Progression of neuroanatomical abnormalities in psychotic disorder and the effect of psychotropic medication

by

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CONTENTS

Author's Declaration

I declare that all the work presented in this thesis was carried out in accordance with the regulations of the National University of Ireland Galway.

All work is original and carried out by Giulia Tronchin at the National University of Ireland Galway within the Centre for Neuroimaging & Cognitive Genomics (NICOG), Clinical Neuroimaging Laboratory, NCBES Galway Neuroscience Centre, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, H91 TK33 Galway, Ireland.

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This thesis has not been submitted previously for any other award.

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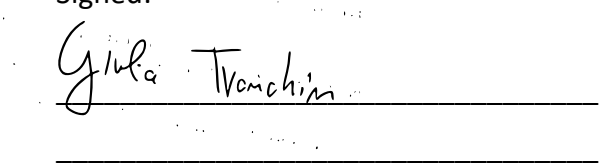

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Contribution Statement

Manuscript 1

Author Colm McDonald designed and revised the manuscript for intellectual content; Dara M. Cannon and Brian Hallahan supervised the general progress of the study; Joanne PM Kenney collected cognitive data; Shane McInerney, John McFarland and Cathy Scanlon recruited and collected data. Peter McCarthy supported acquisition and optimisation of MRI data. Giulia Tronchin and Theophilus N. Akudjedu processed all the MRI data. Giulia Tronchin performed statistical analyses and wrote the manuscript.

Manuscript 2

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Manuscript 3

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List of Abbreviations

American Psychiatric Association (APA)

Body Mass Index (BMI)

Cerebrospinal Fluid (CSF)

Chlorpromazine (CPZ)

Constrained Spherical Deconvolution (CSD)

Continuous Performance Test (CPT)

Diagnostic and Statistical Manual for Mental Disorders 4th Edition text revision (DSM-IV-TR)

Diffusion Magnetic Resonance Imaging (dMRI)

Diffusion Tensor Imaging (DTI)

Echo Planar Imaging (Epi)

Echo Time (Te)

Extrapyramidal Side Effects (EPS)

Family-Wise Error Rate (Fwer)

First episode of Psychosis (FEP)

First-generation antipsychotics (FGA)

Fractional Anisotropy (FA)

Fwer-Corrected P-Values (Pfwe)

Global Assessment of Functioning (GAF)

Healthy Controls (HC)

Intracranial Volume (ICV)

Inversion Time (Ti)

Magnetic Resonance Imaging (MRI)

Magnetisation Prepared Rapid Gradient Echo (MPRAGE)

MATRICS Consensus Cognitive Battery (MCCB)

Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)

Mean Diffusivity (Md)

Multivariate Analysis of Covariance (MANCOVA)

Neuropsychological Assessment Battery (NAB)

Number of Streamlines (Nos)

Positive and Negative Syndrome Scale (PANSS)

Radial Diffusivity (Rd)

Region of Interest (ROI)

Repetition Time (Tr)

Scale for the Assessment of Negative Symptoms (SANS)

Scale for the Assessment of Positive Symptoms (SAPS)

Second generation antipsychotics (SGA)

Standard Deviation (SD)

Statistical Package for the Social Sciences version (SPSS)

Tesla (T)

The Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA)

The John Hopkins University (JHU)

Threshold-Free Cluster Enhancement (TFCE)

Tract-Based Spatial Statistic (TBSS)

Treatment Response and Resistance in Psychosis (TRRIP)

Treatment-Resistant Schizophrenia (TRS)

Wechsler Memory Scale (WMS-III)

White Matter (WM)

World Federation of Societies of Biological Psychiatry (WFSBP)

Abstract

Introduction: Schizophrenia is a particularly severe and disabling mental disorder affecting 20 million people worldwide. The vast majority of structural and diffusion neuroimaging studies on neuroanatomy and cognition have been conducted cross-sectionally and it remains unclear whether risk factors, treatments or associated illness effects are driving changes.
The overarching theme of this thesis is to longitudinally examine elements of neuroanatomical progression and its cognitive or clinical correlates in samples of patients across different phases of psychosis spanning first episode of illness and treatment refractory schizophrenia, using structural and diffusion MRI techniques. Specifically, **Manuscript 1** aims to assess whether impaired executive functioning and emotional intelligence at first presentation of psychotic episode are associated with progressive prefrontal and orbitofrontal cortical thinning and whether negative symptom severity is linked to progressive prefrontal cortical thinning in the years following the first-episode of psychosis. **Manuscript 2** and **3,** using a unique sample of treatment-resistant clozapine-naïve schizophrenia patients, investigate whether subcortical structures, white matter microstructure and structural network organisation demonstrate any progressive changes after 6 months of clozapine treatment and whether any such changes are related to clinical variables including treatment response and amount of clozapine taken.

Method: **Manuscript 1**. 1.5T structural MRI images were acquired at baseline and after 3.5 years for 20 individuals with first-episode psychosis (FEP) and 18 healthy volunteers (HC). At baseline and follow-up, the longitudinal pipeline of Freesurfer was employed to parcellate prefrontal cortex and the MATRICS Consensus Cognitive Battery (MCCB) was used to assess executive functioning and emotional intelligence. At both timepoints the severity of negative and positive symptoms was assessed using the Positive and Negative Syndrome Scale (PANSS). Baseline cognitive performance was compared between diagnostic groups using Multivariate Analysis of Covariance (MANCOVA). Partial correlations investigated relationships between cognition and negative symptoms at baseline and cortical thickness change over time.

Manuscript 2 & 3. Thirty-three patients with treatment-resistant schizophrenia (TRS) and 31 healthy volunteers successfully participated at both baseline, prior to clozapine initiation in patients, and 6 months follow-up clinical assessments and structural MRI scanning (**Manuscript 2**). Of these 64 participants, diffusion MRI data were available at both time points for 22 patients and 23 healthy controls (**Manuscript 3**).

