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Differences in white matter connectivity between treatment-resistant and treatment-responsive subtypes of schizophrenia

Running title: White matter disruption in resistant schizophrenia

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Schizophrenia is a heterogeneous disorder exhibiting variable responsiveness to treatment between individuals. Previous work demonstrated that white matter abnormalities may relate to antipsychotic response but no study to date has examined differences between first-line treatment responders (FLR) and clozapine-eligible individuals receiving first-line antipsychotics. The current study aimed to establish whether differences in white matter structure exist between these two cohorts. Diffusion-weighted images were acquired for 15 clozapine-eligible and 10 FLR participants. Measures of fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) were obtained and between-group t-tests interrogating differences in FA were conducted. To investigate the neural basis of a decrease in FA, the significant cluster from FA analysis was masked and used to obtain mean RD and AD measures for that region. Those who were clozapine-eligible had significantly lower FA in the body of the corpus callosum ($p<0.05$), associated with a significant increase in mean RD compared with FLR ($p<0.001$). No difference in mean AD was observed for this region. These data reveal differences in diffusion measures between FLR and those eligible for clozapine and suggest that lower FA and greater RD in the corpus callosum could exist as a biomarker of treatment resistance in people with schizophrenia.

Keywords: treatment-resistant schizophrenia, magnetic resonance imaging, diffusion tensor imaging, first-line antipsychotics.

1. Introduction

Schizophrenia is a heterogeneous disorder, not only in its symptom profile but in the manner with which it responds to treatment with antipsychotic drugs. A growing pool of research supports the hypothesis that individuals with schizophrenia fall into two distinct categories: those who respond to first-line treatment with typical or atypical antipsychotics; and those who require treatment with clozapine (an agent with unique efficacy in treatment-resistant schizophrenia; this second category may be further divided into those who do and do not respond to clozapine monotherapy) (Farooq et al., 2013; Gillespie et al., 2017; Howes and Kapur, 2014; Howes et al., 2016; Lee et al., 2015). Studies report that only 60% to 80% of individuals initiating treatment with a typical or atypical (non-clozapine) antipsychotic experience a positive clinical response (Agid et al., 2011; Elkis and Buckley, 2016), while response rates to a second non-clozapine antipsychotic are as low as 16% (Agid et al., 2011). Switching individuals who fail two trials of first-line antipsychotics to clozapine results in distinctly higher response rates (Agid et al., 2011; Kane and Correll, 2016; Meltzer, 2010), alluding to a subtype of schizophrenia that is resistant to most antipsychotics but sensitive to the unique pharmacological effects of clozapine. Evidence suggests that treatment-resistance not only develops over time but can occur from the outset (Agid et al., 2011; Lally et al., 2016) making classification by responsivity a valid proposal. Shifting the focus of research to investigate underlying differences between distinct response subtypes may provide a clearer understanding of schizophrenia.

Schizophrenia has been hypothesised to be a disorder of dysconnectivity, caused by neuromodulatory disruptions (attributed to dopamine, serotonin and acetylcholine) in *N*-methyl-d-aspartate (NMDA) receptor-mediated synaptic plasticity (Stephan et al., 2009). NMDA receptor signalling also plays a role in myelination and energy metabolism (Cao and Yao, 2013), promoting myelin induction (Wake et al., 2011) and regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-dependent signalling with surrounding axons (De Biase et al., 2011). Disruptions in white matter architecture are well-documented in schizophrenia (Bora et al., 2011; Cookey et al., 2014; Samartzis et al., 2013; Zhuo et al., 2016). However, despite reporting reliable differences between those with schizophrenia and healthy controls, a meta-analysis of studies investigating recent-onset schizophrenia and clinical high-risk individuals failed to identify a specific underlying neurobiological deficit in white matter (Samartzis et al., 2013). A more recent meta-analysis of 29 independent international studies reported widespread disruptions in 20 of 25 regions of interest representing all major white matter fasciculi in the brain (Kelly et al., 2017). These findings suggest global disruptions in white matter in those with schizophrenia but have not yet identified any specific pathway that is consistently correlated with the disorder.

