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Subtle Structural White Matter Changes Correlate with Positive Symptoms in Individuals at High Risk for Psychosis

Subtile strukturelle Aberrationen der weißen Substanz korrelieren mit positiven Symptomen bei Individuen mit einem hohen Psychoserisiko

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1 Goals of this work

Schizophrenia is a mental disorder affecting almost 1% of the world population that cannot be cured. Efficient preventative measures can only be taken if we understand the pathomechanisms leading to this disease. To this end, it is crucial to assess abnormalities during disease development. Previous research on the prodromal phase including DTI studies have shown abnormal white matter (WM) connectivity and integrity, which are hypothesized to be a major contributing factor to the illness. However, findings are inconsistent and some studies have not reported any differences in early stages. Altered values of fractional anisotropy of the tissue (FA-t) have recently been shown in one study with a new methodology, indicating the presence of cellular aberrations early in the course of the disease. However, given the nonspecificity of DTI measures and even newer methods such as free water analysis, morphological aberrations are difficult to pinpoint in early disease stages.

In the past years, a notion has arisen that the heterogeneity in structural aberrations could correspond to the diversity of symptoms in schizophrenia, thus contributing to the difficulties to compare subtle aberrations within this group and identify reproducible changes when comparing to healthy controls.

As of today, the transformatory trajectory of white matter changes seen in chronic schizophrenia cannot be reconstructed to a satisfactory extent and the underlying pathology remains elusive and necessitates more investigatory efforts.

With the study performed in the context of this work, we are contributing to a disease trajectory in schizophrenia, applying a relatively new method to the earliest stage of the disease. It aimed (1) to identify potential FA, FW, and FA-t aberrations in a largely unmedicated group of HRP compared to healthy controls, thus investigating early changes in structure and extracellular water level and (2) to understand if there is a relationship between positive and negative symptoms assessed with the Positive and Negative Syndrome Scale (PANSS) and FA, FW, or FA-t in HRP.

2 Introduction

2.1 Presentation of schizophrenia

2.1.1 Epidemiology

Schizophrenia, a mental illness presenting with psychosis and disordered thoughts and speech (Gogtay et al. 2011), is a debilitating disease affecting around 1% of the world population (Mueser and Mcgurk 2004). It is a major contributor of direct and indirect health care costs (Gustavsson et al. 2011) due to its predominant chronicity. Stigmatization in schizophrenia is significant and the fear of stigma (Mann and Himelein 2004) and side effects of existing medication (Mccullumsmith et al. 2014) leads to a lower acceptance of treatment among those affected by the disease, contributing to high symptom prevalence. In 1911, Eugen Bleuler collected several different syndromes under the self-coined term of 'schizophrenia', attributing them the same origin while still recognizing their heterogeneity. To the present day, the etiology of schizophrenia is still unclear, but a more heterogenous concept of pathoaetiology is assumed (Feigenson et al. 2014), and therapy remains a difficult task. In order to provide alleviation, the underlying pathological mechanisms need to be identified to create tailored therapeutic concepts and targeted drugs that relieve symptoms early-on and allow affected individuals to participate in life.

2.1.2 Symptom presentation

Schizophrenia onset peaks between late adolescence and early adulthood (Gogtay et al. 2011), most commonly with an acute psychotic phase. Symptoms experienced by affected individuals can be classified in different categories:

positive symptoms, adding to an individual's experience of reality, such as hallucinations and delusion, (2) negative symptoms, referring to deficits including emotional apathy, lack of drive, poverty of speech, social withdrawal, and self-neglect, and (3) cognitive symptoms, e.g., memory deficits, loosening associations, disorganized speech, and a decrease of goal-oriented behavior due to conflicting motivations (Kahn et al. 2015).

The course of the disease is extremely heterogenous. While some subjects (15-20%) recover and never show any further psychotic relapse (Lally and Maccabe 2015), others present with an episodic course that is marked by intermittent psychotic phases with partial or full recovery in between, and for some, symptoms stabilize (Newman 2012). Few measures can be taken to ameliorate the suffering from symptoms and none to attack the disease by its roots. Most antipsychotic drugs alleviate positive, but rarely negative or cognitive symptoms (Gogtay et al. 2011). About half of the affected individuals seem to recover (Harrison et al. 2001). However, those who suffer from a deficit syndrome, presenting with mainly negative symptoms and functional decline, are prone to poor outcome and low social or professional functionality (Strauss et al. 2010).

2.2 Individuals at High Risk for Psychosis

Schizophrenia onset is often preceded by a prodromal phase of attenuated symptoms and functional decline after puberty. This phase takes place in between the premorbid (healthy) and psychotic phase and is only identifialbe as such in retrospect (Perkins et al. 2007). It can extend between two to five years (Perkins et al. 2007) during which insight in disease is usually preserved as altered behavior and experience of increasing severity arise (Perkins et al. 2007). Signs and symptoms preceding full-blown schizophrenia include specific and unspecific symptoms of functional impairment (Wölwer et al. 2006), such as depression, anxiety, and social withdrawal (Gogtay et al. 2011). This is followed by the either abrupt or insidious development of more severe and specific symptoms including

hallucination and delusion, and the gradual loss of insight (Gogtay et al. 2011). Accompanying symptoms consist in anxiety, depression, and sleep disorders (Gogtay et al. 2011). Substance abuse is a common comorbidity, which creates a higher vulnerability to transition to psychosis and poorer outcome (Gogtay et al. 2011).

The concept of standardized risk detection with the goal to provide early intervention and health care to help-seeking individuals experiencing first symptoms was introduced in 1996 (Fusar-Poli 2017). The group of individuals who are likely to experience a psychotic episode has been termed the clinical High Risk state for Psychosis (HRP) population, also Clinical High-Risk for Psychosis (CHR-P), At Risk Mental State (ARMS), and Ultra-high Risk (UHR) population (Fusar-Poli et al. 2012a). Although markers have been sought to identify this heterogenous group, none have yielded a reliable prediction of psychosis outbreak (Fusar-Poli et al. 2012a).

The Early Recognition Inventory for the Retrospective Assessment of the Onset of Schizophrenia (ERIraos) is the most widely used tool for identification of HRP in Germany and Italy.

The Positive and Negative Syndrome Scale (PANSS) contains three categories of positive, negative, and general symptoms. It has a good validity for symptom severity and showed an inverse correlation of functional outcome with negative symptoms (Kay et al. 1987).

Throughout most scales, three different syndromes have been identified among the High Risk population: Individuals experiencing (1) short-lived remittent psychotic episodes (brief (and limited) intermittent psychotic symptoms (B(L)IPS)), (2) attenuated psychotic symptoms (APS), and (3) such with a genetic risk and deterioration syndrome (GRD). The BLIPS category with BLIPS have the highest and the GRD category the lowest probability of transitioning (Fusar-Poli 2017), the latter being close to control groups' risks.

The overall proportion of HRP transitioning to psychosis is believed to be a third (Fusar-Poli et al. 2012a), while most keep experiencing low level symptoms and some recover (Simon et al. 2011). The risk for transitioning to psychosis is highest

in the first two years after being categorized as HRP, then plateaus. Within the first 12 months, 22% of all HRP will experience transition to a first episode of psychosis (Fusar-Poli et al. 2012a) that is commonly severe with an array of quickly developing symptoms. However, the HRP group is heterogenous and transition rates differ according to the assessment instrument that was used (Fusar-Poli et al. 2012b).

To avoid drawn-out periods in which patients are not aided sufficiently, it is of vital importance to identify individuals at risk for schizophrenia as early as possible and be able to take therapeutical measures to postpone or even prevent the outbreak of the first episode of psychosis. However, so far, no exact assessment tools have been developed or biomarkers found that would allow to identify individuals who are going to transition to schizophrenia reliably and differentiate them from those who never experience any psychosis. Being able to tell these two groups apart provides the possibility of administering preventive treatment, both psychotherapy and antipsychotic drugs, which has been shown to have a protective effect (Gogtay et al. 2011). The administration of medication to individuals that will likely not profit from antipsychotic drugs is not justifiable in light of the severity of side effects.

In order to avoid confounders such as late effects of illness or medication and to understand the trajectory and pathological origin of the disease, focus has shifted toward investigating early stages of the disease.

2.3 Pathoaetiology

Although first described in detail as 'dementia praecox' in the late 19th century by Dr. Emil Kraepelin (Kraepelin 1899), the etiology and pathogenesis of schizophrenia remain unclear. It is evident that no one factor alone can explain the development of this complex and heterogenous disease in its entirety and

multifactoral models are being developed, taking into account the influence of individual traits as well as early and late insults and aberrant early development that might contribute to psychosis eruption.

An array of animal models exist to investigate schizophrenia pathology, but they do not reflect the complexity of this disease (Mccullumsmith et al. 2014). It is likely that the illness that is classified based on symptom presentation represents a category summarizing different conditions rather than a single disease state (Feigenson et al. 2014) and different interacting pathological mechanism cause schizophrenia development (Mccullumsmith et al. 2014). This could contribute to the difficulties in finding the biological underpinnings of this disease.

It has generally been accepted that genetics are able to largely predispose for schizophrenia, but environmental factors play a significant role for the outbreak (Misiak 2017). According to the two-hit hypothesis, interacting genetic, epigenetic, and environmental risk factors result in brain abnormalities that continuously contribute to pathology when sufficiently heavy stressors impact an individual's life (De Picker 2017).