The severity of positive and negative symptoms was assessed at both time points using the PANSS, the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS). Social, occupational and psychological functioning was assessed using a Global Assessment of Functioning Score. **In Manuscript 2** the longitudinal pipeline of Freesurfer v.5.3.0 was employed to bilaterally segment eight subcortical regions-of-interest: lateral ventricle, thalamus, hippocampus, caudate, putamen, globus pallidus, amygdala and nucleus accumbens. Two-way repeated MANCOVA was used to assess group differences in subcortical volumes over time and partial correlations to determine association with clinical variables. In **Manuscript 3** the Tract-based spatial statistics approach (TBSS) was used to compare changes over time between groups in fractional anisotropy (FA). Changes in structural network organisation and subnetwork connectivity weighted by FA and number of streamlines (NOS) were assessed using graph theory and network-based statistics.

Results: In **Manuscript 1** we demonstrated that patients in their first-episode of psychotic illness perform significantly worse on several tests assessing different aspects of executive functions compared to healthy controls, including category fluency, attention, working memory and reasoning & problem solving. The poorer performance at baseline in spatial working memory was a significant predictor of loss of total prefrontal cortical thickness in the initial years after illness onset. We also found that impairment of emotional intelligence at illness onset was significantly associated with a progressive reduction of orbitofrontal thickness in patients after their first-episode of psychosis. Finally, we demonstrated a correlation between neuroanatomical progression and clinical variables, specifically, worsening of negative symptoms was associated with prefrontal thickness reduction as the illness progresses.

In **Manuscript 2**, in treatment-resistant schizophrenia patients we showed a substantial progressive volumetric reduction of the thalamus, hippocampus, caudate, putamen and enlargement of lateral ventricles over a 6-month period compared to controls. Furthermore, patients who had the greatest symptomatic and functional improvement displayed the largest thalamo-striatal reductions. We also found that patients who were exposed to higher amounts of clozapine displayed a greater reduction of thalamus volume. In **Manuscript 3**, treatment-resistant schizophrenia patients showed progressive focal FA abnormalities in the white matter of key anterior tracts, such as genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata compared to controls. The brain structural network organisation was preserved in patients compared to controls. The FA reduction was independent of any clinical measures or serum level of clozapine.

Conclusion: Taken together our results indicate that at onset of psychosis working memory and emotional intelligence impairment represents a trait marker of progressive prefrontal thinning as the illness progresses, while worsening of negative symptoms is associated with prefrontal thickness reduction over time, indicating a functional consequence of anatomical progression in psychosis. In those with the chronic treatment-resistant stage of the illness, there is a consistent progressive volume reduction in several subcortical structures as well as progressive focal abnormalities in the white matter microstructure of key anterior tracts, but a preserved brain structural network. However, our findings suggest a divergence of neuroanatomical progression, where progressive atrophy in the thalamo-striatal circuits are linked to clinical and functional improvement, whereas no such association is found with longitudinal progression in lateral ventricles, hippocampus and white matter. This thesis confirms the importance of investigating the neurocognitive dimension at illness onset in order to enhance understanding of the functional consequences of illness progression as well as identifying potential markers at illness onset. It also highlights the potential role of the thalamo-striatal circuits in tracking recovery in treatment-resistant schizophrenia patients, suggesting that its investigation using large scale longitudinal design studies could significantly contribute to the identification of biomarkers in refractory schizophrenia.

List of Figures

Introduction

Manuscript 1. Cognitive and Clinical Predictors of Prefrontal Cortical Thickness Change Following First-Episode of Psychosis

Manuscript 2. Progressive Subcortical volume Loss in Treatment-resistant Schizophrenia Patients After Commencing Clozapine Treatment

List of Tables

Thesis Introduction

1. First-Episode Psychosis

Psychosis is a cluster of symptoms indicating loss of touch with reality characterised by delusions (false beliefs or impressions) or hallucinations (sensory perceptions in the absence of actual stimuli). Several organic and functional types of psychosis exist, and functional types can be further categorised into affective and non-affective. Affective psychotic disorders include bipolar I disorder and major depressive disorder while non-affective psychotic disorders include schizophrenia, schizoaffective disorder, psychotic disorder not otherwise specified, substance induced psychosis and delusional disorder. Psychosis represents a defining characteristic of schizophrenia spectrum disorders, it is common but variable in mood and substance use disorders, and a relatively common characteristic of other disorders, such as degenerative, developmental and medical conditions (Griswold et al., 2015). It can be transient, only present for a limited period of time, in the case of the acute and transient psychotic disorder for instance or it could reach a chronic and stable phase as with chronic schizophrenia (Kaur and Cadenhead, 2010). First episode psychosis is usually defined as the first episode of moderately severe psychotic symptoms for at time period such as seven consecutive days or as the first contact/treatment with mental health services (Breitborde et al., 2009).

2. Schizophrenia

Schizophrenia is a complex and severe disabling mental illness, affecting 20 million people worldwide, associated with an enormous personal, social, and economic burden. The World Health Organisation has ranked schizophrenia in the top twenty causes of disability in the world, with 30% of patients failing to respond to traditional antipsychotic medications. It is characterized by a heterogeneous combination of symptoms that can be broadly divided in positive, negative, and cognitive. Positive symptoms are thoughts and behaviours that reflect a distortion of normal functions and are often described as “loss of contact with reality”, which includes delusions, hallucinations, disorganized speech and disorganized or catatonic

behaviour (Kahn et al., 2015). Negative symptoms represents loss or diminution of normal functioning and include poverty of speech (alogia), reduction of the intensity of emotional expression (flat affective), social withdrawal, diminished capacity to experience pleasant emotions (anhedonia) and lack of ability to initiate and persist in goal-directed behaviours (avolition)(Kahn et al., 2015; Kirkpatrick et al., 2006). There is a distinction between primary negative symptoms that are part of the diseases process itself and secondary negative symptoms that are due to factors not directly linked to the disease, such as depression and anxiety, drug-induced motor symptoms, social deprivation and suspiciousness (Carpenter et al., 1988). Finally, cognitive symptoms are an important but sometimes neglected component of schizophrenia, as the dysfunction spreads across several cognitive domains, from general intelligence, working memory, verbal learning, attention to executive functioning (Gelder et al., 2006). Recently, cognitive impairment has become an important focus due to the significant relationship with both level of function and quality of life, specifically cognitive deficits have been shown to be associated with real world functional performance (Bowie and Harvey, 2006; Green, 1996).

