the 12 left frontal gray matter FreeSurfer ROIs) (Desikan et al. 2006).

Statistical analysis

Statistical Analyses were performed with GraphPad Prism 6 (GraphPadSoftware 2014) and the Statistical Package for Social Sciences version 22.0 (IBMCorp 2013).

To replicate and extend the findings of Rathi et al. (2014), we focused our initial analysis on the association of HFA, FA, MD, and volume with age by calculating correlations (Pearson r) for the entire SCZ and HC group separately, then testing with an F-Test if the correlation coefficients were significantly different from zero (adjusted $p < .0063$).

Next, we separated the entire sample into the two subgroups (patients within three years of onset versus patients with chronic SCZ and their matched HCs).

We analyzed group differences using MANCOVAs (one for each measurement- HFA, FA, MD, volume) for both sub-groups. The independent variable of interest was group affiliation (SCZ versus HC), whereas the dependent variables were HFA/FA/MD/volume of the frontal, parietal, temporal, and occipital regions, while age, gender and scanner update were included as covariates. In the case of a significant group difference, we examined this further using post hoc

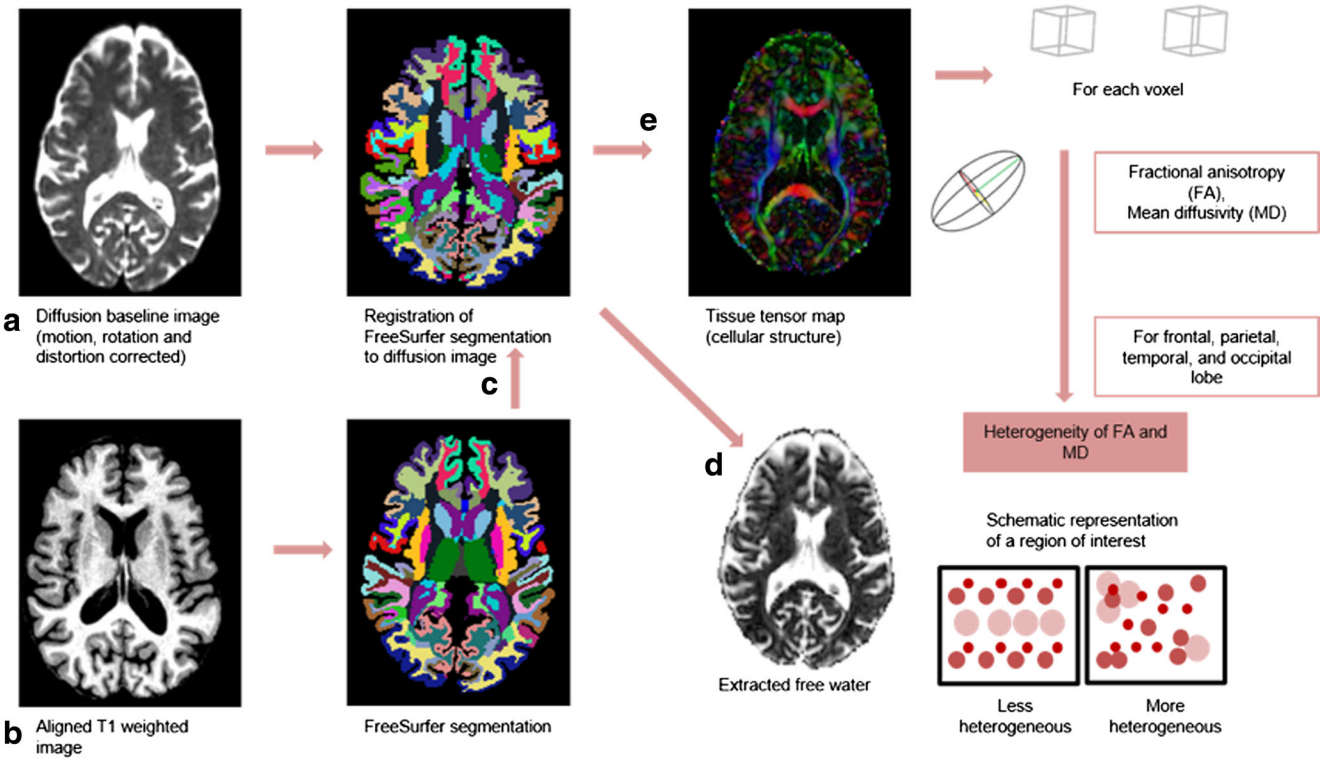

Fig. 1 Image processing (**a**) Diffusion images were motion, distortion and rotation corrected (FLIRT, FSL, Oxford; http://fsl.fmrib.ox.ac.uk/fsl (Jenkinson et al. 2002)) and (**b**) structural images were realigned and parcellated using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). (**c**) This segmentation was non-linearly registered (FNIRT, FSL, Oxford; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FNIRT) to the diffusion images. A free water correction was applied to separate the signal into a "free water" signal (**d**) and the tissue tensor map (**e**). FA and MD were calculated for each voxel of this tissue tensor map. Afterwards heterogeneities of FA and MD were computed for the frontal, parietal, temporal and occipital lobes