2.3.1 Neurotransmitters

Several neurotransmitters have been implicated in the pathoaetiology of schizophrenia. The most prominent amongst them is dopamine in light of the antipsychotic effects of dopamine antagonists and findings concerning the dopaminergic transmission in histological studies. Newer in-vivo imaging studies and genes of the dopaminergic and glutamatergic pathways that were found to be implicated in schizophrenia have backed this hypothesis further.

2.3.2 Neuroinflammation

A number of psychiatric disorders (schizophrenia, depression, bipolar disease) has been linked to infections and autoimmune conditions (Khandaker 2015) and a growing body of evidence suggests that the brain is not shielded from systemic inflammation by the blood-brain-barrier to the extent that was previously believed to be the case (Khandaker 2015). Furthermore, systemic intrauterine, perinatal, and childhood central nervous system infections as well as autoimmune conditions have been identified to coincide with psychosis and schizophrenia development (Khandaker et al. 2012, 2013, 2014).

On a short time scale, inflammation is likely to be healthy and necessary for elimination of invading agents and restitution of the tissue. Prolonged neuroinflammation, on the other hand, might have detrimental effects on neuronal functioning and connectivity.

Currently, positron emission tomography (PET), a functional imaging technique, has facilitated measuring proxies of neuroinflammation in vivo. However, study results are overall highly controversial and newer studies did not confirm the presence of neuroinflammation in schizophrenia (De Picker 2017).

2.3.3 Morphological aberrations of the brain

Gray matter has been found to be reduced and ventricles tend to be enlarged in schizophrenia patients. Especially in frontal and temporal areas, implied to be involved in schizophrenia pathogenesis, gray as well as white matter volume has been shown to be reduced.

2.3.3.1 Trajectories of maturation

Schizophrenia has generally been accepted to be a disease brought about by aberrant neurodevelopment. According to this hypothesis, disruption results from a disturbed organization of the brain that originates in pathological maturation. This highly complex process can be interfered with on many levels, such as faulty neuron migration (Najjar and Pearlman 2015). Another aspect of maturation thought to be implicated in schizophrenia development is the elimination, i.e., pruning, of neuronal cells not deemed vital to functioning, thereby reducing the number of synapses and streamlining connections within the brain during puberty (Boksa 2012).

The genetic findings and the fact that this process mostly takes place overlapping with the age of schizophrenia onset during adolescence further back the hypothesis of an erraneous development laying at the root of this disease. Ergo, the developmental hypothesis postulates a fixed lesion that occurs early in an individual's life and interacts with brain maturation later on.

Conversely, there is also evidence arguing for a deterioration of brain matter in schizophrenia individuals instead of functional impairment being the result of faulty maturation. The course of the disease is commonly (over 60%) deteriorative and in vivo neuroimaging supports the notion that there is accelerated white matter decline (Kochunov 2014).

Considering that there seem to be predisposing factors as well as an ongoing decline, neuroplasticity was thought to be affected and to propulse ongoing alterations throughout the course of the disease (De Picker et al. 2017).

2.3.3.2 Gray matter pathology

Histological as well as in vivo neuroimaging has yielded a body of evidence for gray matter aberrations being present in schizophrenia. Affected individuals have

consistently shown a decreased gray matter volume as well as thickness, aberrant gyrification, and enlarged ventricles (Kochunov and Hong 2014). These changes have been shown to be present, particularly in frontoorbital regions, in prodromes who later convert to full-blown schizophrenia and at disease onset (Kochunov and Hong 2014), and more prominently in chronic cases (Olabi et al. 2011). Specifically the hippocampus shows a reduced size, and all gray matter has been shown to be hypoactive compared to controls (Tay et al. 2018).

2.3.3.3 White matter pathology

While schizophrenia has historically been viewed as a disease developing within the gray matter of the central nervous system, focus has shifted towards white matter in the past decades. Not only technical advancements allowed to investigate white matter functionality better, there is also a large body of evidence hinting at white matter being crucially involved in schizophrenia development.

2.3.3.3.1 Disconnectivity hypothesis

Schizophrenia is believed to encompass structural and functional disconnectivity. There is histological, anatomorphological and functional evidence pointing toward an impaired coupling among neuronal cells (Tay et al. 2018).

In network analyses, altered connectivity and network topology was discovered in schizophrenia with network inefficiency and reduced inter-regional integration in chronic patients (Wheeler and Voineskos 2014). Both strengthened and attenuated correlated variance in anatomical structures of the prefrontal and temporal cortex have been found (Wheeler and Voineskos 2014).

The hypothesis that microarchitectural deficits in the myelin sheaths might contribute to said disconnectivity is backed by neuroimaging results. Furthermore, recent studies with omega-3 polyunsaturated fatty acids (PUFA) required to synthesize myelin sheathing showed a potential protective effect when administered to HRP (Vijayakumar et al. 2016, Schlögelhofer 2014). However, the correlation of blood PUFA levels with white matter imaging parameters proved to be inverse, denoting that their relationship is not yet clarified satisfactorily (Vijayakumar et al. 2016).

2.3.3.3.2 Diffusion imaging

Given the fact that transformation of the brain seems to take place in schizophrenia individuals, magnetic resonance imaging (MRI) has proven to be the technique of choice to be able to appreciate ongoing changes. With the advent of Diffusion Tensor Imaging (DTI) in the 1980s, a novel and unique opportunity emerged to gain insight into the microstructure of white matter in vivo.

Diffusion magnetic resonance imaging utilizes the fact that water molecules tend to diffuse along anatomical structures that hinder them from moving freely (Stejskal and Tanner 1965). The most important measure determined with this technique is fractional anisotropy (FA.) Reduced FA is associated with general microstructural white matter damage, while reduced axial diffusion (AD) implies axonal damage, and increased radial diffusivity (RD) suggests damaged myelin (Song et al. 2003).

In contrast to conventional MRI, in which white matter appears homogenous, diffusion imaging is able to detect changes in organization and coherence of white matter fibers on a micro-architectural level and facilitates tracing their trajectories (Kubicki et al. 2007). Therefore, diffusion imaging has become the most widely used modality for white matter structure analysis.

2.3.3.3.2.1 White matter imaging results

Reduced white matter volume has been found in schizophrenia, predominantly in the frontal cortex (Kochunov and Hong 2014). Fibers traversing it mainly connect the frontal lobe to the thalamus and the cingulate gyrus. By applying diffusion imaging methods, both schizophrenia patients and individuals experiencing pre-psychotic symptoms have exhibited reductions in FA, mostly in the prefrontal and temporal white matter (Karlsgodt et al. 2010). However, reported locations of reduced FA are heterogenous and findings are not consistent throughout literature. Diffusion imaging studies suggest that fronto-temporal and fronto-limbic connections are implicated in schizophrenia, including the superior longitudinal and uncinate fasiculus, cingulum, and corpus callosum. More specifically, in chronic schizophrenia, lowered FA has most frequently been found in fiber bundles connecting frontal and temporal areas, such as the uncinate fasciculus and the cingulum bundle as well as the corpus callosum and the internal capsule (Wheeler and Voineskos 2014). The arcuate fasciculus has been shown to be affected by lowered FA, more so in the left hemisphere, although elevated FA has also been detected in that area (Wheeler and Voineskos 2014). When analyzing the whole brain as opposed to regions of interest, most found changes in interhemispheric fibers, the anterior thalamic radiation, inferior longitudinal fasciculi, inferior frontal occipital fasciculi, cingulum, and fornix (Wheeler and Voineskos 2014). However, replication of these studies proved to be difficult. Some studies did not find any localized abnormalities, but aberrations that were scattered throughout the whole brain (Krakauer et al. 2017), although rather consistent evidence points towards them predominantly being located in the frontal area (Wheeler and Voineskos 2014).

In first episode and early-onset schizophrenia, a population typically defined as being within 3 years of the onset of the first episode of psychosis (Wheeler et al. 2014), aberrations are similar to those found in chronic patients, but found less consistently. Most studies have found alterations in the uncinate fasciculus for this group (Wheeler and Voineskos 2014).

In HRP, white matter has been found to be reduced throughout the brain, especially in interhemispheric fibers, anterior thalamic radiation, inferior longitudinal fasciculi, inferior frontal occipital fasciculi, cingulum and fornix (Bora et al. 2011). Investigations performed in HRP are especially valuable to isolate factors predicting transition to psychosis for early intervention, as well as to identify underlying mechanisms of pathology, risk and protective factors. However, the interpretation of results is difficult, since these individuals are mostly adolescents with ongoing white matter development that are associated with fluctuating FA levels due to increases and decreases in diffusitivity during that period. This is true especially in fiber tracts such as the superior and inferior longitudinal fasciculus and the fronto-occipital fasciculus, which are not fully myelinated at that age (Vijayakumar et al. 2016). There is an array of studies that did not find any significant differences in FA when investigating HRP at baseline (Clemm von Hohenberg et al. 2014, Carletti et al. 2012, Mittal et al. 2014, Peters et al. 2008, Bernard et al. 2015). A substantial part of the ones that do find differences, however, find them inconsistently in the retrospect subgroup that later transitions to psychosis (Mittal et al. 2014) or when longitudinally analyzing group x time interactions (Carletti et al. 2012). An area that has been identified as being affected is the superior longitudinal fasciculus (Karlsgodt et al. 2010, Clemm von Hohenberg et al. 2014, Carletti et al. 2012), a tract that shows a particularly high level of change during the development through adolescence and early adulthood (Wheeler and Voineskos 2014). Additionally, the corpus callosum, the posterior corona radiata (Clemm von Hohenberg et al. 2014, Carletti et al. 2012), the left inferior longitudinal fasciculus, left inferior fronto-occipital fasciculus, right external and internal capsule (Carletti et al. 2012) have been found to be altered. When investigating relatives of affected individuals, parallels could be drawn, several of which depended on the closeness of relation (Wheeler and Voineskos 2014). Although this may reflect the genetic liability, there is not enough data to draw conclusions. There are several possible confounders to DTI studies in general that are not always controled for, which need to be considered when interpreting results. Among these confounders are the chronicity of the illness, treatment with anti-psychotic medication, sex and age of participants, and heavy cigarette smoking (Vijayakumar et al. 2016). The lower level of consistency in first episode and high risk findings has been attributed to the subtlety of neuroanatomical

changes at the time of disease onset as opposed to the accumulation of aberrations during disease progression (Wheeler and Voineskos 2014).