Course of Schizophrenia

The longitudinal course of schizophrenia is usually characterised by different phases of the illness, such as premorbid, prodromal, acute/progressive, and residual/chronic.

In the premorbid phase, several cognitive, academic, and social abnormalities can occur. This includes poor academic achievement, social isolation, emotional detachment, attentional dysfunction, and motor development delays (Tandon et al., 2009). Studies on young nonpsychotic high-risk relatives focusing on neurobehavioral, brain structural, physiological and neurochemical alterations, suggested that the neurobiological abnormalities of the illness may begin in childhood or even earlier (Keshavan et al., 2004).

Successively, the timeframe preceding the onset of psychosis has been defined as the prodromal phase, which could last from weeks to years with an average of 5 years (Klosterkotter et al., 2008., Häfner 2019). Often, many individuals have prior symptoms “At Risk Mental State (ARMS)” on a trajectory to developing their first-episode of psychosis. The prodromal phase might be characterised by “attenuated” psychotic symptoms, full-blown psychotic symptoms that are brief and self-limiting, or significant decrease in functioning in

the context of a genetic risk for schizophrenia (Yung et al., 2008). Initially patients commonly experience negative or non-specific clinical symptoms, such as depression, anxiety, social isolation, school/occupational failure, and cognitive deficits. After this initial period, basic symptoms, attenuated positive symptoms or intermittent attenuated positive symptoms of moderate intensity can emerge. Closer to the onset of psychosis, individuals tend to show greater frequency, duration and intensity of attenuated positive symptoms, and the impairment can culminate with the onset of frank psychosis (Larson et al., 2010). Cannon et al., reported that between 16% to 35% of individuals who were identified as At Risk Mental State had developed a psychotic disorder within an average of 2 to 2.5 years (Cannon et al., 2008).

The first-episode of psychosis can mark the formal onset of schizophrenia and many individuals enter an acute and progressive phase, characterised by exacerbations of psychotic symptoms, with repeated episodes of psychosis as well as intermittent remission. In this phase there is an on-going functional decline, most pronounced in the first 5 years after a first-episode of psychosis (Larson et al., 2010). The progressive worsening of psychosis can continue until reaching a chronic and stable phase, where positive symptoms are less prominent and negative symptoms and cognitive deficits plateau (Kaur and Cadenhead, 2010). 38% of patients who experienced their first episode of psychosis show a full symptomatic and functional recovery afterwards (Lally et al., 2017), while a substantial subset of individuals with chronic schizophrenia, approximately 30%, meet criteria to be considered treatment-resistant (Lieberman et al., 1994; Meltzer, 1997), and with failure to respond to standard antipsychotic treatment they show persistent moderate to severe positive or negative symptoms together with poor global functioning.

3. Cognitive Functioning During the Course of Illness

Although not a necessary diagnostic feature of schizophrenia, neuropsychological dysfunction represents a common characteristic of psychotic disorders and it has been identified as a risk factor for developing schizophrenia (Fusar-Poli et al., 2013). In schizophrenia, patients show a history of cognitive impairment in the premorbid phase of the illness, sometimes with cognitive decline prior to onset of psychosis. Hence, the cognitive functioning seems to deteriorate between pre-morbid phase and the first episode of psychosis (Fusar-Poli et al.,

2013; Keefe, 2014; Lewandowski et al., 2011). It has been shown that individuals at high risk of developing psychosis that perform poorly on several facets of cognition, such as verbal and visual memory, working memory, verbal fluency and executive functioning, is predictive of conversion to psychosis (Bora and Murray, 2014; Fusar-Poli et al., 2013).

A systematic review, based on 24 studies looked at cognitive functions in first episode of psychosis patients across several cognitive domains compared to healthy volunteers, reported a significant impairment in all domains investigated, including general intelligence, executive functions, attention, working memory, verbal and non-verbal memory, processing speed and motor speed (Aas et al., 2014). The largest effect size was reported for verbal memory, executive functions and general intelligence (Aas et al., 2014). Verbal learning and processing speed impairment have been reported as the most prominent in first-episode of psychosis, with processing-speed being the greater predictor of schizophrenia (Dickinson et al., 2007; Mesholam-Gately et al., 2009).

After the first episode of psychosis, the cognitive deficits appear to remain relatively stable over the course of illness and across domains, up to 10 years follow-up (Bora and Murray, 2014; Bozikas and Andreou, 2011) with the exception of verbal memory, whose impairment has been showed to further progress over time (Hoff et al., 2005). A recent ten years follow-up case-control study of patients with schizophrenia (N=65) reported a significant decline in IQ and verbal knowledge and memory, but not in processing speed or executive functions whose impairment remained stable over the years (Zanelli et al., 2019). Furthermore, the cognitive impairment across several domains that first-episode of psychosis patients exhibit seems to be comparable to the chronic phases of the illness (Sponheim et al., 2010).

4. Structural Neuroimaging During the Course of Illness

Structural and diffusion longitudinal magnetic resonance imaging (MRI) studies have been applied to patients at different stages of psychotic illness and often producing heterogenous results. Systematic reviews and recent combination of datasets through meta- and mega-analyses have sought to resolve individual study variation by maximizing statistical power and exploring sources of heterogeneity in large samples. Although this has provided large datasets for cross-sectional studies, longitudinal designs to track intra-individual variation are more sparse, with studies lacking large sample sizes.

