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Cellular and extracellular white matter alterations indicate conversion to psychosis among individuals at clinical high-risk for psychosis

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ABSTRACT

Objectives: It is important to find biomarkers associated with transition to illness in individuals at clinical high-risk for psychosis (CHR). Here, we use free-water imaging, an advanced diffusion MRI technique, to identify white matter alterations in the brains of CHR subjects who subsequently develop psychosis (CHR-P) compared to those who do not (CHR-NP).

Methods: Twenty-four healthy controls (HC) and 30 CHR individuals, 8 of whom converted to schizophrenia after a mean follow-up of 15.16 months, received baseline MRI scans. Maps of fractional anisotropy (FA), FA of cellular tissue (FA_T), and extracellular free-water (FW) were extracted using tract-based spatial statistics after which voxel-wise non-parametric group statistics and correlations with symptom severity were performed.

Results: There were no significant differences between HCs and the combined CHR group. However, prior to conversion, CHR-P showed widespread lower FA compared to CHR-NP ($p_{FWE} < 0.05$). FA changes in CHR-P were associated with significantly lower FA_T and higher FW, compared to CHR-NP. Positive symptoms correlated significantly with diffusion parameters in similar regions as those discriminating CHR-P from CHR-NP.

Conclusions: Our study suggests that cellular (FA_T) and extracellular (FW) white matter alterations are associated with positive symptom severity and indicate an elevated illness risk among CHR individuals.

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Introduction

Patients with schizophrenia and related psychoses suffer from a range of symptoms including hallucinations, delusions, lack of motivation, and cognitive decline (American Psychiatric Association 2013). Early diagnosis and intervention, optimally prior to the onset of psychosis, has the potential to diminish the number of subjects that develop symptoms (Schmidt et al. 2015; Schultze-Lutter et al. 2015). Early identification of at-risk individuals would also be helpful in characterising pathologies that are more closely tied with the aetiology of the disorder, rather than with chronicity effects (Wood et al. 2011; Takahashi and Suzuki 2018).

In the early 2000s, clinical high-risk for psychosis (CHR) criteria (Miller et al. 2003; Yung et al. 2005) were established to address the importance of delineating possible clinical and biological features that are related to an increased risk for developing psychosis (Fusar-Poli et al. 2013). These criteria have been used in many research studies and, more importantly, have been translated into early intervention strategies aimed at preventing psychosis-onset (Schmidt et al. 2015). At the same time, recent analyses suggest that transition rates in CHR individuals are ~30% or even lower (Fusar-Poli et al. 2012; Hartmann et al. 2016), which further highlights the need for identifying

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additional conversion markers (Fusar-Poli et al. 2015; Cannon 2016; Abu-Akel et al. 2018).

Recently, neurodevelopmental alterations of brain white matter have been implicated in the pathophysiology of psychotic disorders (Kochunov and Hong 2014). Diffusion magnetic resonance imaging (MRI) has been employed to investigate neurobiological markers associated with white matter alterations, and may therefore significantly improve prediction of transition outcomes in CHR cohorts (Pettersson-Yeo et al. 2013; Vijayakumar et al. 2016). Specifically, diffusion tensor imaging (DTI) studies have repeatedly shown reductions in white matter fractional anisotropy (FA), a putative measure of microstructural organisation, across different stages of the disorder including at-risk cohorts (Arat et al. 2015; Vijayakumar et al. 2016; Kelly et al. 2018). Support for the presence of abnormal white matter also comes from histopathological and genetic studies which implicate myelin dysfunction in the pathophysiology of psychosis (Tkachev et al. 2003; Takahashi et al. 2011; Vikhrevva et al. 2016). In addition, recent studies show increased evidence for abnormal immune activation as a possible mediator of white matter pathology (Najjar and Pearlman 2015). Reports describing elevated pro-inflammatory cytokines and activated microglia in first-episode psychosis and CHR cohorts further corroborate evidence that immune dysfunction may play a role in the manifestations of psychotic illnesses (Miller et al. 2011; Coughlin et al. 2016; Khoury and Nasrallah 2018). However, traditional DTI metrics are limited in their ability to segregate between evidence of cellular damage to white matter, more likely associated with neurodegenerative or neurodevelopmental changes, and extracellular pathologies that have been proposed to reflect immune dysfunction/activation (Pasternak et al. 2016; Pasternak et al. 2018; Di Biase et al. 2020).

The diffusion MRI-based free-water imaging technique addresses these limitations by decomposing the diffusion signal into two compartments (Pasternak et al. 2009). The first compartment models the fractional volume of isotropic, unrestricted diffusion in the extracellular space (free-water, FW) which can be responsive to pathologies such as edoema and atrophy (Pasternak et al. 2012; Lyall et al. 2018). The second compartment models hindered/restricted diffusion in close proximity to cellular membranes, from which FA of the tissue (FA_T) is derived, reflecting more closely changes in myelination and axonal membrane health than the conventional DTI metric FA (Pasternak et al. 2009). By differentiating these compartments this model has proven to be successful in providing new

insights to potential biological mechanisms underlying the observed FA reductions in schizophrenia (Pasternak et al. 2012, 2015; Lyall et al. 2018).