2.3.3.3.2.1.1 Correlation of white matter alterations with clinical symptoms

Given the heterogeneity of findings, it has been hypothesized that small alterations may be scattered throughout the brain in individual patterns in schizophrenia, similar to multiple sclerosis lesions (Davis et al. 2003). In order to account for this possibility, clinical measures have been correlated with FA values yielding results that very much confirm that FA is related to symptom burden. Positive symptoms in general were associated with lower FA in the uncinate fasciculus (Knöchel et al. 2012, Skelly et al. 2008), the right sagittal stratum, and the left superior longitudinal fasciculus (Skelly et al. 2008). Auditory hallucinations correlated directly with FA in the anterior corpus callosum, cingulum and superior longitudinal fasciculus (Hubl et al. 2004). Negative symptoms were shown to correlate inversely with FA in the uncinate fasciculus and inferior frontal white matter (Wolkin et al. 2003, Szeszko et al. 2008). In deficit patients displaying more negative and cognitive symptoms with low functionality, the left uncinate fasciculus showed lower FA values than non-deficit patients and controls (Kitis et al. 2012, Voineskos et al. 2013) and higher mean diffusivity, which was also shown in the right inferior longitudinal fasciculus (Voineskos et al. 2013). A tendency toward lower FA was reported in the superior longitudinal fasciculus of deficit patients (Rowland et al. 2009). This correlation taking place in frontotemporal tracts lines up with the fact that these tracts are widely thought to be implemented in schizophrenia pathology.

In first episode patients, negative symptoms were found to correlate indirectly and positive symptoms to correlate directly with FA in white matter adjacent to the right lateral ventricle (Moriyaet al. 2010), while positive symptoms also directly correlated with FA in the right frontal lobe, left anterior cingulate gyrus, left superior temporal gyrus, right middle temporal gyrus, right middle cingulate gyrus, and left cuneus (Cheung et al. 2011).

Positive symptoms were inversely correlated with FA in the left medial temporal lobe in HRP that later converted to psychosis and in the superior temporal lobe in the total HRP group (Bloemen et al. 2010). Both positive and negative symptoms were related to lower FA in patterns that were found to have abnormal DTI parameters in HRP. There were fewer patterns in which direct correlation was observed (Krakauer et al. 2017). Studies analyzing correlations between FA and symptoms are scarce in HRP.

Altered FA was furthermore associated with impaired processing speed, visual and verbal learning, intelligence, cognitive performance, social functioning, and poor outcome (Wheeler and Voineskos 2014).

2.3.3.3.2.2 Free water analysis

Despite rapid advancements in the field of neuroimaging, diffusion tensor imaging encompasses severe limitations. (1) One voxel may include crossing fibers, altering and distorting FA results, (2) when calculating the tensor of a diffusion image, diffusion decay is generally not taken into account, and (3) Fractional anisotropy is a descriptive quantity that does not yield any infomation on the underlying pathology leading to its changes.

As of now, white matter research has focused on axon and myelin pathology. FA, the most commonly used marker in DTI, is thought to reflect white matter organization. It is, however, not a specific marker and does not solely represent pathology of the tissue as opposed to the extracellular space (Davis et al. 2003). Reductions in fractional anisotropy may be due to damage to either the structure of the myelin sheathing neurons, its content, the neuronal diameter (Wheeler and Voineskos et al. 2014), or even to altering factors in the immediate vicinity of the neuronal cells including cerebrospinal fluid located in adjacent ventricles or extending into the parenchyma. To address this ambiguity, our methods need to be further developed to be able to differentiate axonal integrity, fiber density, myelin sheathing, but also modulating factors that have so far not been taken into account such as extracellular pathology.

The free water method is thought to yield parameters that are able to differentiate between the signal coming from the tissue and the signal contamination caused by freely diffusing extracellular water. The free water (FW) component is thought to represent extracellular processes, which are hypothesized to be brought about by either atrophy or tissue edema that are potentially evoked by an acute inflammatory response. FA of the tissue (FA-t), on the other hand, is thought to be a parameter more specific to tissue alterations than FA (Pasternak et al. 2009).

When applying the FW method to subjects with chronic schizophrenia, distinct FA-t changes and less pronounced elevated levels of FW were shown (Pasternak et al. 2015, Oestreich et al. 2016). Conversely, studying first episode cases, a massive response in FW was observed (Pasternak et al. 2012), while little FA-t changes were found in one population. Other samples did not show the pronounced FW elevation (Guo et al. 2020, Lesh et al. 2019). The variable finding of fluctuating levels of FW throughout the disease supports the notion that processes leading to elevated FW levels in circumscribed areas might only occur during psychotic episodes in active phases of the disease under certain circumstances and may then give way to the neurodegeneration observed thereafter (Najjar and Pearlman 2015).

Progress in the disease might take on an inverted development, suggesting a decrease in the interstitial pathology while the structural aberrations manifest themselves in a more pronounced way (Pasternak et al. 2015).

2.3.3.3.2.2.1 Free Water in a High Risk for Psychosis population

Tang et al. (TANG et al. 2019) found FA-t aberrations in a group of HRP, along with comparable FA findings and no significant elevation in FW, indicating the presence of cellular aberrations early in the course of the disease. However, given the nonspecificity of DTI measures and even newer methods such as free water analysis, morphological aberrations are difficult to pinpoint in early disease stages, such that the transformatory trajectory of white matter changes seen in chronic

schizophrenia cannot be reconstructed to a satisfactory extent and the underlying pathology remains elusive.

With the study performed in the context of this work we are contributing to a disease trajectory in schizophrenia, applying a relatively new method to the earliest stage of the disease. It aimed (1) to identify potential FA, FW, and FA-t aberrations in a largely unmedicated group of HRP compared to healthy controls, thus investigating early changes in structure and extracellular water level and (2) to understand if there is a relationship between positive and negative symptoms assessed with the Positive and Negative Syndrome Scale (PANSS) and FA, FW, or FA-t in HRP.

3 Methods

The present study was part of a larger project investigating brain connectivity in individuals at high risk for psychosis within the context of the Collaborative Research Centre 936 (“multi-site communication in the brain”, www.sfb936.net, project C6). It was approved by the Ethics Committee of the Medical Association Hamburg and carried out according to the seventh edition of the Declaration of Helsinki (2013). All of the participants provided written informed consent.

3.1 Participants

Twenty-three subjects with clinical high risk of psychosis (HRP) and twenty-two healthy controls (HC) participated in this study. HRP were recruited through the Early Recognition Department for outpatients of the Department of Psychiatry of the University Medical Center Hamburg-Eppendorf and through advertisements on the department's website and on www.psychose.de. HRP approached the department based on a referral by their physicians. Exclusion criteria for all participants were (1) an age below 18 and (2) any preexisting major somatic disorder. Additional exclusion criteria for HC comprised a record or family history of a psychiatric disorder. HRP were identified according to the Early Detection and Intervention program of the German Research Network on Schizophrenia (GRNS) (Wölwer et al. 2006) utilizing the Early Recognition Inventory. The criteria leading to a classification as being at high risk for psychosis comprised a) experiencing basic symptoms: two or more subjective cognitive or perceptual disturbances with a score of 3 or more on the Schizophrenia Proneness Instrument (Schultze-Lutter et al. 2007), or b) a schizotypal personality disorder (or family history of psychotic disorder) and a decline of at least 30% in the Global Assessment of Functioning Scale (GAF) score (American Psychiatric Association 2009), or c) attenuated psychotic symptoms or a brief limited intermittent psychotic syndrome as assessed

with the Structured Interview for Prodromal Syndromes and the Scale of Prodromal Symptoms (SOPS) (Miller et al. 2003).

Individuals at high risk were assessed with the Positive and Negative Syndrome Scale (PANSS) to gauge positive, negative, and global symptoms and the Mini International Neuropsychiatric Interview (Sheehan et al. 1998) was applied in order to rule out schizophrenia spectrum disorders and document any comorbid psychiatric diagnoses. Eleven subjects showed a mood disorder, three an anxiety/obsessive-compulsive disorder, five a substance related disorder (all subjects were abstinent during the process of recruitment), and 6 proved to have a personality disorder. Hyperkinetic, eating, somatoform, and neurotic disorders were represented affecting each one individual. There was a substantial overlap in between comorbid disorders in HRP individuals. Additionally, the Scale of Prodromal Symptoms (SOPS) (Miller et al. 2003) was explored with all HRP. When participating in the study, two subjects were being treated with an antipsychotic drug. One of the HRP had been receiving aripripazol for thirteen months, while the other was receiving the same drug for three months at the time of the study. A third HRP and one HC had been medicated in the past, the HRP had been prescribed seroquel for a year and cipralex for two months, while the HC had formerly taken aripripazol for 4 months. HRP, as well as HC, were recruited in the time period between August 2012 and May 2015 and psychiatric trainees with clinical experience administered all assessments. After finishing the recruitment process, HRP and HC were matched for age and gender (see Table 1).