First-Episode of Psychosis

MRI studies have demonstrated that neuroanatomical abnormalities are already present when the first-episode of psychosis occurs and even beforehand, involving widespread neuroanatomical alterations at global and focal levels. Volume reductions in prefrontal, temporal, cingulate cortices and cerebellum (Dazzan et al., 2012; Fusar-Poli et al., 2012; Leung et al., 2011) have been demonstrated. Specifically, a voxel-wise meta-analysis based on 14 studies reported grey matter reduction in the temporal, limbic and prefrontal cortices in clinical high-risk individuals and grey matter reduction in temporal insular cortex and cerebellum in the first-episode of psychosis (Fusar-Poli et al., 2012). A multimodal meta-analysis of brain changes in first episode psychosis identified both structural and functional differences compared to controls in the insula/superior temporal gyrus and the medial frontal/anterior cingulate cortex bilaterally (Radua et al., 2012).

Diffusion tensor imaging (DTI) provides non-invasive and *in vivo* estimation of the microstructural organization of white matter (Jones, 2008). Fractional anisotropy (FA), the most reported DTI outcome measure, reflects the anisotropic molecular diffusion in white matter. In the white matter the movement of water molecules is anisotropic due to the presence of cellular structures that do not allow free or isotropic diffusivity. FA is sensitive to fibre orientation, coherence, axonal diameter, packing density and degree of myelination of neurons within fibre bundles (Beaulieu, 2002; Pierpaoli et al., 1996; Tournier et al., 2011). Magnetic resonance DTI in individuals experiencing their first episode of psychosis reported extensive FA reduction in white matter tracts compared to controls, including association, commissural and cortico-subcortical fibres (Alvarado-Alanis et al., 2015; Keymer-Gausset et al., 2018; Lee et al., 2013).

Schizophrenia

To date, there is consistent evidence for the presence of grey and white matter brain abnormalities in chronic schizophrenia. A large-scale international collaborative metanalysis (ENIGMA) using standardized methods based on 4474 individuals with schizophrenia and 5098 healthy volunteers reported widespread thinner cortex and smaller surface area, with the largest effect sizes in frontal and temporal regions (van Erp et al., 2018). The same group,

identified smaller hippocampus, amygdala, thalamus and intracranial volume as well as larger pallidum and lateral ventricle volumes in schizophrenia patients (n=2028) compared to healthy controls (n=2540) (Van Erp et al., 2016). Meta-analyses have reported significant white matter abnormalities in schizophrenia compared to controls in the tracts interconnecting the frontal lobe, thalamus, cingulate and tracts traversing left temporal white matter regions (Bora et al., 2011; Ellison-wright and Bullmore, 2009). A large meta-analysis by ENIGMA, based on 1963 schizophrenia patients and 2359 healthy controls reported significant widespread FA reductions in schizophrenia, most prominent in the white matter of the anterior corona radiata and the body and genu of the corpus callosum relative to the control group (Kelly et al., 2017).

First-Episode of Psychosis and Schizophrenia Cross-sectional Comparisons

Ellison-Wright and colleagues' (2008) meta-analysis based on 27 articles looking cross-sectionally at first episode and chronic schizophrenia, reported regions that were affected in both groups, including grey matter decrease in the thalamus, the left amygdala region, the insula bilaterally and the anterior cingulate. Decrease in the grey matter of the caudate head bilaterally was identified in the first episode group but not in chronic schizophrenia, suggesting a normalization of the structure as the illness progresses. The meta-analysis reported evidence of more widespread decreases in cortical regions in chronic schizophrenia (Ellison-Wright et al., 2008).

A cross-sectional DTI study (Friedman et al., 2008) looked at FA differences between 40 individuals with first-episode schizophrenia and 39 matched healthy controls and between 40 with chronic schizophrenia and 40 matched psychiatrically healthy controls. In most regions (right and left forceps minor, right forceps major, left inferior longitudinal fasciculus, and splenium of the corpus callosum) the chronic schizophrenia group showed significant or trend-level lower FA compared to the healthy control group. The group experiencing their first-episode of psychosis instead showed no significant FA reduction compared to healthy controls and only trend-level lower FA in the left and right inferior longitudinal fasciculi. The study suggested that white matter abnormalities are less widespread at illness onset in

schizophrenia, with a spatial or anatomical and quantitative progression with chronicity of illness (Friedman et al., 2008)

Di Base and colleagues (2007) (Di Biase et al., 2017) used network analysis to assess and describe structural connectivity patterns and efficiency of brain networks through mapping the brain as a collection of nodes (subcortical and cortical structures) and edges (white matter connections) (Bullmore and Sporns, 2009). The study looked at the connectome differences between those recently diagnosed with schizophrenia (n=19) and chronic schizophrenia (n=45). A significant reduction in connectivity strength was found in both groups, however, in the recent-onset group the network disruption was limited to frontal and parietal connections (n=7), instead the chronic patient group showed a more widespread disrupted network (n=153). The study suggested a possible anatomical progression of white matter connectivity from early stages to more severe stages of the disease.

Thus direct cross-sectional comparisons between recent onset psychotic illness and chronic schizophrenia both in a spatially discreet and network approach indicates more substantial grey matter and white matter abnormalities associated with psychosis consistent with, but not necessarily confirmatory of, progression of neuroanatomical abnormalities as the illness advances. Other explanations for these findings include selection bias towards more severe illness in chronic schizophrenia samples since a proportion of those with first episode psychosis will recover and leave services. The progression and its correlates can be confirmed by longitudinal study-designs that have the major advantage of assessing the trajectory of the illness course by comparing the same population in various stages of the illness.

Longitudinal Studies: Onset of Psychosis

A recent systematic review based on 19 longitudinal studies looked at the changes in grey matter in the years after the first episode of psychosis compared to healthy controls. The study reported widespread grey matter decrease over time in patients compared to controls, specifically in frontal, superior temporal regions, parietal and subcortical regions (Gallardo-Ruiz et al., 2019).