Specifically, free-water imaging studies in subjects following their first psychotic episode have shown widespread elevations in FW following the onset of psychosis, accompanied with more focal FA_T changes (Pasternak et al. 2012; Lyall et al. 2018). Moreover, two previous free-water imaging studies in independent CHR samples (Wang et al. 2016; Tang et al. 2019) reported reduced FA_T in CHR subjects, but did not identify increased FW (Tang et al. 2019). These studies suggest that structural abnormalities observed in inter-hemispheric and association fibres may already be present in the CHR stage and may reflect a neurodevelopmental predisposition to display prodromal symptoms (Tang et al. 2019). However, these studies have yielded inconclusive results regarding the issue of whether these brain abnormalities are indicative of transition to psychosis, necessitating additional investigation.

In the current study we apply free-water imaging to compare CHR individuals who subsequently developed psychosis (CHR-P) with those who did not (CHR-NP). We hypothesise that CHR-P subjects will show white matter aberrations that are more similar to those previously described in patients with first-episode psychosis (i.e. widespread FW increases and less extensive FA_T reductions) relative to CHR-NP individuals (Pasternak et al. 2012; Lyall et al. 2018). Furthermore, to characterise better the clinical consequences of white matter abnormalities, we investigated whether diffusion MRI (dMRI) measures are correlated with symptom severity, as rated with the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987).

Materials and methods

Participants

CHR individuals, aged 15 to 65 years, were screened for eligibility for participation by clinicians in the Early Recognition Department for Outpatients of the Department of Psychiatry of the University Medical Centre Hamburg-Eppendorf. Healthy controls (HC) who were age-, sex-, and education-matched to the CHR sample were recruited from the same geographic area. This cohort was part of a larger research initiative (Collaborative Research Centre 936) from which other data have previously been reported (Leicht et al. 2016).

Table 1. Demographic and clinical characteristics of the sample.

Variable	HC (n = 24)		CHR (n = 24)		U/ χ^2	p	CHR-NP (n = 16)		CHR-P (n = 8)		$\chi^2/U/T$	p
	11/13		12/12				9/7		3/5			
Sex (F/M)	Mean	SD	Mean	SD	$\chi^2 = 0.083$	0.773	Mean	SD	Mean	SD	$\chi^2 = 0.835^a$	0.659 ^a
Age (years)	23.04	4.32	21.29	3.50	U = 354	0.174	21.50	3.65	20.88	3.36	$\chi^2 = 2.111^a$	0.348 ^a
Education (years)	(n = 23) 14.63	2.83	(n = 23) 13.11	3.00	U = 351.5	0.056	(n = 16) 13.28	2.95	(n = 7) 12.71	3.30	$\chi^2 = 4.031^a$	0.133 ^a
Antipsychotic Medication			(n = 8)				5/16		3/8		$\chi^2 = 0.094$	0.760
CPZE (mg/day)			168.75	148.92			180.00	172.16	150.00	132.29	U = 7	0.879
Duration (months)			3.61	5.58			4.29	6.77	2.23	2.42	U = 8	0.897
PANSS			(n = 23)				(n = 16)		(n = 7)			
Positive			11.61	3.17			11.50	3.39	11.86	2.85	U = 61.5	0.737
Negative			9.65	2.71			9.56	3.03	9.86	1.95	U = 68.5	0.409
Global			26.52	5.63			26.63	6.30	26.29	4.11	T = -0.153	0.880
SOPS												
Positive			(n = 22)				(n = 15)		(n = 7)			
Mean			5.86	4.78			4.73	4.54	8.29	4.68	U = 81	0.047*
Negative			(n = 20)				(n = 14)		(n = 6)			
Mean			5.65	4.68			5.43	4.88	6.17	4.58	U = 48	
Disorganisation			(n = 21)				(n = 15)		(n = 6)			0.648
Mean			2.19	2.16			1.93	2.12	2.83	2.32	U = 57	0.361

Note: F: female; M: male; CPZE: chlorpromazine equivalents; PANSS: Positive and Negative Syndrome Scale; SOPS: Scale of Prodromal Symptoms; HC: healthy controls; CHR: individuals at clinical high-risk for psychosis; CHR-NP: CHR individuals who did not develop psychosis; CHR-P: CHR individuals who developed psychosis; SD: standard deviation

*These test statistics reflect the comparison of three groups (HCs, CHR-P, CHR-NP); *significant at $p < 0.05$

CHR was defined based on the criteria of the Early Detection and Intervention Programme of the German Research Network on Schizophrenia (Wölwer et al. 2006), i.e. as the presence of either: a) basic symptoms: presence of at least two subjective cognitive or perceptual disturbances with a score of ≥ 3 on the Schizophrenia Proneness Instrument (Schultze-Lutter et al. 2007), or b) a schizotypal personality disorder/family history of psychotic disorder plus a decline of $\geq 30\%$ 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) (for details see [Supplementary Material and Methods](#)).

The presence of major neurological or somatic illnesses, as well as current substance abuse, disqualified subjects from study participation. HCs were excluded if they were previously diagnosed with, or treated for, any neurological or psychiatric disorder (including substance use disorders) or had a family history of psychotic disorders.

Ethics statement

The study was approved by the Ethics Committee of the Medical Association of Hamburg. After explaining the objectives and the methods of the study, written

informed consent was obtained from each participant, or, in the case of a minor, from the legal guardian.

Neuropsychiatric assessment

CHR participants underwent a neuropsychiatric assessment within two weeks of the MRI scan and conversion to psychosis was evaluated after a follow-up period of 12 months. Re-assessments after more than 12 months were allowed in order to minimise the number of CHR individuals lost to follow-up. Nevertheless, 6 CHR individuals could not be followed-up (attrition rate: 20%, see [Table S1](#)).