Subtyping individuals with schizophrenia according to treatment response or resistance has shed some light on the issue. Zeng et al. investigated white matter microstructure before and after eight weeks of antipsychotic treatment in first-episode schizophrenia and reported a correlation between changes in the left superior longitudinal fasciculus and improved positive symptoms (Zeng et al., 2016). These authors did not, however, examine features of pre-treatment white matter structure that might predict clinical outcome. An earlier study by Mitelman et al. categorised participants with schizophrenia according to whether they had good outcome or poor outcome, based on their ability to self-care (Mitelman et al., 2006). Those with poor outcome had deficits in white matter in both hemispheres compared with healthy controls, compared to only lateralised deficits in those with good outcome (Mitelman et al., 2006). More recently, Reis Marques et al. conducted an investigation in first-episode psychosis to determine whether pre-treatment fractional anisotropy (FA, the degree of restricted water movement in tissue) could distinguish responders from non-responders to a 12 week course of antipsychotics (Reis Marques et al., 2014). They identified lower FA in non-responders compared with responders at 12 weeks in several white matter tracts, including the uncinate fasciculus, stria terminalis, superior frontal-occipital fasciculus, corpus callosum, internal and external capsule and corona radiata (Reis Marques et al., 2014). Response was measured at 12 weeks to ensure at least one drug was administered for an appropriate length of time (6-8 weeks) according to clinical recommendations (Taylor et al., 2007). Diagnostic criteria for treatment-resistant schizophrenia (i.e. schizophrenia eligible for treatment with clozapine), however, requires failure to respond to at least two 6-to-8-week trials of first-line antipsychotic drugs (McGorry, 2005; Mcilwain et al., 2011). Therefore, although studies to date have reported white matter disruptions in those not responding to treatment, none have investigated the difference between treatment responders and those with clinically confirmed treatment-resistant schizophrenia (clozapine eligibility) receiving first-line antipsychotics.

We hypothesise that the discrepancies seen in the literature to date are a result of inadequate patient subtyping and may be rectified by classifying individuals according to whether they respond to first-line antipsychotics or require treatment with clozapine. In the current study, we applied this subtyping framework to investigate differences in structural brain connectivity. The study employed tract-based spatial statistics to investigate FA, axial diffusivity and radial diffusivity in individuals with schizophrenia receiving treatment with first-line antipsychotics. Based on previous work exhibiting increased glutamate levels (a downstream effect of NMDA receptor hypofunction) in individuals with treatment-resistant schizophrenia compared with first-line treatment responders (Demjaha et al., 2014), we expected white matter integrity to be lower in individuals who are eligible for clozapine compared to those who respond well to first-line therapy.

2. Methods

2.1. Participants

Details from the functional imaging component of the study have been reported previously (McNabb et al., 2018). Fifteen individuals who were eligible for clozapine (with treatment-resistant schizophrenia) and ten first-line responders (FLRs) were recruited from inpatient and outpatient clinics within the Waitemata, Counties Manukau and Auckland District Health Boards of New Zealand as well as from mental health support groups and social media (FLR only). Participants in the FLR group were required to be between 18 and 45 years of age, have a history of schizophrenia or a psychotic episode according to DSM-5 criteria (American Psychiatric Association, 2013), no history of treatment with clozapine, and be clinically stable on a first-line antipsychotic drug, with a Positive and Negative Syndrome Scale (PANSS) score of <50 during screening. Participants in the clozapine-eligible group were required to meet criteria for treatment resistance current at the time of the study (McGorry, 2005). Participants were to be between 18 and 45 years of age, meet DSM-5 criteria for schizophrenia (American Psychiatric Association, 2013), have failed at least two six-week trials with first-line antipsychotic drugs, still be receiving treatment with at least one of the aforementioned antipsychotics, be able to give informed written consent (determined by their treating clinician), and present with persistent positive or negative symptoms contributing to a PANSS score of ≥50 during screening. Exclusion criteria for both groups included diagnosis of another psychiatric disorder, co-morbid neurological illness, self-reported low treatment adherence to current antipsychotic medication, claustrophobia, history of traumatic brain injury resulting in loss of consciousness greater than three minutes, active substance dependence and standard contraindications to magnetic resonance imaging (MRI). Participants in the clozapine-eligible group should not have had a trial of clozapine within three months of the screening visit. The study was approved by the Northern A Regional Ethics Committee and all participants gave informed written consent.

Screening for clozapine eligibility consisted of a semi-structured interview with a study psychiatrist to confirm diagnosis, as well as a PANSS assessment. Participants in the FLR group were screened by a study psychiatrist or nurse using the PANSS. Diagnosis was confirmed by a psychiatrist or general practitioner based on clinical notes.

Participants were requested to provide a urine sample for drug screening (Medix Pro-Split Integrated Cup, Multi Drug Screening Test; Sobercheck Ltd) during the study visit. Urine was screened for the

presence of amphetamine, methamphetamine, benzodiazepines, cocaine, opiates and tetrahydrocannabinol (THC). One participant in the clozapine-eligible group refused drug screening but was later excluded due to excessive head motion during scanning.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 23. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using a Student's t-test. For those variables that violated assumptions of normality and/or homoscedasticity, the Mann-Whitney U test was employed. Z scores were calculated for demographics that were better described using proportions.