Table 1 - Participants

	Healthy Controls (n = 22)	**HRP Individuals** (n = 23)	**p**
Sex (f / m)	11/11	12/11	0.8841
Age (SD)	23.59 (4.19)	21.43 (4.41)	0.0977
Handedness (right / left / N/A)	20 / 1 / 1	20 / 2 / 1	0.5775
Years of Education (SD)	14.88 (2.97)	13.38 (3.01)	0.0879
Positive Symptoms* (SD)		11.48 (3.22)	
Negative Symptoms* (SD)		9.67 (2.77)	
Global Symptoms* (SD)		26.05 (5.61)	

3.2 Diffusion-weighted imaging data acquisition

Diffusion weighted MRIs were acquired on a 3 T Siemens Magnetom Trio scanner with a 12-channel head coil with a b-value of 1000 s/mm^2, 60 non-collinear gradients, and 10 b-0s. Each volume consisted of 64 slices with a TR of 7700 ms and a TE of 85 ms. The flip angle was 90°, the FOV 216 x 256 mm^2, and the matrix 108 x 128. The slice acquisition was interleaved with a slice thickness of 2 mm.

3.3 Image analysis

After preprocessing, Free Water Analysis, a diffusion tensor imaging method, was applied as described elsewhere (Pasternak et al. 2009). Consecutively, Tract-based Spatial Statistics (TBSS) (Smith et al. 2006) was performed. All procedures will be described in the following.

3.3.1 Preprocessing

All images underwent visual quality control in 3DSlicer (Norton et al. 2017). Images were screened for greater scale artifacts and for missing significant parts of the brain. One participant of the healthy control group was excluded due to large-scale structural aberrations, i.e. enlarged ventricles. Utilizing FSL (Functional MRI of the Brain Software Library, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), brain masks were generated, then manually edited in 3DSlicer to exclude non-brain areas. Images were corrected for head motion and eddy currents using in-house scripts

(https://github.com/pnlbwh/pnlutil/blob/master/scripts-all/dwiPipeline-nofilt.py).
After calculating a motion parameter (Ling et al. 2012) for each individual, one participant of the HRP group was excluded due to a motion parameter that was greater than two standard deviations above the mean.

3.3.2 Diffusion Tensor Imaging

A magnetic field established in a magnetic resonance scanner streamlines the alignment of hydrogen atoms in water molecules in the scanned tissue in question. Small pulsed field gradients that alter the orientation of protons, first in one, then back the opposite direction, are used in diffusion imaging and the signal sent off by the protons during reorientation is registered. Attenuation of this signal is directly dependent on the freedom of diffusitivity in the direction of the small magnetic pulse. When repeated for different directions, these images can be integrated to give an account of diffusion restriction in a three-dimensional space (Hahn 1950).

In theory, with no restrictions, water molecules starting out at a specific place will diffuse outward on the surface of a sphere. This kind of isotropic diffusion will be found in water compartments, such as ventricles in the brain, where no structures hinder water molecules from freely diffusing. Diffusion is anisotropic when it is influenced by a medium's orientation, by spatially ordered macromolecules and membranes (Stejskal and Tanner 1965). Diffusivity is thus higher along fibers than perpendicular to them. In a human body, these traits are present in kidneys, skeletal and cardiac muscle, and white matter (Stejskal and Tanner 1965). In white matter, these structures consist of axon bundles and myelin sheaths (Kubicki et al. 2008). The shape diffusing water molecules form in anisotropic diffusion will become an ellipsoid as opposed to a sphere, elongated in the direction of least resistance, the direction of fiber orientation. For diffusion tensor imaging, these ellipsoids (tensors) are calculated from the multitude of images showing diffusion and its specific direction in the form of signal attenuation for each voxel (image element in three-dimensional imaging) (Basser and Pierpaoli 1996). Departing from there, different measures can be derived from the tensor.

These parameters are defined by the vectors within the tensor, where fractional anisotropy (FA) is computed from the scalar mesasures axial, radial, and mean diffusivity. Axial diffusivity (AD) represents diffusivity along the length of the tensor or its main axis, while radial diffusivity (RD) quantifies diffusion perpendicular to the axis in any direction, and mean diffusivity (MD) or the apparent diffusivity coefficient (ADC) (Krakauer et al. 2017) is a nonspecific parameter indicating bulk diffusivity that, if elevated, can point towards increased tissue water content (Pasternak et al. 2009).

3.3.2.1 Free Water Analysis

In conventional diffusion MRI, signal attenuation accounts for the total sum of diffusion taking place in one voxel. The average magnitude of molecular displacement has been found to be a few tens of microns, which are distances too large to exclusively take place intracellularly or in the adjacency of cells (Pasternak et al. 2015).

Processes in the extracellular space can lead to excitotoxic or vasogenic edema, causing the water volume in the tissue to rise creating larger extracellular spaces such that diffusion can take place uninfluenced by the proximity of axon bundles. This might interfere with the accuracy of the tensor model that holds up for homogeneous voxels, but averages diffusion parameters throughout the voxel in case of different matters being repesented, such as white matter bundles and cerebrospinal fluid (CSF) (Pasternak et al. 2009). The model then loses its accuracy and might miss subtle changes, especially in areas where parenchyma and ventricles adjoin. These CSF-contaminated voxels show a similar diffusion pattern to the ones that hold white matter with vasogenic extracellular edema. When the signal of diffusion taking place intracellularly and in direct adjacency to cell membranes overlap with freely diffusing water, the isotropic free water was theorized to mask the more anisotropic signal of the underlying tissue and, hence, cause loss of specificity (Pasternak et al. 2009).

The signals stemming from diffusion taking place within or adjacent to cells on one hand and the signal from the extracellular space on the other hand need to be separated in order to obtain more specific information on myelin or axonal pathology as opposed to extracellular processes. One approach to parsing out the mixed diffusion signal is observing diffusion in a multi-compartment space by deconstructing it and detracting the free water signal as done by Pasternak et al. (Pasternak et al. 2009). The tensor calculation with this method, opposed to conventional DTI calculations, aims at predicting the signal attenuation factor of the freely diffusing water. As opposed to other approaches that aim to eliminate CSF contamination, the free water method is able to address edema contamination and it can be integrated in a conventional diffusion modeling pipeline such that it does not require an increased scan time and is widely applicable (Pasternak et al. 2009).

According to the free water method, two tensors were calculated for each voxel instead of one in this study. The bi-tensor model assumes that there is no exchange between the tissue compartment and the extracellular compartment. The signal attenuation for contaminating free water was calculated and thus, the FA signal was deconstructed into two compartments that represent freely diffusing water in the extracellular space, free water (FW), and FA of the tissue (FA-t) that seeks to visualize the microscopic architecture of white matter, alignment of axons, and myelin properties more specifically. For each voxel, we obtained a FA, FW, and FA-t value that we utilized for the following analyses.

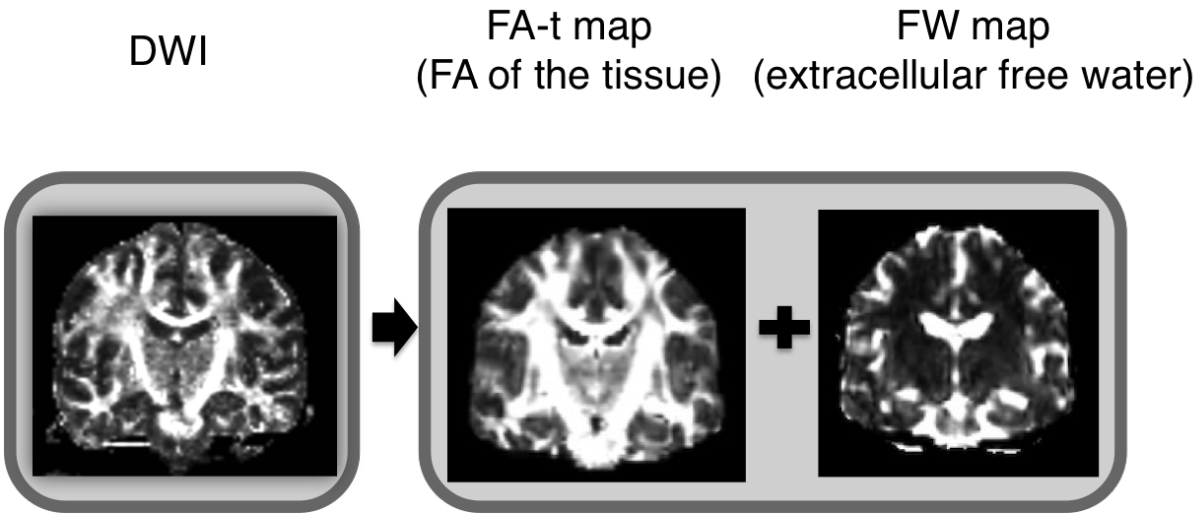

Figure 1 - Free Water Analysis - *Diffusion weighted images were separated into FA-t and FW maps*

3.3.2.2 Tract-based spatial statistics

In order to successfully compare diffusivity parameters in brain structures across different subjects, the images need to be aligned in a way that diffusivity measures such as FA values can be subtracted in any given point. Each individual image is usually adjusted to fit a template. This template can either be chosen from a number of existing ones, or constructed utilizing the normalized FA images of the group that is to be investigated. Then, specific areas or the whole white matter of the brain can be compared.

Tract-based spatial statistics represents an approach to merge the advantages of both hypothesis-driven and -free methods.