Psychiatric comorbidities in the CHR population were recorded after the administration of the Mini Neuropsychiatric Interview (Sheehan et al. 1998) and a comprehensive review of each individual's psychiatric medical history. Symptom severity was assessed at baseline utilising both the PANSS and the SOPS (Kay et al. 1987; Miller et al. 2003).

Conversion to psychosis was defined as reaching a score of 6 on any of the positive symptom domains of the SOPS. The Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) was subsequently used to specify the type of psychosis.

Final dataset

In the present study, we included 30 CHR subjects and 27 HCs based on the availability of baseline diffusion weighted MRIs (dMRI). Twenty-four (24) HCs

remained in the study, after 3 HCs were excluded following visual inspection of all MRI scans (severe signal dropouts, $n = 2$; hydrocephalus, $n = 1$). Clinical follow-up information was available for 24 of the 30 CHR individuals.

Image acquisition

All dMRI scans were acquired on a 3T MRI scanner (Siemens Magnetom Trio, Siemens Erlangen) at the University Medical Centre Hamburg-Eppendorf using a 12-channel head coil. Throughout the study, the same scanner and software was used. A spin-echo single-shot echo-planar imaging sequence (EPI) was implemented with the following parameters: repetition time (TR) = 7.7 s, echo time (TE) = 85 ms, flip angle = 90°, 64 interleaved axial slices, slice thickness = 2 mm, field of view (FOV) = 216 × 256 mm², matrix = 108 × 128, 30 non-collinear gradient directions with $b = 1000$ s/mm² collected twice within the same acquisition and 10 images with $b = 0$ s/mm². The acquisition time was 9 min and 22 s, using 6/8 phase partial Fourier, as well as generalised autocalibrating partial parallel acquisition (GRAPPA) with an acceleration factor of 2. In addition, a T1-weighted magnetisation prepared rapid gradient echo (MPRAGE) data set was obtained to rule out neurological/structural abnormalities.

Image processing

Quality control consisted of visually inspecting the dMRIs (3D Slicer v4.5, www.slicer.org, (Fedorov et al. 2012)) and semi-automatically removing gradient direction affected by severe artefacts or signal dropouts with an in-house tool. The groups did not differ in the number of removed gradients (maximum = 8, $p = 0.882$). Further, all scans were corrected for motion and eddy currents by affine registration of all volumes to the first $b = 0$ volume using FLIRT (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT>, (Jenkinson et al. 2002)). The gradient vector table was updated accordingly and a relative motion parameter was obtained for each scan from the transforms (Ling et al. 2012). There was no significant difference between groups for relative motion during the scanning session ($p = 0.236$). Finally, brain masks for skull-stripping were automatically generated within 3D Slicer and then manually edited to ensure anatomical accuracy.

Diffusion tensor and FW estimation

For comparison purposes with previous studies, we chose to include the traditional FA metric in all analyses alongside our primary outcome measures FA_T and FW. Diffusion tensors were estimated for each voxel from the preprocessed images using a least-squares fit. Next, scalar maps for FA were derived from the tensors. In addition, the free-water model was fitted to the diffusion images (Pasternak et al. 2009). The fit was performed using a regularised non-linear fit, resulting in FW maps, and FW-corrected diffusion tensors, from which FA_T was calculated. Of note, biophysical components other than free-water may confound diffusivity in white matter and might not be resolved by the free-water model. Moreover, it is possible that an inaccurate model fit, as well as image artefacts may bias the estimation of the volume of the free-water compartment. Nevertheless, FA_T and FW will be interpreted as surrogate markers for the tissue and extracellular compartment, respectively (Pasternak et al. 2012, 2015; Lyall et al. 2018).

Tract-based spatial-statistics

We adapted the standard tract-based spatial-statistics (TBSS) pipeline (Smith et al. 2006) in terms of the registration procedure. For this purpose a study-specific template, created with `antsMultivariateTemplateCreation2.sh` (www.github.com/ANTsX/ANTs, (Avants et al. 2011; Keihaninejad et al. 2012)), was used as the registration target. After averaging all registered FA images, a white matter skeleton of the mean FA image was created and thresholded at $FA > 0.25$. All FA, FA_T and FW maps were then skeletonised according to the TBSS pipeline.

Statistical image analysis

Non-parametric, voxel-wise group comparisons and correlation analyses were performed on the entire skeleton in Randomise (Winkler et al. 2014) using threshold-free cluster-enhancement (Smith and Nichols 2009). For each contrast described below, 5000 permutations were carried out to obtain family-wise error-corrected (FWE) statistical maps with the significance level set to $p < 0.05$. Age, sex, and motion were included as covariates in *all* voxel-wise analyses.