2.2. Image acquisition

Magnetic resonance images were acquired on a Siemens Magnetom Skyra 3T scanner at the Centre for Advanced MRI, University of Auckland, New Zealand. All participants were imaged using a 32-channel head coil. Diffusion-weighted images were acquired using a multiband gradient-echo pulse sequence (University of Minnesota (Moeller et al., 2010)). One image without diffusion gradients (b=0 s/mm^2) was acquired, in addition to 5 images with b=5 s/mm^3 and 100 images with unique diffusion-encoding directions isotropically distributed in space at b values ranging from 995 to 2010 s/mm^2 (9 values in total). Seventy-two slices were acquired in the anterior to posterior direction, with the following parameters: repetition time (TR) 3600 ms; echo time (TE) 92.4 ms; echo spacing 0.67 ms; echo planar imaging (EPI) factor 108; multiband slice acceleration factor 3; flip angle 78°; field of view (FOV) 220 mm, voxel size 2 x 2 x 2 mm. Gradient distortion images were acquired using a gradient echo pulse sequence with the following parameters: TR 704 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.0 mm; phase-encode direction A >> P; FOV 220 mm.

2.3. Data analysis

Image preprocessing and analysis were performed using the FMRIB Software Library (FSL) version 5.0.9 (Jenkinson et al., 2012). Raw diffusion-weighted images were corrected for head motion and eddy current distortions using FSL's eddy tool (Andersson and Sotiropoulos, 2016). Slices with average intensity at least four standard deviations lower than the expected intensity were interpolated with predictions made by the Gaussian Process (Andersson et al., 2016). In-scanner head motion was determined for each slice and averaged across volumes to give a single mean for each participant. Any participant with mean motion greater than two standard deviations away from the group mean was excluded. Gradient distortions were corrected using FSL's fugue function and output registered to gradient-free images using the linear registration function (FLIRT) (Jenkinson et al., 2002).

A single FA image was created for each participant whereby diffusion tensors were independently fit to each voxel. Output yielded voxelwise maps of FA, λ1 (axial diffusivity; AD), λ2 and λ3 (combined to give radial diffusivity; RD) for each participant. FA is a measure of the shape of an ellipsoid, providing information about the degree of anisotropy in a voxel. High FA values represent areas of high anisotropy, and consequently restricted water movement, whereas low FA values represent areas of low anisotropy where water molecules diffuse freely. Related measures of diffusion are AD and RD, which represent diffusion in the principal direction (ε_1) and perpendicular directions (ε_2 and ε_3), respectively. In vivo measures of white matter integrity possess limitations in terms of interpretation, especially with regard to low FA measurements in areas of crossing or kissing fibres; however, previous work (Nair et al., 2005) supports the assumption that FA represents the degree of diffusion orientation coherence, AD, the degree of axonal shrinkage and RD, the degree of myelination within a voxel.

Voxelwise statistical analysis of the FA data was carried out using FSL's tract-based spatial statistics (TBSS) (Smith et al., 2006). First, FA images were eroded and end slices zeroed to remove likely outliers from the diffusion tensor fitting. All subjects' FA data were then aligned to a white matter (FMRIB58_FA) template and then to MNI152 1mm standard space using nonlinear registration (Andersson et al., 2007). A mean FA image was created and thinned to produce a mean FA skeleton representing the centres of all tracts common to the group. A threshold of 2000 (corresponding to FA>0.2) was applied to exclude voxels containing grey matter and cerebrospinal fluid. Each subject's aligned FA data were then projected onto the skeleton and fed into voxelwise cross-subject statistics. Between-group t-tests interrogating differences in whole-brain FA over 5000 permutations were conducted using the Randomise tool (Winkler et al., 2014). Though the difference in the proportion of male and female participants between groups was not statistically significant, gender was added as a covariate to account for the low number of female participants included in the clozapine-eligible group. Output contained statistical maps corrected for multiple comparisons (family-wise error corrected) at the cluster level using threshold-free cluster enhancement (Smith and Nichols, 2009). These were further corrected for multiple comparisons (contrasts: FLR>clozapine eligibility and clozapine eligibility>FLR) using the false discovery rate (FDR).

Mean FA within the significant cluster was determined for each participant and Glass's delta (Δ) effect size for differences in FA (Ialongo, 2016) was calculated using the following equation:

$$\Delta = \frac{mean_{FLR} - mean_{Clozapine}}{standard\ deviation_{FLR}}$$

To investigate the neural basis of a decrease in FA, the significant cluster from whole-brain FA analysis (covaried for gender) was masked and back projected into native space for each participant.