In hypothesis-driven image analysis, regions of interest are defined a priori and their mean FA values are compared among groups. The regions that are determined based on a neuroanatomical atlas can be selected manually or as an automated process by applying tractography. Then, the mean FA value of the voxels in the analyzed region is computed and finally compared with the mean of corresponding regions. If regions of interest are selected by hand, there is

inherently a certain amount of subjectivity to the process, especially when observing small fiber tracts (Smith et al. 2006), and even when identified automatically, anatomical differences can interfere significantly with the accuracy and comparability of the areas (Melonakos 2008). As even automated systems can only detect fiber bundles that have previously been well-described, small tracts may not be taken into consideration, resulting in a loss of valuable information on other areas of the brain and, consequently, in a bias due to this selectivity.

Conversely, in voxel-based morphometry (VBM) and VBM-style analyses, a hypothesis-free whole brain approach, the FA image is registered to a common template. Each voxel is assigned a value and compared to every single voxel in the same position across the entirety of the subjects' brains (Melonakos 2008). This process can be largely automated and is therefor superior concerning practicality. It is useful to detect dispersed aberrations, as are suspected to be present in schizophrenia. Voxels are analyzed independently from each other and no assumptions are made about the position and boundaries of affected brain areas. However, even careful registration protocols are likely not capable of guaranteeing the same localized areas of the brain are being compared (Smith et al. 2006). Another issue that arises with voxel-based approaches is the amount of smoothing an image should undergo. It is, again, arbitrary and has the potential of influencing the results significantly (Smith et al. 2006). The likelihood of generating false-positives is relatively high (Caprihan et al. 2011).

We utilized Tract-based spatial statistics of the FSL (FMRIB Software Library) toolbox (Functional MRI of the Brain Software Library, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), thus analyzing the whole brain without making a priori assumptions about the localization of white matter tracts. This was accomplished by first calculating a whole brain FA map for each individual. Secondly, a white matter skeleton was generated by thinning the tracts identified on these maps. Along white matter tracts, the perpendicular area was searched for the voxel with the maximum FA, which was defined as the center of the bundle. All other voxels were consecutively suppressed, such that each tract was represented

by either a thin sheet or a line following the center of major fiber bundles, indicating their direction and position (Smith et al. 2006). In a third step, skeletons with maximum FA values of all probands were warped to align with an FA skeleton template in order to mitigate any residual misalignment after preprocessing. The FA target image and skeleton were provided by the Enhanced Neuroimaging Genetics by Meta Analysis (ENIGMA) DTI Working Group (Jahanshad 2013, Thompson et al. 2014). This template was created by merging 400 FA maps of healthy adults aged 18 to 85 in order to create a common reference space for enhanced comparability across different populations (Jahanshad 2013). This procedure ensures that diffusion measures can be compared in the same bundles without having to warp the image to an extent that increases the likelihood of losing information.

The FA values of the individual skeletons were projected onto the template and a voxelwise comparison was performed (Smith et al. 2006). Then, 43 regions of interest (ROIs) based on the Montreal Neurological Institute (MNI) 152 space, Johns Hopkins University ICBM-DTI-81 white matter labels atlas were defined and FA, FA-t, and FW averages for these regions determined.

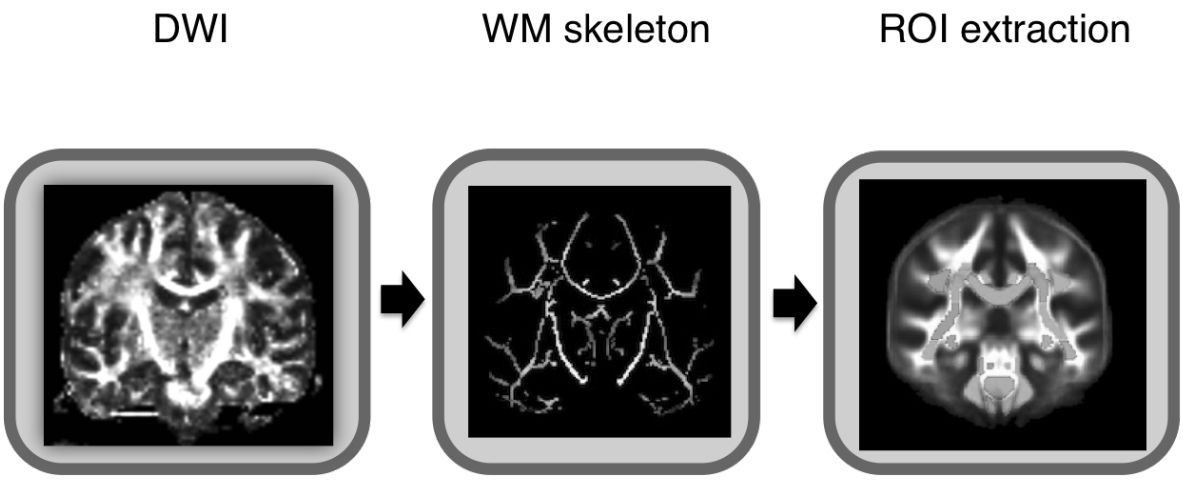

Figure 2 - ENIGMA-TBSS (Tract-Based Spatial Statistics) automated pipeline - *A white matter skeleton was calculated from diffusion weighted images and regions of interest defined according to the John Hopkins University White Matter Atlases*

3.4 Statistical analysis

Statistical Analyses were performed in R studio (version 0.99.484, https://www.rstudio.com) and Prism (version 5, https://www.graphpad.com/scientific-software/prism/). Nonparametric two-sided t-tests (Mann-Whitney-U-test) were conducted to detect differences in age and the number of years of education. Chi-square test was applied to analyze differences in the distribution of sex and handedness.

Analyses of covariance (ANCOVAs) were performed to compare the diffusivity parameters of average whole-brain and ROI FA, FA-t, and FW between HRP and HC with the covariates ‘group’, ‘sex’, and ‘age’.

A Spearman correlation was utilized to assess the relationship of positive, negative, and global PANSS scores and average whole-brain FA, FA-t and FW in HRP only. For the correlation, two HRP individuals were taken out of the analysis due to missing values for the symptom assessment with the PANSS.

ANCOVAs and Spearman correlations were Bonferroni-corrected to account for multiple comparisons. Significance was assumed upon obtaining a p-value < 0.05 after correction.

4 Results

4.1 Demographic and clinical characteristics

There were no significant differences between HRP and HC for age (p=0.0977), distribution of sex (p=0.8841) and handedness (p=0.5775). Education was measured in years and, likewise, showed no significant difference between HRP and HC (p=0.0879). For IQ, 3 values were missing in the control group and 8 values were missing for HRP. When compared without these, there was no significant difference in IQ. Both groups exceeded the average IQ.

4.2 Tract-based spatial statistics – whole brain and region of interest analysis

No significant differences were found in average whole-brain FA, FW, or FA-t between HRP and HC. Regions of interest (ROIs) were defined according to the Johns-Hopkins University DTI-based white-matter atlases. When comparing these, likewise, no differences were seen in FA, FW, or FA-t. However, FA-t showed an increase in HRP on a sub-threshold level in the fornix/striatum of the left side (uncorrected p = 0.0159). When comparing FW in different regions of interest, the inferior frontooccipital fasciculus on the right side (uncorrected p = 0.0051) and the posterior limb of the internal capsule on both sides (uncorrected p for the left side = 0.0212, for the right side = 0.0328) showed higher FW in HRP than HC.

4.3 Correlations

4.3.1 **Average whole-brain parameters**

When correlating the overall positive symptom scores as assessed with the PANSS with FA in HRP, there was a significant indirect correlation ($r = -0.5894$, corrected $p = 0.0441$). When correlating positive symptoms with FA-t and FW, a significant correlation between FA-t and overall positive symptoms was seen ($r = -0.6144$, corrected $p = 0.027$). Average FA-t and global symptoms correlated indirectly on a subthreshold level.

Table 2 - Average FA was correlated with positive, negative, and global symptoms as assessed with the PANSS (Spearman's correlation). Thereafter, FA-t and FW were also correlated with each symptom measure to determine if potential correlations with FA were driven by one or the other. *Significance was assumed at a p-value of < 0.05. P-values of correlations with FA were corrected for the performance of three individual tests (FA and each of positive, negative, and global symptoms). P-values of correlations with FA-t and FW were corrected for six individual tests.