Using FSL Randomise, we performed two-sided *T*-tests comparing the HC ($n = 24$) and CHR ($n = 30$) groups at baseline regarding FA, FA_T and FW. Furthermore, we conducted two-sided *T*-tests

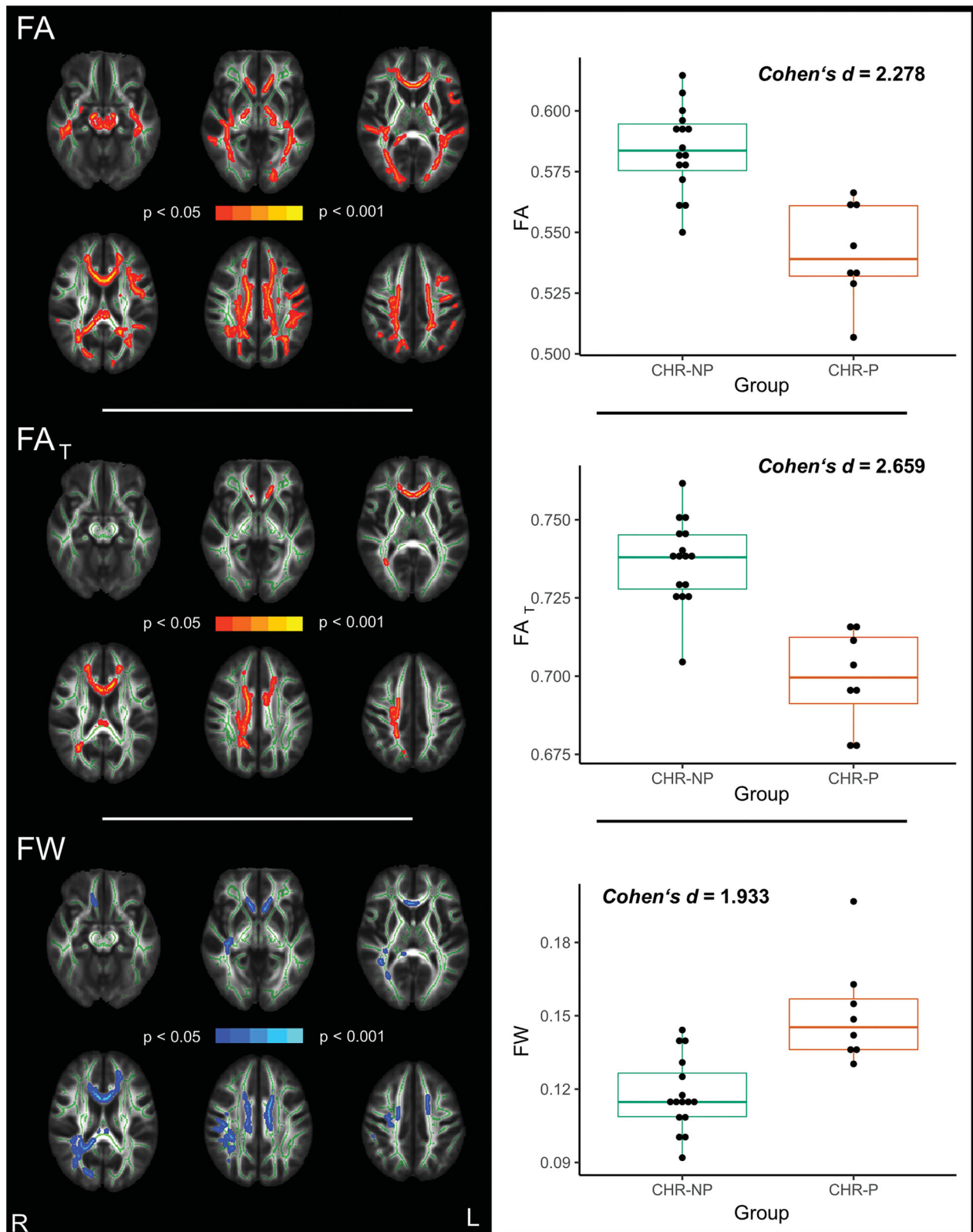


Figure 1. White matter alterations in CHR-P compared to CHR-NP ($p_{FWE} < 0.05$). Global FA reductions in CHR-P comparing to CHR-NP appear to be explained both by FA_T reductions and FW increases in CHR-P as there is a considerable overlap between significant clusters (left panels). Diffusion measures averaged across corresponding significant clusters are presented as boxplots on the right.

Note: CHR: clinical high-risk for psychosis, CHR-P: CHR individuals who developed psychosis, CHR-NP: CHR individuals who did not develop psychosis, FA: fractional anisotropy, FA_T: fractional anisotropy of the tissue, FW: free-water

comparing HCs ($n = 24$), CHR-NP ($n = 16$) and CHR-P subjects ($n = 8$) with one another.

Moreover, voxel-wise, non-parametric permutation-based correlations were conducted in the CHR group ($n = 28$) to investigate associations between symptom severity as rated with the PANSS subscales (positive, negative and global) and dMRI measures.

Once significant clusters were identified, the dMRI measures were averaged within the clusters for which Cohen's d was calculated for cross-sectional group differences and Pearson's r was calculated for correlations with symptoms. Post-hoc Spearman's ρ correlations were conducted in CHR-P and CHR-NP separately when associations with symptoms in the combined CHR group were significant. Further, we chose to use the percentage of the white matter skeleton affected as a proxy for a "spatial effect size" as previously conducted by our group (Tang et al. 2019). Lastly, anatomical locations of significant clusters are reported for our primary outcome measures FA_T and FW utilising the JHU ICBM-DTI-81 atlas (Mori et al. 2005) if significant voxels attributable to the respective region represented ≥ 1 % of the total number of voxels in the cluster.