Individualised masks were applied to λ1, λ2 and λ3 output and mean values for masked regions obtained. Mean λ1 from the masked region provided a mean value of AD for the significant cluster from FA analysis. Mean RD was determined by taking the combined mean of masked λ2 and λ3 maps. It was not appropriate to investigate mean diffusivity (MD), as it is not independent of AD and RD. Between-group t-tests were conducted in SPSS (IBM). This method was adapted from previous work by Bennett et al., who investigated the neural underpinnings of FA loss in aging adults (Bennett et al., 2010).

Including only those voxels demonstrating statistically significant differences between groups, post-hoc assessments of the relationships between callosal FA and symptom severity (PANSS score) and between collosal FA and antipsychotic drug dose (chlorpromazine equivalents) were performed using Pearson's correlation analyses in Matlab 2016a (Mathworks, USA).

Our hypothesis focused primarily on the assumption that aberrant NMDA receptor function affects the integrity of white matter fibres in those with schizophrenia and that underlying causes of NMDA receptor dysfunction differ between those whose symptoms respond to treatment and those whose symptoms are resistant. Previous work suggests that those who respond well to treatment exhibit higher levels of dopamine synthesis capacity compared to those who fail to respond (Demjaha et al., 2012). If differences in response to first-line antipsychotic drugs are attributable to structural disruptions in dopaminergic pathways, limiting FA analysis to those tracts previously identified in the pathology of schizophrenia (i.e. mesocortical and mesolimbic pathways (Stahl, 2013)) may demonstrate differences between groups. To test this theory, we employed probabilistic tractography to identify likely structural connections between the ventral tegmental area (VTA), bilateral striata, ventromedial (VM) and dorsolateral (DL) prefrontal cortices (PFC) and compared FA values within these tracts.

Analysis was carried out using FSL. First, DLPFC and VMPFC masks were created in Neurosynth.org (Yarkoni et al., 2011) using an automated meta-analysis of 489 and 199 studies, respectively. Thresholds were specific to each mask and were selected such that areas outside the PFC (e.g. precuneus) were no longer included (threshold=5 for DLPFC and 7 for VMPFC). Masks were transformed to 1mm MNI space from 2mm MNI space using linear registration. The VTA mask was obtained from the Harvard Ascending Arousal Network Atlas (www.martinos.org) and the striatal mask was created using the Harvard Oxford subcortical atlas in FSL, using a threshold of 0.5. All masks were binarised for use in tractography.

Using whole-brain diffusion-weighted data, crossing fibres were modelled using Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTX (Behrens et al., 2007)). Probabilistic tracts between VTA, striata and DLPFC masks and between VTA, striata and VMPFC masks for each participant were determined using FSL's PROBTRACKX (Behrens et al., 2007; Behrens et al.,

2003). Using multiple seed masks in this way, PROBTRACKX repeatedly samples tracts from every seed mask in the list, and retains only those tracts that pass through at least one of the other seed masks. Tractography output for each participant was then constrained using a binarised mask of the thresholded (FA>0.2) skeletonised group FA map. Voxels included in the group comparison were limited to those selected by probabilistic tractography in all participants, meaning that every participant contributed an FA value to all voxels in the voxel-wise group comparison. Between-group t-tests interrogating differences in FA over 5000 permutations were conducted for both pathways using the Randomise tool (Winkler et al., 2014).

3. Results

3.1. Participant information

One participant from the clozapine-eligible group was excluded from analysis due to excessive head motion during scanning. After exclusion of this participant, mean movements from the first volume and from the previous volume were calculated. No significant differences in head movement were found between response groups (root mean square motion from first volume 0.95±0.37 mm vs 1.07±0.45 mm [p=0.52] and from previous volume 0.51±0.16 mm vs 0.50±0.24 mm [p=0.91] for FLR and those who were clozapine eligible, respectively). Participant demographics for the remaining participants are reported in Table 1.

3.2. Diffusion-weighted imaging results

Results of the mean FA skeleton between-groups t-tests are presented in Figure 1. Significantly lower FA was observed in the body of the corpus callosum in individuals who were clozapine-eligible compared to FLR ($p<0.05$, FDR corrected). No other cluster was identified as showing differences in FA between groups.

Glass's Δ was 0.353 for the difference in mean FA between groups (FLR=0.87 versus clozapine eligibility=0.83, see Figure 2), representing a small to moderate effect size. Post-hoc investigation of AD and RD in this region revealed a significant increase in mean RD in those who were clozapine-eligible ($2.1x10^{-4}$) compared with FLR ($1.6x10^{-4}$; $p<0.001$). No difference in mean AD was observed for this region.