ANCOVAs for the four cortical lobes with group as independent variable, HFA as dependent variable, and age, scanner update, and gender as covariates.

In the case of a significant ANCOVA, receiver operating characteristics (ROC) curves were created for HFA, FA, MD, and volume and then the areas under the ROC curve (AUCs) were calculated. ROC curves provide a way to quantify how well a measure, for instance heterogeneity, can discriminate between two groups, in our case the HC and patient groups. AUCs were interpreted following Hosmer and Lemeshow (Hosmer and Lemeshow 2000), where higher values stand for better prediction.

To further elucidate the association of group differences with clinical variables, we used partial correlation analysis (corrected for age) in case of significant group differences between diffusion measurements and duration of illness, medication dose, and age of onset for patients with early course SCZ.

Results

Correlation of HFA with age

There was a positive association between both HFA and FA with age, whereas MD and volume exhibited negative correlations with age in patients and HC (Table 2).

Difference between SCZ and HC

Patients with chronic SCZ

None of the MANCOVAs showed a significant group difference ($p < .013$, Bonferroni corrected for four tests) for HFA, FA, MD, and volume between patients with chronic SCZ and controls (Table 3).

Patients with early course SCZ

The MANCOVA for HFA showed significant group differences ($p < .013$, Bonferroni corrected for four tests) ($F = 5.50$, $df1 = 4$, $df2 = 26$, $p = .0020$), whereas the MANCOVAs for FA, MD, and volume did not (Table 3).

Due to the significant group effect, we conducted post hoc ANCOVAs for the frontal, parietal, temporal, and occipital regions with group as the independent variable, HFA as the dependent variable, and age, scanner update, and gender as covariates. Only the HFA of the frontal region showed significant ($p < .013$, Bonferroni corrected for four tests) group

Table 2 Correlation coefficients (Pearson) for patients with schizophrenia (SCZ) and healthy control individuals (HC) of HFA, FA, MD and Vol with age

	HFA SCZ	HFA HC	FA SCZ	FA HC	MD SCZ	MD HC	Vol SCZ	Vol HC
Frontal	0.63*	0.77*	0.54*	0.66*	-0.69*	-0.58*	-0.58*	-0.46*
Parietal	0.67*	0.51*	0.71*	0.54*	-0.57*	-0.48*	-0.41*	-0.44*
Temporal	0.41*	0.34	0.48*	0.56*	-0.50*	-0.40	-0.47*	-0.47*
Occipital	0.63*	0.25	0.67*	0.48*	-0.55*	-0.21	-0.032	-0.39

*indicates statistically significant correlation with $p < .0063$ (Bonferroni Correction, $n = 8$)

HFA = heterogeneity of fractional anisotropy; FA = fractional anisotropy; MD = mean diffusivity (in 10-3 mm^2 / s); Vol = gray matter volume

differences (F = 10.68, df = 1, p = .0030) while the HFA of the occipital (F = 6.44, df = 1, p = .017), parietal (F = .15, df = 1, p = .70) and temporal (F = 6.44, df = 1, p = .28) regions were not significant. For descriptive statistics please see Table 4.

Furthermore, HFA of the frontal region demonstrated excellent discrimination (AUC = 0.82, p = .023) between patients with SCZ and HC (Fig. 2). Whereas AUC analyses of FA frontal (AUC = 0.73, p = .43), MD frontal (AUC = .58, p = .43) and volume frontal (AUC = .55, p = .64) showed poorer discrimination.

Additional analyses

The correlation analysis between HFA and age of onset (r(10) = .24 $p < .45$), duration of illness (r(10) = .38, $p < .22$), and medication dose (r(10) = .24, $p < .45$) did not show any significant results.