Clinical and demographic data analysis

Statistical analyses of the clinical and demographic data, as well as the correlation analyses, were conducted in R (version 3.5.2, <https://www.R-project.org/>). All tests were two-sided, and the level of significance was set to $p < 0.05$. Depending on the distributions, either Student's T -tests/one-way analyses of variance or Mann-Whitney-U/Kruskal-Wallis tests were used to compare means between groups. For dichotomous variables we used χ^2 -tests. Pearson's r was used to test for medication effects by correlating chlorpromazine equivalents (CPZ equivalents in mg/day) with average FA, FA_T , and FW of significant clusters in medicated subjects.

Results

Participants

Detailed information about clinical and demographic characteristics of HCs and CHR individuals that received a *clinical* follow-up to confirm diagnosis are presented in Table 1. There were no significant differences between HCs and CHR individuals with respect to age (range: 18–33 years, 17–30 years, respectively; $p = 0.174$) and sex ($p = 0.773$). However, we observed

a close to significant difference in years of education ($p = 0.056$).

Conversion status

Eight out of 24 CHR individuals (33 %) converted to schizophrenia as diagnosed by the SCID-I (DSM-IV). The mean duration of the follow-up was 15.16 months ($SD = 5.54$). There were no significant differences in age, sex, or education between HCs, CHR-NP, and CHR-P individuals at baseline (Table 1). Finally, SOPS positive symptoms scores were significantly higher in CHR-P comparing to CHR-NP individuals prior to conversion ($p = 0.047$). However, other symptom scores (SOPS negative and disorganisation, as well as PANSS subscores) did not differ significantly between groups at baseline (Table 1).

Medication

At the time of the study, 8 CHR individuals (5 CHR-NP, 3 CHR-P) were medicated with one or two atypical antipsychotics. The relative number of subjects treated with antipsychotics ($p = 0.760$) and the total average treatment duration ($p = 0.897$), as well as CPZ equivalents ($p = 0.879$) did not differ significantly between CHR-NP and CHR-P subjects (Table 1). Furthermore, 2 CHR subjects (1 CHR-NP, 1 CHR-P) also received antidepressants (for more details see [Supplementary Material and Methods](#)).

Comorbidities

The majority of CHR subjects in this study were previously diagnosed with a psychiatric comorbid disorder ($n = 27/30$). The most prominent diagnoses were mood disorders ($n = 23$), followed by anxiety and stress-related disorders ($n = 8$), personality disorders ($n = 7$), and substance use disorders ($n = 7$: 3 CHR-NP, 2 CHR-P, 2 CHR lost to follow-up; all subjects were currently abstinent).

Image analysis: cross-sectional group differences

There were no significant voxel-wise differences between HCs and the *combined* CHR group with respect to FA, FA_T , or FW ($p_{FWE} > 0.1$).

When the CHR group was divided according to conversion status, CHR-P subjects showed significantly lower FA ($0.05 > p_{FWE} \geq 0.002$) in 17.12 % of the white matter skeleton compared to CHR-NP subjects (Figure 1, left side). For FA, the estimated effect size across