Assessing only those voxels demonstrating statistically significant differences in FA between groups at the whole-brain level, PANSS score was significantly negatively correlated with FA (Figure 3). No significant correlation was observed between collosal FA and chlorpromazine equivalents (Figure 4).

No differences in FA were found between FLR and those eligible for clozapine when FA analysis was restricted to tracts between VTA, striata and DLPFC or between VTA, striata and VMPFC.

4. Discussion

This study examined whether variations in white matter microstructure could account for differences in the response to antipsychotic treatment in people with schizophrenia. Tract-based spatial statistics revealed significantly lower FA in the body of the corpus callosum in individuals who were clozapine-eligible compared to FLR. Post-hoc investigation of AD and RD in this cluster revealed greater RD in those who were eligible for clozapine compared with FLR, with no difference in AD observed between groups.

These results are in line with a number of studies reporting reduced FA in the corpus callosum of individuals with schizophrenia (Henze et al., 2012; Lener et al., 2015; Reis Marques et al., 2014; Whitford et al., 2010; Zhuo et al., 2016). Though most studies have focused on differences in the genu and splenium of the corpus callosum in those with schizophrenia compared with healthy controls (Zhuo et al., 2016), abnormalities in the callosal body have also been reported (Henze et al., 2012; Reis Marques et al., 2014; Zhang et al., 2018). Of greatest significance to the current study are findings by Reis Marques et al. demonstrating reduced FA in the body of the corpus callosum in non-responders compared with responders to a 12-week course of antipsychotic treatment (Reis Marques et al., 2014). These authors also identified regions of lower FA in tracts such as the uncinate fasciculus and fornix (Reis Marques et al., 2014) that were not seen in the current study. This may be a consequence of our stricter criteria for defining treatment failure; whereas those in the study by Reis Marques et al. failed to respond to 12 weeks of treatment, participants in the current study met criteria for treatment-resistant schizophrenia. Therefore, findings from this study expand on those by Reis Marques et al. and suggest that low FA in the callosal body, specifically, is associated with clozapine eligibility.

Assessing only those voxels showing statistically significant differences in FA between response groups, FA was shown to be significantly negatively correlated with PANSS score. Given that treatment response in our study was defined by PANSS score of less than 50 (and treatment resistance by PANSS greater than or equal to 50 after two adequate trials of first-line antipsychotics), the linear relationship between PANSS and FA is difficult to disentangle from the primary hypothesis that these two response groups represent distinct pathophysiological subtypes of schizophrenia. This is a limitation of conducting cross-sectional research using this subtyping regime; to better separate the influence of symptom severity from the binary measure of response or resistance, longitudinal assessment of treatment response following baseline evaluation of symptom scores is necessary. Including baseline PANSS score as a

covariate (along with follow-up length) in their assessment of treatment response at 12 weeks, Reis Marques et al. demonstrated a minimal effect of baseline scores on FA, with an overlap in affected regions compared to when no covariates were included (Reis Marques et al., 2014). The relationship between FA and PANSS score in the current analysis may therefore be an anomaly of the response criteria employed. By conducting more research into the pharmacological and symptomatic boundaries between treatment response and resistance, it will become clearer as to which FA properties result from a predetermined resistance to medication and which are due to symptom severity at the time of assessment.

To investigate the neural underpinnings of lower FA in the clozapine-eligible group, AD and RD were measured in the cluster of voxels that exhibited a significant difference in FA between groups. Greater RD in the absence of any change in AD likely reflects reduced myelination in the clozapine-eligible group compared with FLR (Budde et al., 2007). This hypothesis is supported by work demonstrating that myelin-deficient shiverer mice exhibit increased RD but unchanged AD compared with normal age-matched controls (Song et al., 2002). A similar pattern of increased RD in healthy aging adults (Bennett et al., 2010) as well as increased age-related FA decline in the corpus callosum of people with schizophrenia (Kochunov et al., 2013) suggest that the difference in RD observed could be attributable to accelerated age-related decline in those eligible for clozapine. Alternatively, these white matter deficits may be more static in nature and potentially due to disruptions in myelination that occur during adolescence (Whitford et al., 2012).

It is currently unclear how deficits in white matter relate to treatment response; however, there may be an association with NMDA receptor function in oligodendrocytes. In addition to the role of NMDA receptors in synaptic plasticity and neuronal communication, NMDA receptor signalling in oligodendrocytes is thought to play a crucial role in myelination and energy metabolism (Cao and Yao, 2013). NMDA receptor hypofunction (as discussed by Howes et al. (Howes et al., 2015)) in those eligible for clozapine but not (or to a lesser degree) in FLR may account for the lower FA and higher RD compared with FLR, in addition to the poor response to D_2 antagonists observed in this population.