Correlation Positive Symptoms

	r	p^{corr}
Average FA	- 0.5894	0.0441*
Average FW	0.4262	0.486
Average FA-t	- 0.6144	0.027*

Correlation Negative Symptoms

	r	p^{corr}
Average FA	0.1108	1.0000
Average FW	- 0.2643	1.0000
Average FA-t	- 0.04005	1.0000

Correlation Global Symptoms

	r	p^{corr}
Average FA	- 0.4164	0.5436
Average FW	0.2346	1.0000
Average FA-t	- 0.5339	0.1143

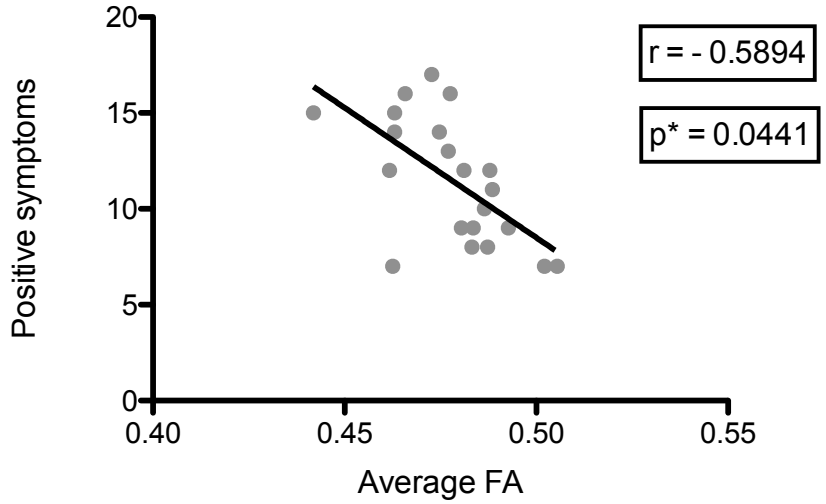

Figure 3 - Correlation of Positive Symptoms with Average FA in HRP - *FA correlates directly with overall positive symptoms in HRP - *corrected p-value*

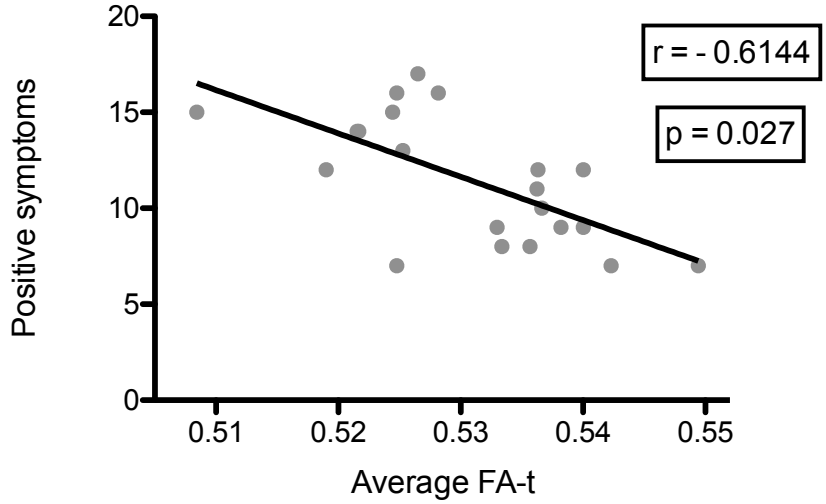

Figure 4 - Correlation of Positive Symptoms with Average FA-t in HRP - *FA-t correlates directly with overall positive symptoms in HRP - *corrected p-value*

4.3.2 **Regions of interest**

4.3.2.1 Correlations with positive symptoms

When analyzing the relationship of FA with positive symptoms in specific regions of interest, we found an indirect subthreshold correlation on the left side in the internal capsule, the posterior thalamic radiation, retrolenticular part of the internal capsule, and the sagittal stratum as well as the corona radiata, anterior limb of the internal capsule, and posterior part of the corona radiata on the right side. Other regions of interest showed weaker correlations (sagittal stratum, posterior thalamic radiation, posterior corona radiata, and the anterior limb of the internal capsule on the right side, the posterior corona radiata and the anterior limb of the internal capsule on the left side, the genu of the corpus callosum).

There were inverse correlations of positive symptoms with FA-t in circumscribed ROIs that did not survive correction for multiple testing in interhemispheric fibers, i.e., in the area of the genu of the corpus callosum and projection fibers, such as the left internal capsule, the right posterior corona radiata, and the posterior thalamic radiation bilaterally. Additionally, there was a subthreshold correlation in the left sagittal stratum.

None of the correlations of FW with positive symptoms were significant after correction for multiple testing, however, on a subthreshold level, average FW showed a direct correlation with positive symptoms ($r = 0.43$, uncorrected $p = 0.054$). Other ROIs that also showed trend-level direct correlation were interhemispheric fibers (corpus callosum, specifically its body), projection fibers, i.e., the left corona radiata, the superior part of the corona radiata bilaterally, and connection fibers, such as the superior longitudinal and uncinate fasciculus on the right side. Finally, positive correlations were also found in the left cingulate gyrus, fornix and striatum.

4.3.2.2 Correlations with negative symptoms

There were direct subthreshold correlations of FA with negative symptoms in two projection fibers, the left inferior fronto-occipital and the right superior longitudinal fasciculus, while FW correlated inversely with negative symptoms in the left cingulate gyrus and the external capsule on a subthreshold level.

4.3.2.3 Correlations with global symptoms

Global symptoms correlated weakly with DTI parameters before correcting for multiple analyses in a few regions. We found an inverse correlation with FA in the left internal capsule and the left posterior thalamic radiation. The FA-t of the left cingulum/hippocampus area had an inverse relationship, and the FA-t of the left uncinate gyrus had a direct relationship with global symptoms. When analyzing FW, direct correlations were found in the left cingulate gyrus, the fornix, and striatum.

5 Discussion

In the present study, we analyzed if there were any differences in FA, FA-t, or FW when comparing 23 HRP with 22 HC. The second goal of this study was to investigate the relationship between positive, negative, as well as global symptoms as assessed with the PANSS and white matter FA, FA-t, as well as FW aberrations.

We did not find any significant differences in FA, FA-t, or FW when comparing the two groups. However, we identified a significant inverse correlation of whole-brain average FA and positive symptoms that showed to be driven by a correlation of FA-t with positive symptoms.

5.1 White Matter Characterization

In the process of gathering information about schizophrenia development, it is important to obtain more specific information on morphological white matter aberrations than has so far been possible utilizing DTI. One of our goals was to contribute to the delineation of a trajectory of these changes with the relatively new Free Water analysis method. With its aid, a more precise account of structural aberrations can be taken compared to the traditional analytical tools for white matter and it is possible to further investigate if these aberrations are grounded on structural changes such as axonal or myelin damage.

This method has been applied to few chronic schizophrenia (Pasternak et al 2015, Oestreich et al. 2016), several first episode populations (Pasternak et al. 2012, Lyall et al. 2018, Bergé et al. 2020, Guo et al. 2020, Kraguljak et al. 2019, Lesh et al. 2019), and two HRP sample (Tang et al. 2019, Wang et al. 2017). FW, reflecting processes taking place outside of the cellular structure and its immediate vicinity, showed pronounced increase in a part of the mentioned first episode populations (Pasternak et al. 2012, Lyall et al. 2018, Bergé et al. 2020), while not showing any difference in others (Guo et al. 2020, Lesh et al. 2019). In individuals with chronic schizophrenia, very limited or no FW elevation was seen (Pasternak

et al. 2015, Oestreich et al. 2016). Neither did Tang et al. (Tang et al. 2019) nor did we see any differences in FW in our sample, which seems to suggest that if processes leading to an increased FW level were taking place during a first episode, this would and not be seen before the onset of schizophrenia and attenuate with time.

FA-t, the counterpart, possibly identifying structural aberrations such as axon or myelin damage, does not show a marked difference when comparing healthy controls to individuals undergoing a first episode of psychosis, but is drastically reduced in the chronic state, while small-scale FA-t aberrations were seen before the onset of schizophrenia. Based on this, we hypothesize that FA-t changes accumulate over the course of the disease, as was postulated for FA changes already. Therefore, no large-scale reductions in FA-t were predicted in the present study. Finding no changes in neither FA-t nor FW was not entirely expected, however. Firstly, if FW is able to mask the directedness of diffusion caused by structural aberrations, separating it from the signal would lead to more precise results and improved sensitivity for structural changes reflected by FA-t. Secondly, FA aberrations have been reported in many HRP studies (Vijayakumar et al. 2016) and FA-t reductions in the group mentioned above (Tang et al. 2019). Notedly, the latter was a much larger sample, such that the present study could be short in power to demonstrate subtle differences that were seen when analyzing a group twice the size. Nonetheless, findings throughout HRP are inconsistent. There is a subset of studies conducted in HRP that also did not find any significant FA differences (Clemm von Hohenberg et al. 2014, Carletti et al. 2012, Mittal et al. 2014, Peters et al. 2008, Bernard et al. 2015). Some among these have shown more conclusive findings only after separating the sample into individuals that converted to psychosis after a follow-up assessment and those who did not (Mittal et al. 2014) or when longitudinally analyzing group by time interactions (Carletti et al. 2012). Although this was not the case for the HRP population showing FA-t alterations (Tang et al. 2019), it could imply that morphological disease correlates predate psychosis in those individuals that will have worse outcome or experience a transition in the future. If this is the case, developing more sensitive methods to assess aberrations within the brain could lead to improved risk stratification and a more accurate disease model.

5.2 Correlations

Reduced as well as elevated FA have been reported to be associated with symptoms in schizophrenia (Vijayakumar et al. 2016). Decreased FA in the right superior temporal lobe (Bloemen et al. 2010) and other patterns (Krakauer et al. 2017) were associated with positive symptoms in HRP. Whole-brain average FA-t was reported to correlate with functional decline in a HRP population before (Tang et al. 2019).

Among other regions of interest, we found an inverse subthreshold correlation of positive symptoms with FA as well as FA-t in the genu of the corpus callosum and a direct trend-level correlation with FW across the entirety of the corpus callosum. This structure represents the main interhemispheric connecting tract and seems to be implicated in disease development. In high risk individuals, lower FA-t (Tang et al. 2019), lower FA (Katagiri et al. 2015), and higher MD (Clemm von Hohenberg 2014) have been found in that area, and FA has been shown to decrease over time in individuals who later converted to psychosis (Carletti et al. 2012). In schizophrenia, aberrant white matter parameters (Wheeler and Voineskos 2014) and FA correlations with positive as well as negative symptoms (Kubicki et al. 2008) were predominantly found in the anterior portion, the genu, which connects the prefrontal cortices of the two hemispheres, which are, as well as the temporal lobes, the regions most heavily affected in structure and function in schizophrenia (Davis et al. 2003). Maturation of the genu of the corpus callosum takes place during early adulthood, which is a typical time of disease onset. This might be an expression of a developmental aberration caused by the lack of communication between the two hemispheres that contribute to a faulty development of each of these. Finding average FA-t in that area to be lower depending on symptom severity supports the theory of impaired interhemispheric connectivity in schizophrenia (Kubicki et al 2008).