Discussion

The results of the present study show that patients with early course SCZ exhibit significantly greater HFA in the frontal lobe compared to HC and that HFA is very sensitive in group discrimination. We did not find any group differences for any other parameter, nor for the patients with chronic SCZ. Heterogeneity is a way of statistically determining the distribution of diffusion properties. Please keep in mind that the term "heterogeneity" has widely been used to describe genetic or clinical "heterogeneity" in patients with chronic schizophrenia (Dacquino et al. 2015; Jouan et al. 2013; Liang and Greenwood 2015). However, we refer to our statistical method of data evaluation which we believe can provide insights into the microstructure of gray matter.

Higher heterogeneity in frontal areas in early course SCZ patients could be indicative of aberrant maturational processes that preceded the transition to psychosis. A number of post mortem studies have reported that patients with SCZ have fewer inhibitory synapses and display excessive pruning of excitatory synapses in prefrontal areas, resulting in increased neuronal density and fewer dendrites and synapses (N. C. Andreasen 2010; Rapoport et al. 2012; Roberts et al. 2015). These changes, in turn, could result in cortical microstructural disorganization in patients with SCZ, occurring during brain maturation which might be reflected as increased heterogeneity in the gray matter.

The findings of early microstructural alterations in SCZ are in line with the neurodevelopmental theory, which postulates that genetic susceptibility and early environmental risk factors may alter the normal maturational trajectory of the brain development, which then leads to the onset of SCZ (Feinberg 1983; Lewis and Levitt 2002; Nonaka et al. 2013; Rapoport et al. 2005; Rapoport et al. 2012; Sipos et al. 2004; Sullivan et al. 2003; Thermenos et al. 2013; Weinberger 1987). It is also well established that frontal regions are the last to develop and that the elimination of synapses in frontal lobe regions continues beyond adolescence into the

Table 3 Group differences between patients with schizophrenia (SCZ) and healthy control individuals (HC) demonstrated by MANCOVAS

	Patients with chronic SCZ	Patients with early course SCZ
HFA	F = 1.78, df1 = 4, df2 = 41, p = .15	F = 5.50, df1 = 4, df2 = 26, p = .0020*
FA	F = 2.67, df1 = 4, df2 = 41, p = .046	F = 2.83, df1 = 4, df2 = 28, p = .045
MD	F = .33, df1 = 4, df2 = 41, p = .86	F = 3.02, df1 = 4, df2 = 26, p = .036
Volume	F = 1.30, df1 = 4, df2 = 41, P = .29	F = 2.04, df1 = 4, df = 26, p = .12

*indicates statistically significant correlation with $p < .013$ (Bonferroni Correction, $n = 4$)

HFA = heterogeneity of fractional anisotropy; *FA* = fractional anisotropy; *MD* = mean diffusivity (in 10-3 mm^2 /s); Vol = gray matter volume

Table 4 Descriptive statistics (mean ± standard deviation) of heterogeneity for patients with early course schizophrenia (SCZ) and healthy control individuals (HC)

	Patients with early course SCZ	HC
HFA frontal	.1190 ± .006474	.1113 ± .005676
HFA parietal	.1047 ± .005366	.1043 ± .005810
HFA temporal	.1140 ± .006606	.1148 ± .007776
HFA occipital	.09391 ± .004783	.09668 ± .007865

HFA = heterogeneity of fractional anisotropy

second decade of life (Gogtay et al. 2004; Huttenlocher and Dabholkar 1997; Lebel et al. 2008; Petanjek et al. 2011). Our findings are in line with these studies, showing group differences in the frontal lobe only. Heterogeneity may thus reflect these maturational changes and it may therefore be a potential biomarker for early disease stages, or maybe even increased risk for transition to psychosis.

More importantly, heterogeneity is also a useful tool because it allows excellent discrimination between patients with early course SCZ and HC, and in this regard outperforms classical measurements, such as FA, MD and volume. It is crucial to find early diagnostic biomarker of SCZ, because intervention strategies are more likely to be successful in early disease states (McEvoy 2007), and as such, heterogeneity might be a promising in vivo biomarker for increased risk for schizophrenia.