Although FA and RD provide a reliable measure of white matter integrity in areas of coherent fibre orientation, DTI studies suffer uncertainty in areas of crossing fibres (Oouchi et al., 2007). This problem can be minimised by employing large b values, many diffusion-encoding directions and smaller voxel size (as was done in the current study); however, the issue cannot be completely eliminated. As such, the decreased FA and increased RD observed in the clozapine-eligible group compared with FLR could also be indicative of reduced axon packing density (Beaulieu, 2002) or increased crossing fibres in the corpus callosum of those eligible for clozapine, rather than demyelination as previously discussed. This

would denote a greater level of axon crossing in the clozapine-eligible group, potentially signifying greater disorganisation of structural connectivity. Future work may benefit from employing myelin water imaging, which assesses myelin changes in cerebral white matter by employing a multiexponential T2 relaxation time (Alonso-Ortiz et al., 2015).

In a recent study by Zeng et al., changes in FA following eight weeks of treatment with antipsychotic treatment were discovered in the left superior longitudinal fasciculus and correlated with changes in positive symptoms and processing speed (Zeng et al., 2016). The current study's cross-sectional nature prevents determination of causality with regard to the differences in FA observed here. However, the observation of decreased FA in the body of the corpus callosum in those eligible for clozapine is supported by prospective findings (Reis Marques et al., 2014), suggesting these differences may be present prior to the onset of treatment. Further work is needed to determine whether FA can be used as a predictor of clozapine eligibility in drug-naïve patients.

This study benefited from several strengths that contribute to the generalisability of these findings, including well-matched duration of illness, age of onset, chlorpromazine equivalents and drug class between groups. The effect size for the difference in FA was small to moderate, though still provides evidence of lower FA in people eligible for clozapine compared to FLR. This modest effect size may relate to the small sample size used in the study. Small sample size was attributable to inherent difficulties in recruitment of individuals experiencing little-to-no symptoms associated with schizophrenia (FLR) as well as those who were eligible for clozapine and able to provide informed written consent. Fifty-one individuals were screened for the study, of which only 25 were eligible and willing to participate; therefore, the rate of consent in the current study was lower than that previously reported for individuals with psychosis (Patel et al., 2017). Of particular note was the difficulty with which FLRs identified by their previous psychiatrist/psychologist were able to be contacted after leaving mental health services and the proportion of those contacted who no longer met criteria for treatment response. This highlights an increased need for follow-up in mental health services once clients are referred back to their general practitioners.

Small sample size also resulted in an uneven ratio of females to males ($p>0.15$) between the two groups. Differences in FA between healthy male and female participants have been reported in several regions of the brain, suggesting that the difference in gender ratios between groups could affect these results (Hsu et al., 2008). As a precaution, gender was added as a covariate in the general linear model to reduce any likely effect on the results.

FA values reported in the current study are higher than those typically reported for voxels within the corpus callosum, both in healthy individuals and those with schizophrenia (Henze et al., 2012; Knöchel et al., 2012). However, values reported here are only for those voxels demonstrating statistically significant differences between groups. It is therefore possible that only those voxels with high FA exhibit differences between response subtypes of schizophrenia. Future work may benefit from applying pre-specified masks to the corpus callosum to measure differences in FA between these cohorts.

No psychiatrically healthy controls were included for comparison in the study. Comparison of individuals with schizophrenia and healthy controls introduces an additional confounder of antipsychotic drug exposure, from which healthy controls are naïve. Antipsychotic drug exposure is correlated with a reduction in age-related FA decline in the corpus callosum in those with schizophrenia (Xiao et al., 2018), potentially via facilitation of oligodendrocyte regeneration and myelin repair following injury (Zhang et al., 2012). Although in the current study we found no correlation between chlorpromazine equivalents and corpus callosum FA, a comparison with healthy controls would likely suffer from some degree of interference from antipsychotic drugs. By comparing those with treatment resistance to a very well-responding but drug-exposed cohort, a more relevant measure of white matter disruption indicative of the pathology associated with treatment-resistance can be obtained. Although we have not assessed FA in healthy subjects in the current study, after 12 weeks of treatment, Reis Marques et al. showed reduced FA in multiple white matter tracts in non-responders (but not responders) to antipsychotics compared with psychiatrically healthy controls (Reis Marques et al., 2014). These findings suggest that the lower FA observed in those eligible for clozapine compared with FLR in the present analysis represents aberrant structural connectivity in clozapine-eligible participants rather than in those who respond to treatment.