A longitudinal structural MRI study looked at cortical changes over the 2 years following the first episode of psychosis. The study based on 27 patients and 25 controls, reported progressive cortical thinning in the superior and inferior frontal and superior temporal cortex to a lesser extent (Gutiérrez-Galve et al., 2015). Sun and colleagues, investigated progressive brain structural changes in 35 individuals at ultra-high risk for developing psychosis. Twelve out of 35 experienced first episode of psychosis within 1 year. The study found a significant volume loss in the right prefrontal regions in those who experienced psychosis onset, indicating ongoing pathological processes during the transition stage to illness (Sun et al., 2009). Ho *et al.,* in a 3-year longitudinal MRI study on 73 individuals with recent-onset schizophrenia and 23 psychiatrically healthy controls showed accelerated enlargements in cortical sulcal cerebrospinal fluid (CSF) spaces and progressive white matter volumetric reduction in the frontal lobe in the schizophrenia group compared to healthy controls. The study suggested that the neurodevelopmental progression was already active during the onset of illness and continues during the early years of the illness (Ho et al., 2003).

Longitudinal Studies: Chronic Schizophrenia

A meta-analysis of 27 longitudinal volumetric studies (Olabi et al., 2011) in patients with schizophrenia (n=928) and healthy controls (n=867) over durations ranging from 1 to 10 years, revealed that schizophrenia is associated with significant volumetric declines in whole brain (- 0.07% annual volumetric change), whole brain gray matter (-0.59%), frontal grey and white matter (-0.32%), temporal (-0.39%) and parietal (-0.32%) white matter volume over time, as well as increases in the lateral ventricles (+0.36%) compared with healthy control.

Kempton and colleagues (2010) performed a meta-analysis on 13 longitudinal MRI studies, looking at the volumetric change of lateral ventricles in schizophrenia patients over the course of illness. The study reported a progressive ventricular enlargement after illness onset compared to controls. A subgroup of the chronic schizophrenia group with a mean duration illness of 7.6 years showed a more significant progressive ventricular enlargement (Kempton et al., 2010).

Van Haren et al (2007) in a longitudinal voxel-based morphometry study investigated differences between 96 subjects with schizophrenia and 113 healthy controls in focal grey and white matter over the course of 5 years. The analysis revealed a decrease in grey matter density in compared to controls, specifically in the left superior frontal area, left superior temporal gyrus, right caudate nucleus, and right thalamus. The progression in left frontal density loss appears to be related to an increased number of psychotic episodes (van Haren et al., 2007). When looking at white matter, the study failed to report any differences in change in white matter density during the scan interval between patients and controls. Additionally, Van Haren and colleagues looked also at the change in cortical thickness in the same cohort over a 5-year period. They reported a significant widespread cortical thinning, more pronounced bilaterally in the temporal cortex and the left frontal areas. More pronounced thinning was associated with poorer outcome (van Haren et al., 2011).

Mitelman et al. 2009, combining diffusion-tensor and structural magnetic resonance, assessed in a 4-year period, the changes in white matter in 49 chronic schizophrenia patients, subdivided into good-outcome (n=23) and time poor-outcome (n=26) groups, compared to 16 healthy controls (Mitelman et al., 2009). The white matter FA analysis, for frontal, parietal and temporal lobes revealed a lesser progression in patients with poor outcome compared to good outcome, especially in the prefrontal and anteroposterior cingulate regions. The analysis of white matter volumes showed an overall pattern of greater volume expansion in patients with poor outcome compared to the good outcome group (Mitelman et al., 2009).

Taken together this data suggest that there is a robust body of studies showing cortical and subcortical grey abnormalities at onset of psychosis with a progressive deficit over the later course of the illness. Widespread white matter abnormalities are also reported during different stages of the illness, however, due to the lack of large sample longitudinal studies, the white matter abnormalities trajectory is uncertain.

5. Association of Neuroanatomical Abnormalities with Antipsychotic Medications

5.1 Antipsychotic Medications

Typical antipsychotic medications were first introduced in the 1950's when the antipsychotic actions of chlorpromazine were discovered, revolutionising the treatment of psychoses, and especially the management of schizophrenia (Delay et al., 1952). Since then, dozens of antipsychotics drugs have been developed and to date, we can broadly divide them in two categories: typical antipsychotics or first-generation (FGA) and atypical antipsychotics or second generation (SGA).

Typical antipsychotics, such as chlorpromazine and haloperidol, act by strongly blocking dopamine D_2 receptors in mesolimbic and mesocortical pathways of the brain and are very effective in the treatment of positive symptoms. The dopaminergic receptors are highly expressed in basal ganglia and due to their location extrapyramidal side effects are very common (EPS) (Tarazi, 2001). The side effects include involuntary muscle spasms knows as dystonia, parkinsonisms that include tremors at rest, slow movements, limb-rigidity and progressively the long-term use of typical antipsychotics can result in tardive dyskinesia, with uncontrolled involuntary movements. The clinical response to the medication is higher when 60% of the dopamine receptors are occupied by the drug, while at over 80% occupancy extrapyramidal side effects are likely to manifest (Kapur et al., 2000).

Atypical antipsychotics, such as clozapine, olanzapine, risperidone, quetiapine, amisulpride, and zisprasidone became available in the 1990's. This new generation has been developed to achieve equivalent clinical efficacy but with less extrapyramidal side effects. These drugs have been showed to be more tolerable and compared to typical first antipsychotics work better in the treatment of negative and cognitive symptoms as well as positive (Lehmann and Ban, 1997). Due to the lower affinity and occupancy for dopamine receptors, and high occupancy for serotoninergic receptors 5-HT_{2A} (Meltzer et al., 1989) or through partial agonism of D_2 receptors (Abi-Dargham and Laruelle, 2005) atypical antipsychotics induce less extra-pyramidal effects. Side effects such as weight gain, hyperlipidemia, drowsiness, agitation,

anxiety, and insomnia often accompany the use of second-generation antipsychotics (Üçok and Gaebel, 2008).