Furthermore, we found direct subthreshold correlations of FW with positive symptoms in several projection fibers, the corona radiata, and other connection fibers, one of them being the superior longitudinal fasciculus, in which FA has

been shown to correlate with subthreshold psychotic symptoms in non help seeking individuals (DeRosse et al. 2017). Furthermore, FA aberrations of the superior longitudinal fasciculus have been shown consistently in schizophrenia (Wheeler and Voineskos 2014). According to the Free Water theory, which suggests that extracellular pathology is reflected by increased FW levels, a direct correlation between free water and symptom severity as a measure of psychopathology would support this notion.

As expected with the lack of large-scale FW changes in another HRP population (Tang et al. 2019) additionally to the present sample, we did not find any correlation between whole brain average FW and positive symptoms. Nor did we detect any correlations of FA-t or FW with negative symptoms, although correlations of FA with negative symptoms have been found that were either direct (Moriya et al. 2010) or indirect (Wolkin et al. 2003, Szeszko et al. 2008) in schizophrenia.

Besides the inverse correlations of FA and positive symptoms, some studies have also reported indirect correlations of FA with negative symptoms in HRP (Krakauer et al. 2017, Wang et al. 2016). Specifically, these regions comprised the inferior fronto-occipital, the inferior longitudinal fasciculus, and the superior longitudinal fasciculus, the latter being a tract frequently associated with schizophrenia (Wheeler and Voineskos 2014).

Although the regions of interest in which we found correlations did not overlap with those described before, our results are in line with the general concept that FA or FA-t aberrations are more pronounced with augmenting symptom severity. The direction of the correlation implies that reduced FA that has been used as a marker for reduced connectivity and white matter pathology, if driven by FA-t changes, might be associated with higher symptom burden. This is in keeping with the disconnectivity hypothesis linking aberrant brain signal synchronization to schizophrenia development.

5.3 Interpretation

FW is a relatively new method that aims to remove potential signal contamination stemming from extracellular processes that might mask aberrations specific to axons and myelin. As of yet, research has focused on white matter structures while the extracellular space has been given little attention, although it influences diffusion properties within the tissue. A number of different processes can lead to an altered overall diffusion signal. Decreased neuronal size could alter properties of the tissue itself (Boksa 2012), resulting in FA-t changes, while excessive synaptic pruning could result in atrophy (Rajskowska et al. 1998) and might be able to alter FW. Moreover, cerebral edema poses the possibility of tissue signal contamination by enlarging the extracellular space. The kind of edema most likely taking influence on FW is the vasogenic subtype, which can be elicited by a number of different processes, an acute immune response being one of them (Lyall et al. 2018).

The immune system has been thought to be a driving force behind psychotic relapses and macroscopic brain alterations. Based on previous immunological findings and individual disease trajectories, it has been hypothesized that primed microglia induce a process interfering with physiological neuroplasticity and resulting in morphological and chemical aberrations that progress throughout the course of an individual's life (DePicker et al. 2017).

Conversely, Lyall et al. found better outcomes in individuals who showed higher levels of FW and speculate that this may correlate with a very subtle form of neuroinflammation, possibly representing a protective reaction to an unknown stimulus (Lyall et al. 2018). This stimulus might be an insult with sequelae that infringe on functionality to a lesser degree if said immune reaction or another unknown protective process leading to raised FW levels is commenced.

Neuroinflammation represented a promising concept, meeting the criteria of being an insult that can befall an individual at any time, and being able to elicit neurodegeneration that is seen later on in the course of the disease (Najjar and Pearlman 2015). However, more recent studies that aimed to confirm findings

concerning microglial activation as an essential part of the immunological activation in the brain, failed to show the changes in question (van der Doef et al. 2016, Di Biase et al. 2017, Hafizi et al. 2017a, Hafizi et al. 2017b). In light of the inconsistent findings regarding the FW level in white matter throughout the disease states, it is possible that rather transient unspecific imbalances of the immunological system being most pronounced during periods of florid psychosis may be sparking the changes seen in chronic schizophrenia.

5.4 Limitations

The present study has several limitations. Our findings might reflect distortion due to the small sample size and age of HRP individuals that does not allow to assume a representative population or to extrapolate and apply to the general HRP population. The small sample size furthermore did not permit to control for sex and age to a satisfactory extent, two factors that might have influence on diffusion parameters and confound the results, even though the high risk and healthy control group were closely matched. Age has a significant impact on white matter microstructure. The aging brain shows a decrease in FA (Inano et al. 2011), commonly attributed to physiological deterioration. Kochunov et al. suggest an inverse-U trajectory of Fractional Anisotropy with its peak in a person's third decade of life (Kochunov and Hong 2014) to which the present sample was closer than other HRP populations might be. White matter volume decreases around the age of 50 and shows a more steep decline after the age of 60. Regions in which age-related differences were shown were the corpus callosum, parts of the internal capsule, and frontal regions, among others (Hsu et al. 2010).

Sex differences also showed to have substantial influence on white matter microstructure in certain brain regions, such as the cerebellum and left superior longitudinal fasciculus where men showed higher FA than women and vice versa in the corpus callosum (Kanaan et al. 2012). Other regions in which differences

correlated with sex were the cingulum and a small part of the anterior limb of the internal capsule (Inano et al. 2011).

Besides sex and age differences, the information whether a High Risk for Psychosis individual later transitions to psychosis or not is a very important parameter to further narrow down the sample of 'real' prodromal individuals and help define a schizophrenia trajectory. The only way to parse out this group is to conduct longitudinal studies and retest for psychosis after a certain period of time. Then, after separating into converters and non-converters, informed analyses comparing these two groups are needed. Individuals declared as non-converters, however, are apt to later transition to psychosis regardless, such that even after follow-up, the group investigated is not necessarily a homogenous sample of prodromal individuals. Without this important piece of information, however, the group regarded is very heterogeneous and might not allow for the detection of subtle changes across those who do convert. In light of the fact that symptoms are extremely heterogeneous as well, not only in HRP but also in schizophrenia, detection of white matter aberrations is further complicated by the notion that they might manifest in individual patterns and differently in each subject, not unlike the ones seen in multiple sclerosis (Davis et al. 2003). Consequently, averaging DTI parameters across a group would not yield any specific results and aberrations would escape detection in a small sample.

In addition to above-mentioned confounders, the possibility of a bias inherent to investigated samples needs to be taken into consideration. Being a very heterogenous population a priori, selection of HRP individuals necessitates great care and standardized procedures. However, recruitment methods vary greatly across different studies such that risk enrichment fluctuates in different HRP groups (Fusar-Poli et al. 2016).

Only a small part of first episode patients have institutional contact during their prodromal phase (van Os and Guloksuz 2017). Hence, only a small proportion of prodromal individuals sought help and were recruitable for studies. It is difficult to rule out the possibility that this lead to a certain subgroup of prodromal individuals being represented too strongly in research. This selection bias might contribute to a skewed image of the prodromal phase, which possibly only reflects a restricted

pool of underlying pathologies, if these are, in fact, different in each individual. Furthermore, a large proportion of HRP (over 73%) have additional psychiatric diagnoses, the most common among them being depression, anxiety, and substance abuse (Fusar-Poli et al. 2014). Including individuals affected by these conditions holds the risk of identifying morphological aberrations that pertain to a different diagnosis instead of being inherent to schizophrenia. Excluding them, on the other hand, does not only lead to massive restriction of already hampered power by producing much smaller sample sizes, but further might exclude a group that could represent an important part of pathology development. Psychosis occurs in the context of different phenomenological frameworks and only reflects one aspect of psychiatric disease presentation without necessarily forming part of a schizophrenia syndrome (van Os and Guloksuz 2017). Hence, belonging to the HRP group does not necessarily predict clinical or functional outcome, as other conditions and mental health problems predispose for psychosis to a similar extent (Conrad et al. 2017), while a great proportion of those seeking help recover over time. Recent research has shown that outcome after 38 months is predicted correctly only in a fourth of this population (Fusar-Poli et al. 2016). The HRP group is therefore not a simple and homogeneous study subject that can easily be used to extrapolate and generalize a disease trajectory valid for the entire schizophrenia population.

Another difficulty pertaining to the HRP concept is that while genetic markers and affected relatives play an important role in the assessment, the great majority of individuals at risk are identified by assessing attenuated symptoms (van Os and Guloksuz 2017). Being at risk for psychosis on one side and the transition to psychosis – marked by the severity of positive symptoms – on the other side, are both measured on the same scale, such that the greatest risk factor for developing schizophrenia symptoms is already showing these to a certain extent. The shift from 'risk' to 'psychosis' is only a quantitive, not a qualitative one (van Os and Guloksuz 2017). The binary concept of transition with a prodromal period is likely simplified in light of the complex mechanisms leading to psychiatric diseases that have not fully been understood to date. The lack in knowledge might contribute to the existing deficit in implementing early identification and prevention of psychosis in preventive medicine. Furthermore, without the basic understanding of the

illness, screening tools are inherently inaccurate as discussed above. There is no known effective curative treatment for schizophrenia as of now, such that prevention efforts are not streamlined and incited by an ultimate procedural goal. It was suggested that risk be better interpreted as baseline differences in severity of psychopathology, which can be regarded as a fluid multidimensional concept instead of a rigid categorization (van Os and Guloksuz 2017). Alleviation of HRP as well as a better characterization of the group could be achieved by following the whole cohort of help-seeking persons and addressing present problems while backing the individual's position through a continuous and reliable relationship with the caregiver (Conrad et al. 2017).