Three participants in the FLR group and two in the clozapine-eligible group tested positive for THC on the day of the MRI scan. Chronic cannabis use has been reported to reduce FA in several brain regions, including the corpus callosum, both in healthy adults and in people with early-phase schizophrenia (Cookey et al., 2014). Given that a greater proportion of THC-positive urine screens were observed in the FLR group, it is unlikely that these results are confounded by the effects of cannabis use. However, it cannot be discounted that the effects of longer term cannabis use or life-time exposure may have been greater in the clozapine-eligible group. Unfortunately, it would be impractical to measure life-time exposure and self-reported use may be inaccurate.

These data reveal differences in diffusion measures between FLR and those eligible for clozapine and suggest that lower fractional anisotropy and greater radial diffusivity in the corpus callosum may be a

biomarker of treatment resistance in people with schizophrenia. More work is needed to determine whether the differences observed during treatment are present in treatment-naïve individuals and whether these differences are substantial enough to accurately predict clozapine eligibility.

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Contributors

Carolyn McNabb contributed to the design of the study, wrote the protocol, contributed to the procurement of ethical approval, collected and analysed the data and wrote the first draft of the manuscript. Rob Kydd contributed to the design of the study, contributed to the procurement of ethical approval, interviewed participants and provided professional opinions regarding their eligibility for clozapine. Frederick Sundram and Ian Soosay interviewed participants and provided professional opinions regarding their eligibility for clozapine. Bruce Russell contributed to the design of the study, contributed to the procurement of ethical approval and obtained funding for the study. All authors contributed to and have approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest

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	FLR (n=10)	Clozapine-eligible (n=14)	
Age (years)	29.1 (8.4)	25.3 (5.3)	t=-1.372; p=0.184,
Gender (number female)	4	2	Z score=-1.434; p=0.153
Duration of illness (years) ǂ	5.5 (5.9)	5.3 (4.5)	Mann Whitney U=66; p=0.841
Age of onset (years) ǂ	23.9 (5.5)	21.1 (3.4)	Mann Whitney U=90; p=0.259
PANSS score			
Positive	8.0 (3.0)	19.5 (10.0)	
Negative	11.0 (6.0)	20.0 (17.0)	
General psychopathology	20.9 (2.8)	37.3 (8.1)	
Total	40.1 (6.3)	79.1 (17.4)	
Current prescribed antipsychotic	Amisulpride=1 Aripiprazole + olanzapine (low dose)=1 Olanzapine=4 Quetiapine=1 Risperidone=3	Aripiprazole=2 Aripiprazole + olanzapine=2 Olanzapine=3 Olanzapine + quetiapine=1 Palideridone=3 Risperidone=3	
Chlorpromazine equivalents	438.9 (304.2)	561.6 (267.4)	t=1.047; p=0.307
Positive drug screen (THC; number participants)	3	2	Z score=-0.935; p=0.352

Table 1. Demographic data for participants included in the analysis. Values are presented as mean (standard deviation), unless denoted by a ǂ, indicating a non-parametric statistical comparison for

which results are presented as median (interquartile range). Age of onset was defined as age at first recorded contact with mental health services. THC=tetrahydrocannabinol

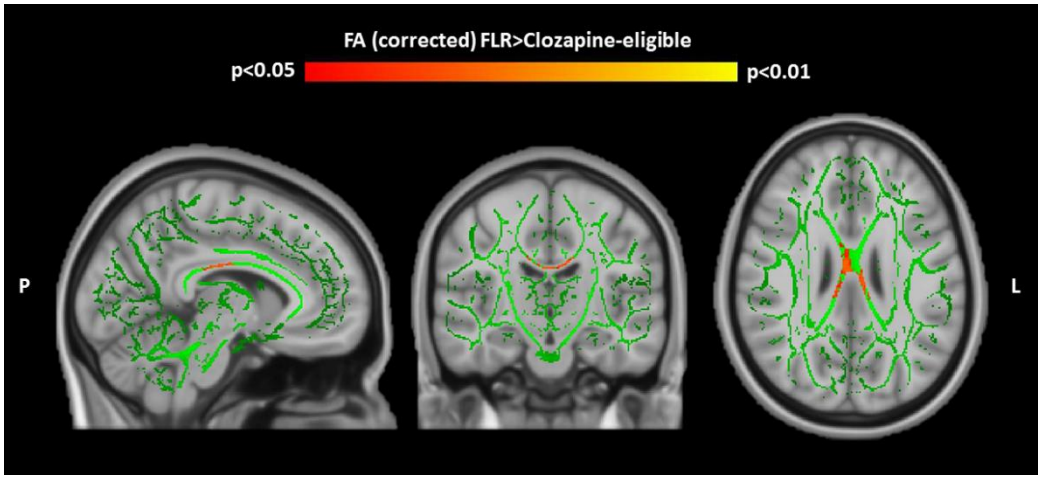

Figure 1. Tract-based spatial statistics results showing FA difference in red-orange (FLR>clozapine-eligibility; p<0.05, FDR corrected) in the body of the corpus callosum (peak MNI coordinate 5 -10 26 mm) overlaid onto mean FA skeleton and MNI152 1mm brain.