5.2 Association Between Neuroanatomical Changes and Antipsychotics

To date, the effect of antipsychotic medications on brain structures is still not fully understood. Cross-sectional studies which investigated this relationship through correlational analyses are susceptible to multiple confounds, whereas longitudinal studies, especially with a randomised approach design, can better disentangle disease effect from medication effect but are difficult to implement.

Cross-Sectional Studies

A large meta-analysis by ENIGMA, based on 1963 schizophrenia patients and 2359 healthy controls assessed the potential impact of antipsychotic medications on white matter changes, specifically whether chlorpromazine (CPZ) score was correlated with FA changes in the white matter of anterior corona radiata and corpus callosum. The study failed to find any significant associations, moreover the study was underpowered to compare patients based on their atypical or typical antipsychotic use (Kelly et al., 2017).

A meta-analysis of over 18,000 subjects based on 317 cross-sectional MRI studies looked at brain volumes differences between medicated-schizophrenia patients (8327), antipsychotic-naïve patients (771) and healthy controls (9231). The study reported a significant total brain volume reduction, explained by a marked reduction in total grey matter. The brain changes in the antipsychotic-naïve sample were similar to the one showed in the medicated patients but to a lesser extent. The effect size for total brain and gray matter volume in antipsychotic-naïve patients was 30% lower compared to medicated patients. White matter reduction was similar in medicated and medicated-naïve patients, suggesting that the white abnormalities present before the treatment had a slow progression compared to the significant grey matter reduction. Moreover, higher dose of antipsychotics was associated with a large volumetric loss in grey matter and not white matter (Haijma et al., 2013).

Navari and Dazzan's (2009) systematic review, reported results from 10 cross-sectional MRI studies investigating the association between antipsychotic treatment and brain structure. The authors highlighted the limitation of cross-sectional studies, which evaluate brain structures at one single time point and often type and dose of antipsychotic medications are not reported, which makes it difficult to elucidate the role of medical treatment on the structural brain changes. Overall, the review reported that, even after short-term treatment, antipsychotics medications, especially typical antipsychotics, have an effect on basal ganglia. There was also evidence of a drug-specific action at cortical level (Navari and Dazzan, 2009).

Dazzan and colleagues (2005) investigated the different effects of typical and atypical antipsychotics on grey matter in first episode of psychosis patients. Grey matter differences were compared between 22 drug-free patients (13 neuroleptic-naïve), 32 on treatment with typical antipsychotics and 30 with atypical antipsychotics. Drug-free patients were considered those subjects that had not taken any antipsychotic medication in the 3 weeks prior the MRI scan. The study did not report significant differences between the three groups for total grey matter volume. Patients on typical antipsychotics compared to drug-free patients showed volumetric increase of putamen and volumetric decrease of lobulus paracentralis, anterior cingulate gyrus, superior and medial frontal gyri, superior and middle temporal gyri, insula, and precuneus. Patients taking atypical antipsychotics compared to those who were drug-free showed bilaterally enlargement of the thalamus (Dazzan et al., 2005).

Longitudinal Studies

Vita et al., (2015) in a meta-analysis of longitudinal MRI studies on 1155 patients with schizophrenia and 911 healthy controls reported that reduced cortical grey matter volume was related to cumulative exposure and mean daily dose of antipsychotics medications. The greater grey matter loss was displayed by patients treated with first-generation antipsychotics compared with second-generation (Vita et al., 2015).

Van Haren and colleagues (2011), in a 5-year MRI longitudinal study looking at cortical thickness changes in schizophrenia, showed an association between higher cumulative dose of first-generation antipsychotics over time and more pronounced cortical thinning, while a

higher dose of second-generation antipsychotics was associated with less marked cortical thinning (van Haren et al., 2011). Another study from the same cohort, with a focus on volumetric changes, reported that a higher cumulative dose of second-generation antipsychotics during the scan interval was related to lesser decrease of grey matter in the left superior frontal gyrus (van Haren et al., 2008).

A 3-year MRI longitudinal study on 73 recent-onset schizophrenia patients and 23 controls, did not find any significant correlation between volumetric changes over time and cumulative antipsychotics dose, total duration of antipsychotics treatment, percentage of time treated with atypical antipsychotics or percentage of time treated with typical antipsychotics. The study concluded that the progressive volumetric brain change was occurring despite the ongoing treatment with antipsychotics (Ho et al., 2003).

Randomised Design Approach

Crespo-Facorro et al., (2008), in a randomized controlled one-year longitudinal study looked at the different effect of haloperidol (N=18), risperidone (N=16) and olanzapine (N=18) on cortical and subcortical brain structure in first-episode drug-naïve patients with non-affective psychosis compared to healthy controls (N=38). The study found that there were not significant volumetric differences in the course and magnitude between patients with non-affective psychosis undergoing treatment with risperidone, olanzapine, or low dose of haloperidol. The study found that patients on typical antipsychotic medication displayed no change over time in caudate volume, but there was a slight volumetric reduction of the caudate in patients on atypical antipsychotic medications. Typical and atypical antipsychotics did not significantly differ in their effect on whole brain white matter (Crespo-Facorro et al., 2008). In the same cohort the effect of the three antipsychotic medications on cortical thickness during 1-year follow-up period was investigated. No significant differences in cortical thickness were observed between patients treated with haloperidol, risperidone or olanzapine (Roiz-santiáñez et al., 2012).

An MRI longitudinal randomized controlled double-blind study based on 161 patients with first episode psychosis and 52 controls looked at the effect of olanzapine and haloperidol on

brain structures after 12 and 24 weeks of treatment. A subgroup of patients was also scanned after 52 and 104 weeks. The study showed a significant between-treatment difference in volumetric changes. Patients on haloperidol treatment showed significant reduction in grey matter volumes between weeks 12 and 24, whereas olanzapine was not associated with brain volume changes. A specific effect of haloperidol bot not olanzapine was also found, with volumetric increase of caudate (Lieberman et al., 2005).