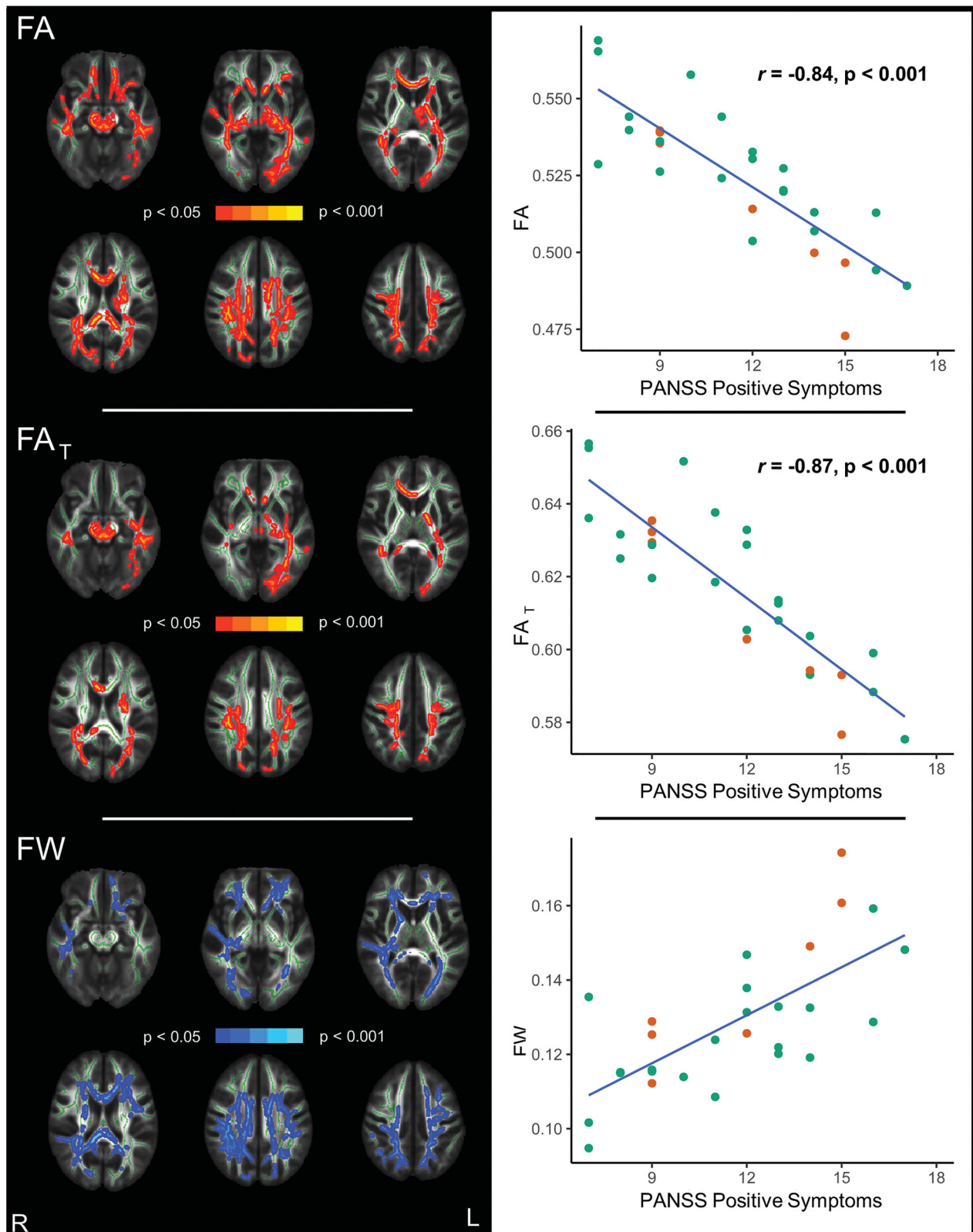


Figure 2. Correlations between positive symptoms and diffusion measures in CHR individuals ($n = 28$, $p_{FWE} < 0.05$). FA and FA_T (red) are inversely correlated, while FW (blue) is positively correlated with positive symptoms (left panels). Pearson's correlations using diffusion measures averaged across corresponding significant clusters are shown on the right. Data points for CHR-P subjects are orange-coloured.

Note: CHR: clinical high-risk for psychosis, FA: fractional anisotropy, FA_T: fractional anisotropy of the tissue, FW: free-water, PANSS: Positive and Negative Syndrome Scale

significant voxels was Cohen's $d=2.278$ (Figure 1, right side). When applying free-water imaging, CHR-P subjects showed significantly lower FA_T ($0.05 > p_{FWE} \geq 0.006$) in 3.65 % of the skeleton, as well as higher FW ($0.05 > p_{FWE} \geq 0.035$) in 5.35 % of the white matter skeleton, in comparison to CHR-NP subjects (Figure 1, left side). FA_T reductions, with Cohen's $d=2.659$ across significant voxels (Figure 1, right side), were primarily localised to the corpus callosum, the right corona radiata (anterior, posterior and superior), the left anterior corona radiata and the right posterior thalamic radiation. In contrast, FW elevations were more widespread and mainly found in the corpus callosum, right superior longitudinal fasciculus, right retrolenticular part of the internal capsule, right posterior thalamic radiation, right tapetum, and right (anterior, posterior and superior) and left corona radiata (anterior and superior) (Figure 1, left side). Cohen's d across the significant FW cluster was $d=1.933$ (Figure 1, right side).

Differences between HC and CHR-P subjects did not reach the significance threshold (FA: $p_{FWE} \geq 0.065$; FA_T : $p_{FWE} \geq 0.060$; FW: $p_{FWE} \geq 0.109$), although these differences were in similar regions and in the same direction as differences between CHR-P and CHR-NP individuals (Figure S1). Comparing CHR-NP with HCs showed small areas with significantly higher FA and FA_T in CHR-NP (FA: $0.5 > p_{FWE} \geq 0.045$ in 0.16% of the white matter skeleton; FA_T : $0.5 > p_{FWE} \geq 0.035$ in 0.14% of the white matter skeleton; Figure S2), localised to the body of the corpus callosum, as well as the right posterior and superior corona radiata.

Image analysis: correlation of symptom scores and diffusion measures

Voxel-wise correlation analyses revealed significant and widespread associations between positive symptom severity (PANSS) and diffusion measures among the combined group of CHR individuals (Figure 2, left side). Specifically, positive symptoms were inversely correlated with FA in 23.7 % of the white matter skeleton ($0.05 > p_{FWE} \geq 0.005$). The Pearson correlation coefficient across significant voxels was $r = -0.84$ ($p < 0.001$; Figure 2, right side). Further, by testing for associations of positive symptoms with free-water imaging metrics, we find a significant inverse relationship with FA_T in 11.4 % of the white matter skeleton ($0.05 > p_{FWE} \geq 0.007$), mainly localised to the corpus callosum, bilateral superior longitudinal fasciculus, left internal capsule, bilateral corona radiata, bilateral sagittal stratum, left posterior thalamic radiation, as

well as left external capsule (Figure 2, left side). For FA_T , the Pearson correlation coefficient across significant voxels was $r = -0.87$ ($p < 0.001$; Figure 2, right side).

FW was significantly *positively* correlated with positive symptoms in 18.5 % of the white matter skeleton in the combined CHR group ($0.05 > p_{FWE} \geq 0.017$) with a Pearson's correlation coefficient of $r=0.70$ ($p < 0.001$) across significant voxels (Figure 2, right side). These associations were more extensive comparing to those with FA_T and primarily found in the corpus callosum, bilateral corona radiata, bilateral superior longitudinal fasciculus, bilateral posterior thalamic radiation, right retrolenticular part of the internal capsule, and right sagittal stratum (Figure 2, left side).