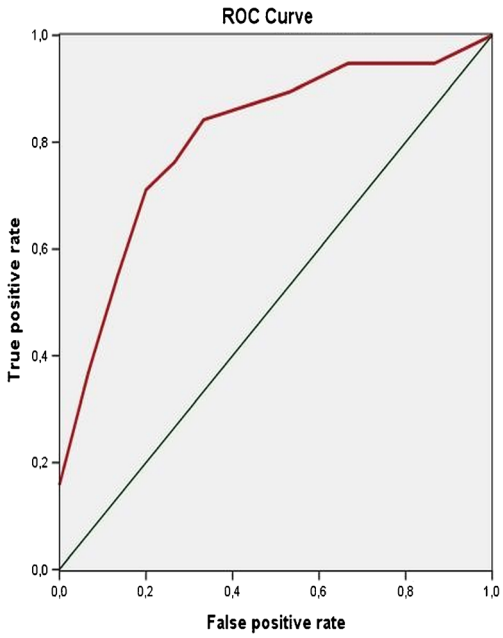

Fig. 2 Area under the curve analysis. HFA of the frontal region demonstrated excellent discrimination between patients with SCZ and HC

The absence of group differences in the chronic SCZ patients may indicate a lack of progressive cellular pathology in the gray matter. The existence of progressive, neurodegenerative cellular pathology continues to be the matter of active debate in SCZ. Although there are no classical neurodegenerative signs such as gliosis (G. Roberts and Harrison 2000) or neurofibrillary tangles reported (Bozikas et al. 2002), cortical thinning in SCZ has been suggested to be the result of accelerated aging (Arnold 2001). Present MRI evidence, however, does not clearly support the neurodegenerative hypothesis. Multiple studies show progressive gray matter reductions (in widespread cortical areas), while others find either stable or minimal gray matter group differences with age (Brans et al. 2008; Hulshoff Pol and Kahn 2008; Kubota et al. 2011; Olabi et al. 2011; van Haren et al. 2008). However, even this progressive volume loss, if present, is being argued to be more accentuated during first few years after disease onset, and then normalizing afterwards (N. C Andreasen et al., 2011; Bose et al. 2009; Kubota et al. 2011). This view is also consistent with the clinical profile of the disease, where after the stabilization of symptoms, no observable cognitive deterioration is seen (Jeste et al. 2011; Napal et al. 2012).

Limitations and future directions

Our study has several limitations. First, we employ a cross-sectional study design, which limits our ability to draw conclusions about the developmental trajectories of SCZ pathophysiology. Second, our population does not include at-risk subjects, it is thus possible that the changes described herein might reflect acute alterations related to disease-onset, stress, or an acute reaction to antipsychotic treatment, rather than a neurodevelopmental pathology.

Additionally, using diffusion imaging in gray matter may lead to partial volume effects. As described in the method section, we have taken multiple precautions to minimize this effect. We used high resolution diffusion data and a free water correction. This correction minimizes partial volume effects, and therefore ensures that changes in gray matter heterogeneity are less influenced by edema, atrophy or neuroinflammation (Pasternak et al. 2009). However, even though free water has widely been used (Bergamino et al. 2016; Metzler-Baddeley et al. 2012; Pasternak et al. 2014; Pasternak et al. 2015) the model has limitations itself. The assumption that there is no exchange between compartments is a simplification not accounting for potential differences in membrane permeability (Kochunov et al. 2014). Further studies are therefore needed to combine our model with

more sophisticated approaches (Kochunov et al. 2014; Zhu et al. 2014).

Additional longitudinal studies are needed to determine the role of heterogeneity as a potential biomarker for alterations relating to onset of SCZ. Furthermore, it is unclear which effect chronic administration of medication has on heterogeneity. It is therefore possible that a longer period of medication could influence the lack of findings in the chronic group (Ho et al. 2011). Future studies that include a group of unmedicated patients with SCZ are thus needed.

Finally, heterogeneity may be useful not only for the investigation of SCZ, but also in other diseases, such as brain tumor or Alzheimer's disease, to help detecting early changes in cellular organization. In fact, a recent study (Walker-Samuel et al. 2011) has shown subtle changes in tissue organization in brain tumor, measured through heterogeneity of MR signal.

Conclusion

In summary, we observed higher heterogeneity, a novel measure of microstructure, in the gray matter of the frontal lobe in patients with early course SCZ, whereas commonly used measures, such as FA, MD or volume lacked sensitivity. These findings suggest that gray matter alterations in early course SCZ may be associated with abnormalities in synaptic organization or pruning. Future studies are needed to establish heterogeneity as a neuroimaging marker of SCZ risk.

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Compliance with ethical standards

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Disclosure of potential conflicts of interest The Authors Seitz Johanna, Rathi Yogesh, Lyall Amanda, Pasternak Ofer, del Re Elisabetta C, Niznikiewicz Margaret, Nestor Paul, Seidman Larry J, Petryshen Tracey L, Mesholam-Gately Raquelle I, Wojcik Joanne, McCarley Robert W, Shenton Martha E, Koerte Inga K, and Kubicki Marek have declared that there are no conflicts of interest in relation to the subject of this study.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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