Taken together these results, coming from different study designs, suggest an effect of antipsychotic medications on grey matter, specifically volumetric reduction and cortical thinning associated with antipsychotics exposure. However the possibility of confounding factors, such as those with more severe illness receiving higher amounts of antipsychotic medication, are difficult to exclude in observational studies; and the small number of randomised studies indicate an attenuated effect for atypical antipsychotic medication. The effect of antipsychotics on white matter seems to be less straightforward and is still not fully understood, due to the lack of studies investigating this relationship or lack of large sample size studies.

6. Treatment-Resistant Schizophrenia

Approximately 30% of patients with schizophrenia meet criteria to be considered treatment-resistant (Lieberman et al., 1994; Meltzer, 1997). Refractory schizophrenia is usually defined by the failure to respond to at least two trials of classic antipsychotic medication, of adequate dose and duration, with patients showing persistent moderate to severe positive, or disorganisation, or negative symptoms together with poor global functioning over a prolonged period of time (Barnes et al., 2020; Meltzer, 1997). This definition has been employed by the Treatment Response and Resistance in Psychosis (TRRIP) group (Howes et al., 2017), the National Institute for Health and Care Excellence (Nice et al., 2014), the American Psychiatric Association (APA) (Lehman et al., 2004) and the World Federation of Societies of Biological Psychiatry (WFSBP) (Hasan et al., 2012). To date, it is still unclear whether treatment-resistant schizophrenia represents a different subtype of schizophrenia group or simply a more severe end of the illness spectrum.

A recent systematic review based on 19 studies tried to shed a light on this debate, showing that overall treatment-resistant patients show glutamatergic abnormalities, a lack of dopaminergic abnormalities, and a significant decrease in grey matter compared to treatment-responsive patients (Gillespie et al., 2017). Recently, a study looking at the neurobiology of treatment-resistant schizophrenia proposes two theories to explain treatment-resistant schizophrenia: on one hand TRS is characterized by normal dopamine function, while glutamate abnormalities contribute to the neurobiology of treatment-resistance; on the other the dopamine super sensitivity leads to the development of treatment-resistance over time (Potkin et al., 2020). The dopamine super sensitivity theory, first introduced in the 1978 (Chouinard and Jones, 1980), states that continuous blockade of dopamine D_2 receptors results in dopaminergic changes that lead to break-through symptoms, where the initial dose of antipsychotics medication is no longer effective in the treatment of symptoms. The authors also suggest that the two models could explain different presentations of treatment-resistant schizophrenia, where the normal dopamine hypothesis explains the treatment resistance from the illness onset and the dopamine super sensitivity hypothesis explained the development of the treatment-resistance in some patients (Potkin et al., 2020). Lally and colleagues (Lally et al., 2016) in a 5 years follow-up study assessed the clinical outcomes in a cohort of 246 first-episode schizophrenia spectrum. The study reported that 23% of the total study population was treatment-resistant, 70% of which was treatment-resistant from illness onset.

To date, the most effective and gold-standard treatment for patients with treatment-resistant schizophrenia is clozapine, one of the first atypical antipsychotic medications introduced in the market. The association between clozapine and brain morphometry in schizophrenia is still uncertain (Mouchlianitis et al., 2018) and longitudinal studies are necessary to enhance the understanding of its effect on neuroanatomy over time.

7. Clozapine

Clozapine is a dibenzodiazepine derivate and has an established superior clinical effect in treating refractory schizophrenia, with 60-70% of patients showing a positive response (Chakos et al., 2001; Kane et al., 1988; Stroup et al., 2003). However, some patients with

treatment-resistant schizophrenia fail to respond to clozapine or all other available treatments, resulting in so-called ultra-resistant schizophrenia. Clozapine was withdrawn from the market in the 1970s as it caused lowered white blood cells count (agranulocytosis) in patients and reintroduced in the 1980s with the requirement of weekly/monthly blood monitoring tests.

The mechanism by which clozapine exerts its superior clinical effect on the treatment of symptoms is still unknown. The dopamine hypothesis (Davis et al., 1991) provides us a neurobiological and clinical explanation of how presynaptic dopamine dysfunction plays a key role in leading to symptoms in patients with schizophrenia and confirms the efficacy of antipsychotic medication whose action target dopamine D_2 receptors (Howes et al., 2016). However, this hypothesis might not apply to clozapine that shows low affinity for D_2 receptors. It has been suggested that treatment-resistant schizophrenia patients present an abnormal glutamate system, and the ability of clozapine to modulate brain glutamine release could contribute to its efficacy in treatment-resistant schizophrenia patients (Amitai et al., 2012). Neuroimaging studies showed higher glutamate levels in the anterior cingulate cortex, with relatively normal dopamine functioning in patients with treatment-resistant schizophrenia compared to treatment-responsive (Demjaha et al., 2014, 2012). Another study reported higher levels of glutamate + glutamine in the putamen in treatment-resistant schizophrenia patients compared to first-line responders, suggesting that the total level of glutamate + glutamine could represent a biomarker of response to clozapine (Goldstein et al., 2015). A recent longitudinal study examined the effects of clozapine on brain glutamate in 37 treatment-resistant schizophrenia patients, before and 12 weeks after switching to clozapine. The study found that 12 weeks of clozapine treatment was associated with a longitudinal reduction in glutamate in the caudate nucleus. The percentage of glutamate reduction in the caudate was significantly associated with improvement in symptoms (McQueen et al., 2020), suggesting a key role of the glutamate in the action of clozapine and the symptoms improvement during the first months of clozapine exposure.

8. Rationale & Thesis Outline

The overarching theme of this thesis is to examine elements of neuroanatomical progression and its cognitive or clinical correlates in samples of patients across different phases of psychosis spanning first episode of illness and treatment refractory schizophrenia. The vast majority of structural and diffusion neuroimaging studies on neuroanatomy, and cognition have been conducted cross-sectionally and it remains unclear whether risk factors, treatments or associated illness effects are driving changes. While on one hand sources of the heterogeneity in cross-sectional case-control studies have been explored using *post-hoc* statistical analyses, such as through correlations, assessing interactions, and meta-regression analyses, this field of research requires longitudinal studies to directly tease these factors apart. Although randomised studies are the optimal approach to separate out the impact of one element (e.g. antipsychotic treatment), these studies are difficult and sometimes unethical to perform. However, observational longitudinal studies are powerful in being able to track intraindividual variation over time and examine whether this variation is associated with clinical and cognitive metrics contemporaneously acquired.