Post-hoc Spearman's correlation analyses – performed within the CHR-NP and CHR-P groups separately – showed significant correlations between positive symptoms and dMRI measures in the same directions as analyses of the combined CHR group (see Table S2).

Neither negative symptom scores (FA: $p_{FWE} \geq 0.164$; FA_T : $p_{FWE} \geq 0.058$; FW: $p_{FWE} \geq 0.524$) nor global symptom scores (FA: $p_{FWE} \geq 0.211$; FA_T : $p_{FWE} \geq 0.322$; FW: $p_{FWE} \geq 0.1378$) were significantly correlated with FA, FA_T or FW.

Medication effects

CPZ equivalent dosage did not correlate significantly with average diffusion measures of clusters distinguishing CHR-P and CHR-NP subjects ($p > 0.6$).

Discussion

In this study, we demonstrate that prior to illness-onset, clinical high-risk individuals who subsequently converted to psychosis exhibit significantly lower FA in commissural and association tracts when compared to those who did not convert. Moreover, when we apply free-water imaging, we show that FA reductions in those who convert to psychosis can be explained both by lower FA_T , and also, to a greater spatial extent, by significant elevations in extracellular FW. In addition, we find that both FA_T reductions and FW increases are associated with positive symptom severity in CHR individuals. Our study thus demonstrates that the development of, and transition to, psychosis may be characterised by two different biological mechanisms and that free-water imaging may be useful for stratifying risk for conversion to psychosis

within a CHR population (Smieskova et al. 2010; Chung and Cannon 2015).

Our finding of lower FA_T , taken together with the two previous publications that employed free-water imaging in a CHR population (Wang et al. 2016; Tang et al. 2019), suggests that FA_T abnormalities are a consistent finding across CHR and CHR-P individuals. Similar to our finding, Wang et al. (2016) also report lower FA_T in the corpus callosum of subjects who subsequently converted to psychosis. While Tang et al. (2019) did not identify FA_T differences between CHR-P and CHR-NP, they did report significantly lower FA_T in the corpus callosum and right corona radiata associated with decline in GAF when comparing the entire CHR group to HCs. Such findings, taken together, lend further support to the notion that early FA_T changes in CHR individuals may reflect a neurodevelopmental vulnerability to psychosis (Tang et al. 2019). Whereas the exact pathologies that drive FA_T changes are still not known, the free-water model suggests that these are tissue-based, e.g. myelin-related or axonal pathologies which have been proposed previously (Davis et al. 2003; Kubicki and Shenton 2014). Neurodevelopmental white matter pathologies may alter the maturational trajectories during adolescence (Kochunov and Hong 2014) and potentially serve as one of the key mechanisms in the emergence of psychotic symptoms. Longitudinal free-water imaging studies would be helpful in delineating the influence of environmental insults and inflammatory factors on white matter maturational trajectories (DeRosse et al. 2014; Prasad et al. 2015; Bennet et al. 2018; Fu et al. 2019; Wassenaar et al. 2019) among individuals developing psychosis.

In terms of FW, the present study diverges from the studies by Wang et al. (2016) and Tang et al. (2019) in that we show that individuals who subsequently develop psychosis also exhibit higher FW compared with those who do not convert to psychosis. This increase in CHR-P individuals more closely aligns with findings from two previous studies of first-episode/recent-onset psychosis populations, which have also described increases in extracellular FW (Pasternak et al. 2012; Lyall et al. 2018). These studies suggest that FW elevations in psychotic patients might represent an acute brain response, which may serve as an indicator of immune activation taking place around the time of illness onset. Our current finding of increased FW in CHR-P indicate that such an acute response to psychosis-onset, reflected by FW increases, may begin already in the CHR stage in only those who subsequently convert to psychosis, although not to

the same extent and magnitude as observed after illness-onset. While the pathological origins of FW increases have yet to be biologically validated, a recent pre-clinical study (Di Biase et al. 2020) showed significant FW elevations in the frontal white matter of a rodent model of maternal immune activation widely used in schizophrenia research (Meyer and Feldon 2012). These clinical and pre-clinical free-water imaging studies align with mounting evidence for immune activation in individuals with schizophrenia and individuals at CHR (Monji et al. 2013; Najjar and Pearlman 2015; Goldsmith et al. 2016; Khoury and Nasrallah 2018; Wang and Miller 2018). For example, a meta-analysis of first-episode and acutely-relapsing patients with schizophrenia showed that both patient groups exhibited increased peripheral levels of inflammatory cytokines, such as interleukin(IL)-6, IL-1 β , and transforming growth factor- β (TGF- β), which the authors interpret as 'state markers for acute exacerbation' (Miller et al. 2011). Our study further encourages biological validation studies to establish the relationship between FW and pathological traces of an acute immune response.

In line with previous free-water imaging studies (Lyall et al. 2018; Tang et al. 2019), it is evident here that differences in FA_T and FW between CHR-P and CHR-NP individuals were more focal than differences in the conventional DTI metric FA. While free-water imaging metrics gain biological specificity relative to FA as noted in the introduction, the model likely loses some sensitivity due to decomposing the diffusion signal into two compartments, encouraging large-scale/multi-centre investigations with greater statistical power in the future.