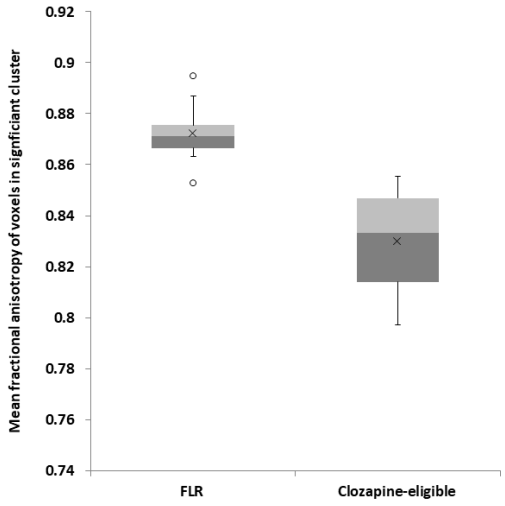

Figure 2. Mean FA of significant cluster identified during tract-based spatial statistics analysis. Upper and lower boxes represent 25th and 75th percentiles, respectively; lines through each box represent the median; mean of the sample is represented by an 'x'; outliers are shown as rings.

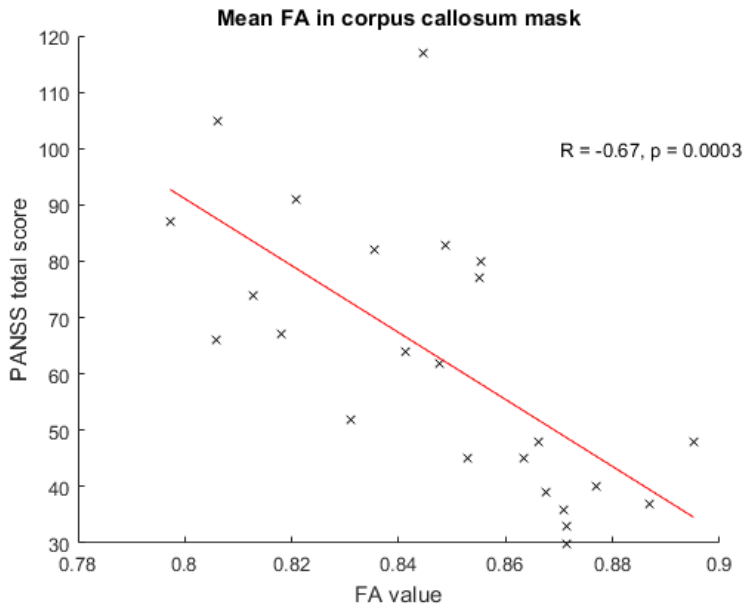

Figure 3. Pearson's correlation between mean FA value in significant cluster (from tract-based spatial statistics analysis) and PANSS score.

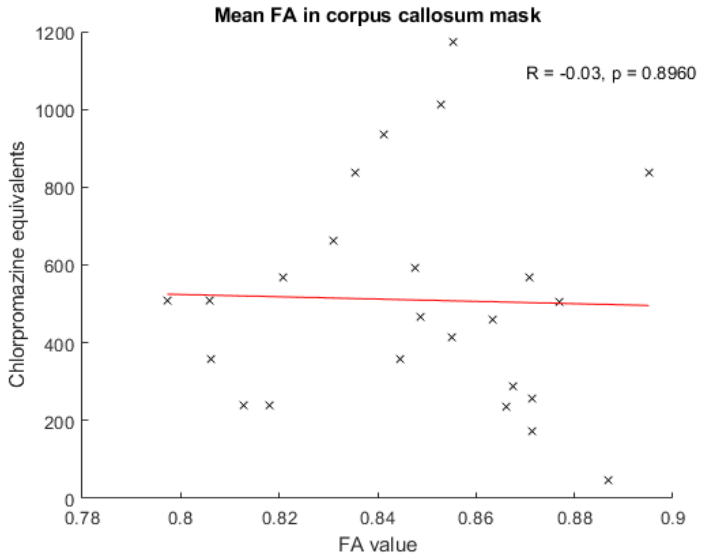

Figure 4. Pearson's correlation between mean FA value in significant cluster (from tract-based spatial statistics analysis) and antipsychotic dose in chlorpromazine equivalents.