5.5 Conclusions

The findings of the present study support the notion that microstructural white matter differences may develop over the course of the illness and might be very subtle before the transition to psychosis. The inverse correlation between average FA and severity of positive symptoms we found that seemed to be driven by FA-t implies that, although not yet detectable in our sample, structural aberrations may be linked to symptoms in HRP.

The process of psychopathology manifesting in cerebral structure might be a gradual one, depending on the number and frequency of acute psychotic phases, medication, etc.

Further, as Tang et al. (Tang et al. 2019) and we reported no increases in FW in HRP, we could hypothesize that FW is only elevated in a part of the patients that are actively experiencing acute psychosis. Our results, however, do not suggest large-scale extracellular water pathology in our population of HRP.

In order to elucidate the causality of extracellular water changes during disease progression and its level of significance in schizophrenia development, substantial gaps of knowledge need to be closed in the future and the missing links between

the different existing hypotheses need to be clarified and put into context. De Picker et al. suggest that this might best be accomplished by integrating different modalities, such as multimodal imaging in vivo, immunological markers in vivo, and histological studies (De Picker et al. 2017).

The developments in this field is not conclusive of FW aberrations being due to neuroinflammatory processes, which was postulated upon its development (Pasternak et al. 2009). This method has not been applied to many samples and the exact source of signal alteration is a subject of speculation. It was shown that rats showed a higher level of FW in white matter later in life when their pregnant mothers were injected with Poly I:E, a robust inductor of inflammation, while the individuals were in utero (DiBiase et al. 2019). However, this specific method has not widely been applied to models with induced pathologies to achieve validation yet. Hence, a high level of care is imperative when interpreting results obtained with this method. One of the conclusions that can be drawn from results so far is that even though it is not certain if FW measures neuroinflammation, edema of another origin, atrophy, or a decline in neuronal size, there are likely processes taking place in the extracellular space accompanying or even preceding psychosis that need to be differentiated from aberrations related to axons and myelin seen later on in the course of the disease and thought to be reflected in FA-t changes.

The development of the FW method has instigated further discussions about processes other than neurodegeneration that underlie schizophrenia development and has opened up a new field of investigation. With its application, new possibilities of monitoring and exploration are gained.

Little is known about the pathology underlying schizophrenia and we are still far from understanding this disease. One of the reasons for the difficulties investigating schizophrenia is the variability in its presentation. Different approaches have been suggested to overcome this challenge. In addition to phenomenology, endophenotypes are being investigated such as the trajectory of white matter that is characterized on a more detailed level thanks to advancing technology in the field. Changes are seen very distinctly in advanced stages, but factors predisposing for or eliciting the disease are difficult to determine. Since prevention is not feasible without an accurate risk stratification, studies are being

conducted with the goal to find more tangible and categorizable criteria to predict outcome early-on and thus gauge the risk more effectively that an individual carries to develop psychosis. It is expected to thus minimize heterogeneity and facilitate the administration of preventive medication to groups at very high risk for transition without taking unreasonable risks with individuals displaying a low risk. On these grounds, further investigation is needed in the field of high risk individuals. However, besides the problem of small numbers and a reporting bias, unspecific symptomatology, and the lack of detailed knowledge of the disease trajectory, heterogeneiety persists in this group. Since criteria to define this population are not based on quantifiable biological markers, the interpretability of results is limited. A closer observation of the development in the HRP paradigm is needed as more information is collected.

In order to gain more insight in disease development and to integrate the existing hypotheses concerning the biological underpinnings of schizophrenia, it will be of crucial importance to utilize multiple modalities and take advantage of all the instruments available. This will not only comprise serological markers and genetic analyses, but also different approaches to cerebral imaging. Spectroscopy to investigate aberrant levels of substances, functional imaging including MRI and electroencephalography as well as diffusion MRI to further explore microstructural aberrations will need to be concerted. Diffusion MRI offers growing possibilities to investigate white matter aberrations, among them the free water method that we have utilized in this study. However, since it is not yet known which processes lead to changes in the free water level, validation ex vivo is needed to align histological findings with imaging. Subsequently, the application to larger samples than those already analyzed might contribute to further clarification of schizophrenia pathology. This, in turn, will improve prevention, care, and alleviation of individuals affected by this decapacitating disease.

6 Summary

Microstructural white matter (WM) alterations found in schizophrenia are hypothesized to contribute to the disease pathology. They are, however, not found consistently in subjects at high risk for psychosis (HRP). This might be due to the limited specificity of the applied diffusion analysis and the heterogeneous nature of the group.

In this study, we were able to apply a relatively new method to HRP, which yields a parameter reflecting tissue damage (FA-t) more specifically than commonly used methodologies and further reveals extracellular free water (FW) changes related to extracellular processes that might contribute to disease development.

Diffusion magnetic resonance imaging (MRI) was acquired from 23 HRP and 22 healthy controls (HC). FW Analysis was used to deconstruct the diffusion signal into FW and tissue FA (FA-t) maps. We then employed the ENIGMA approach to Tract-Based Spatial Statistics, which creates and aligns WM skeletons for optimal comparability of diffusion measures.

When comparing HRP to HC, no significant differences were found in FA, FW, or FA-t. This may indicate that gross WM alterations develop over time. Negative correlations between FA and positive symptom scores assessed with the Positive and Negative Syndrome Score (PANSS) were mirrored by significant correlations between FA-t and positive symptoms, suggesting that early subtle structural WM aberrations may contribute to symptom development.

However, the HRP population remains a very heterogenous group that may not allow to draw generalizable conclusions. A more careful investigation of the HRP group, greater samples, and longitudinal analyses are needed to parse it into individuals that do and do not convert to psychosis in order to further elucidate early schizophrenia pathology.

Zusammenfassung

Mikrostrukturelle Veränderungen der weißen Substanz (WM) von Menschen mit Schizophrenie könnten nach Erkenntnissen der letzten Jahre zur Entwicklung der Erkrankung beitragen. Diese Alterationen werden bei Personen mit hohem Psychoserisiko (HRP) nicht konsistent gefunden. Dies könnte an der limitierten Spezifität der applizierten Diffusionsanalysen und der heterogenen Natur dieser Gruppe liegen.

In dieser Studie wandten wir eine relativ neue Methode auf HRP an, welche einen Parameter erbringt (FA-t), der Schäden am Gewebe möglicherweise spezifischer darstellt als üblicherweise verwendete Methoden, und Veränderungen des extrazellulären freien Wassers (FW) identifiziert, das mit extrazellulären Prozessen in Zusammenhang steht, die zur Pathogenese beitragen könnten.

Bei 23 HRP und 22 gesunden Kontrollpersonen (HC) wurden Diffusions-Magnet-Resonanz-Tomografien (MRI) durchgeführt. Die FW-Analyse wurde angewandt, um das Diffusionssignal in FW- und FA-t-Maps zu dekonstruieren. Nachfolgend verwendeten wir den ENIGMA-Ansatz für *Tract-Based Spatial Statistics*, welches Gerüste für Fasertrakte der weißen Substanz generierte und für eine optimale Vergleichbarkeit der Diffusionsparameter aufeinander ausrichtete.

Im Vergleich von HRP mit HC fanden wir keine signifikanten Unterschiede in Bezug auf FA, FW oder FA-t. Dies könnte bedeuten, dass relevante Veränderungen der weißen Substanz sich über die Zeit hinweg entwickeln. Wir sahen negative Korrelationen zwischen FA sowie FA-t und positiven Symptomen nach dem *Positive and Negative Syndrome Score* (PANSS), welches suggeriert, dass frühe und subtile strukturelle Veränderungen der weißen Sustanz zu der Entwicklung von Symptomen beisteuern könnten.

Die HRP-Population bleibt eine höchst heterogene Gruppe, welches erschwert, an ihr gewonnene Erkenntnisse zu generalisieren. Eine sorgfältigere Untersuchung dieser Gruppe, größere Stichproben und longitudinale Analysen sind vonnöten, um diejenigen Personen, welche im Verlauf eine Psychose erleiden, von denen ohne eine solche Entwicklung zu unterscheiden und die pathogenetischen Mechanismen der Schizophrenie besser zu verstehen.

7 List of Abbreviations

AD	axial diffusivity
ADC	apparent diffusivity coefficient
APS	attenuated psychotic symptoms
B(L)IPS	brief (and limited) intermittent psychotic symptoms
DTI	diffusion tensor imaging
ENIGMA	Enhancing NeuroImaging Genetics through Meta-Analysis
ERIraos	early recognition inventory for the retrospective assessment of the onset of schizophrenia
FA	fractional anisotropy
FA-t	fractional anisotropy of the tissue
FOV	field of view
FW	free water
GRNS	German Research Network on Schizophrenia
HC	healthy control
HRP	individuals at High Risk for Psychosis
MD	mean diffusivity
MO	mode of anisotropy
MRI	magnetic resonance imaging
PANSS	positive and negative syndrome scale
PET	positron emission tomography
RD	radial diffusivity
ROI	region of interest
SOPS	scale of prodromal symptoms
TBSS	tract-based spatial statistics
T	Tesla
TE	echo time
TR	repetition time
VBM	voxel-based morphometry

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10 Curriculum vitae

The curriculum vitae has been omitted due to privacy reasons.

11 Declaration on Oath

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