Our findings of associations of positive symptom severity and diffusion measures highlight the clinical significance of microstructural imaging findings in CHR populations (Karlsgodt et al. 2009; Bloemen et al. 2010; Wang et al. 2016). Along with associations with FA, we find significant inverse correlations of positive symptoms with FA_T similar to the study by Wang et al. (2016). Our study goes further, however, as it also shows a significant widespread relationship between positive symptoms and FW. While PANSS symptom scores alone did not differ significantly between CHR-NP and CHR-P prior to conversion, many of the regions which we found to be significantly correlated with positive symptoms, such as the corpus callosum, corona radiata, posterior thalamic radiation and superior longitudinal fasciculus, were also among those differentiating CHR-P from CHR-NP. Taken together with the increased FW and decreased FA_T in

CHR-P, our findings suggest that CHR individuals who present with higher FW and lower FA_T values at baseline are more likely to suffer from a greater burden of positive symptoms and are more likely to eventually transition to psychosis. While these findings are promising, the relationship between symptomatology and dMRI measures in patients with schizophrenia is still inconclusive (Cheung et al. 2011; Mulert et al. 2012; Yang et al. 2017). Therefore, studies that use multimodal imaging techniques might be helpful to delineate more comprehensively biological factors that influence outcomes in symptom severity and transition to psychosis (Moser et al. 2018; Tognin et al. 2020).

Divergences from the two previous CHR studies (Wang et al. 2016; Tang et al. 2019) are likely due to a few key factors. First, the overall sample size of the current study was small compared to the two previous studies. Paired with the small percentage of converters in our sample and attrition of CHR participants at follow-up, this likely limited our power to detect significant differences between CHR-P subjects and HCs (Bloemen et al. 2010; Rigucci et al. 2016), which we acknowledge as a limitation of our work. However, we note that the regions that are implicated in the previous studies, such as the corpus callosum, corona radiata and superior longitudinal fasciculus, are very similar to those showing trend-level differences between CHR-P and HCs in our study (see Figure S1).

Second, unlike previous free-water imaging studies (Wang et al. 2016; Tang et al. 2019), where CHR were defined based on the SOPS and Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria, in the current study, in addition to the SOPS criteria, inclusion was based on the presence of at least two subjective cognitive or perceptual disturbances (basic symptoms) (Schultze-Lutter et al. 2007). Differences in inclusion criteria can add to the heterogeneity of CHR individuals, in addition to impacting both clinical outcomes and transition rates (Fusar-Poli et al. 2016). Our CHR sample was also approximately two years older and exhibited less severe symptoms at baseline than the population in the Tang et al. (2019) study, which could account for some differences in the findings, as both age and symptomatology have been shown to affect white matter microstructure (Kyriakopoulos et al. 2009; Krakauer et al. 2017).

Third, and finally, the current study used a single-shell dMRI acquisition, while Tang et al. (2019) used a multi-shell acquisition. Implementing multi-shell dMRI would have improved the sensitivity to detect subtle group differences and, moreover, would have further increased the confidence in the interpretation of our

findings due to a more robust fit of the bi-tensor model and the relaxation of the regularisation that is required to fit data from single b-value dMRIs. (Pasternak et al. 2012; Bergmann et al. 2020).

While CHR-NP individuals exhibited significantly higher FA and FA_T relative to HCs, these increases were spatially very confined. For example, FA_T increases were localised to the corona radiata only, a region in which crossing fibres might bias diffusion parameters (Tuch et al. 2003). When tracts along the principal direction of diffusion stay intact, increases in FA_T , for instance, could occur despite selective degeneration of crossing fibres (Chad et al. 2018). Moreover, pilot analyses in a sub-sample of our cohort with follow-up dMRIs indicate that these FA/ FA_T elevations are no longer present after ~19 months ($n_{HC} = 6$, $n_{CHR-NP} = 6$, $p_{FWE} > 0.05$; data is presented in the [Supplementary Material and Methods](#)). Altogether, it is conceivable that our finding of increased FA_T in CHR-NP at baseline might only be temporary or even incidental, making future investigations of larger samples utilising advanced dMRI techniques with higher anatomical accuracy than TBSS (Glenn et al. 2016; Pasternak et al. 2018; Jeurissen et al. 2019) necessary to probe its clinical and biological relevance.

Of further note, whether antipsychotic medication affects white matter is still debateable, with some studies suggesting deteriorating effects on white matter (Szeszko et al. 2014) and others proposing a beneficial role in myelination (Bartzokis 2011). However, in agreement with a recent large scale investigation (Kelly et al. 2018), we did not observe a significant association between diffusion measures and CPZ equivalence in our limited sample of medicated CHR individuals (8 out of 24). In addition, the two previous free-water imaging studies of CHR individuals demonstrated that white matter alterations were present in mostly unmedicated CHR individuals comparing to HCs, suggesting that the observed differences are not due to medication effects (Wang et al. 2016; Tang et al. 2019).

Our study demonstrates that co-occurring decreased FA_T and increased FW contribute to reductions in FA that precede illness-onset in CHR individuals who subsequently develop psychosis. Furthermore, we show that diffusion measures are correlated with positive symptoms in similar regions as those differentiating CHR-NP from CHR-P. Taken together, our results lend support to the notion that in addition to putative tissue-related changes that have previously been shown, the involvement of an extracellular, possibly immune-related, acute response should be

considered in the subsequent development of psychosis. Although larger longitudinal studies are needed to replicate our findings, we believe that free-water imaging biomarkers are relevant indicators of increased illness risk and might therefore facilitate improved outcome prediction in CHR cohorts.

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Statement of interest

The authors report no conflict of interest.

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