This thesis employs structural and diffusion MRI techniques to examine longitudinal samples acquired for studies into first-episode of psychosis and treatment-resistant schizophrenia, in order to advance the understanding of neuroanatomical progression and its relationship with clinical and cognitive variables in different stages of the psychotic illness. This is a thesis by publication and incorporates 3 studies that are published (manuscript 1, 2 and 3). The specific aims and hypotheses are presented in detail in each chapter. Briefly, the aims of each study are outlined below.

- Manuscript 1 is based on a cohort of First-Episode Psychosis Patients

This study is a 3.5-year follow-up study of a first episode psychosis sample, with comprehensive clinical, cognitive, and structural MRI data. The broad aim of the study is to identify the extent of progressive brain structural abnormalities associated with the early stages of psychotic illness and their clinical, cognitive, medication and course predictors. Previously, Kenney and colleagues (Kenney et al., 2015) investigated the longitudinal course

of cognitive deficits in the cohort and the relationship of neuropsychological performance with clinical course. At the onset of psychosis patients showed significant cognitive deficits compared to controls across all cognitive domains tested, such as speed of processing, attention, working memory, verbal learning, visual learning, reasoning, and problem solving and social cognition. The widespread cognitive deficit was largely stable over 3.5 years compared to controls, but showed progressive deterioration for verbal learning and processing speed.

Subsequently, Akudjedu and colleagues (Akudjedu et al., 2020) investigated progression of ventricular and cortico-subcortical regional brain volumes over the course of 3.5 years in the same sample. This study demonstrated localised progressive prefrontal cortical thinning, progressive volume deficits in the dorsal striatum and thalamus, and right lateral ventricular enlargement after the onset of psychotic illness. The progressive changes in lateral ventricles volume were associated with indices of poorer outcome amongst patients, as evidenced by worsening negative symptoms and functioning scores over the 3.5-year period.

Developing on these prior studies, I explored the interplay between neuroanatomical progression, cognitive and clinical deterioration after first-episode of psychosis. Specifically, Manuscript 1 entitled **"*Cognitive and Clinical Predictors of Prefrontal Cortical Thickness Change Following First-Episode of Psychosis*"** I explored whether impaired executive functioning, emotional intelligence and negative symptom severity at the onset of psychosis (markers of a potentially more severe illness process) are predictors of prefrontal cortex thinning at follow-up, and whether impaired emotional intelligence at onset of psychosis is associated with loss of orbitofrontal cortical thickness at follow-up. Additionally, through exploratory analyses, this study investigated the relationship of cognitive and clinical change over time with prefrontal cortical thickness change (Figure 1). The decision to focus specifically on emotional intelligence rather than more broadly on social cognition is based on the cognitive battery used in this study. The Mayer-Salovey-Caruso Emotional Intelligence Test is designed to measure the four branches of emotional intelligence, namely: perceiving emotions, facilitating thought, understanding emotions and managing emotions (Mayer et al, 2002).

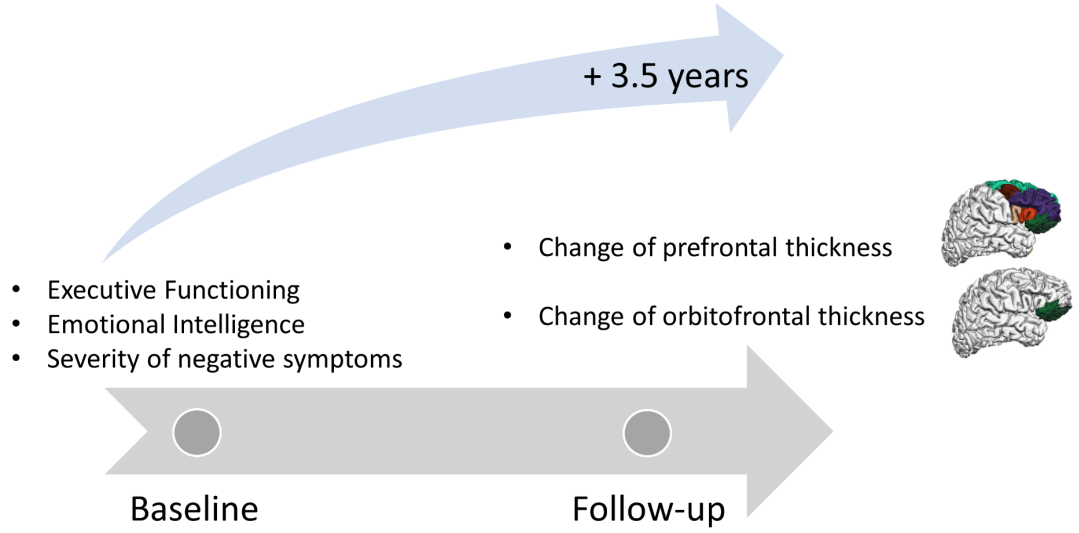

Figure 1. Visual representation of longitudinal study-design in Manuscript 1.

- Manuscript 2 and 3 are based on a cohort of Treatment-Resistant Clozapine-Naïve Schizophrenia Patients.

These follow-up studies avail of comprehensive longitudinal clinical, structural and diffusion MRI data acquired on patients with treatment resistant schizophrenia just before commencing clozapine and again after 6 months taking this medication. The therapeutic effect gained with clozapine is not immediate and beneficial effects are observed up to 6 months after commencing treatment (Lieberman et al., 1994). The studies aim to assess the impact of switching to clozapine monotherapy on neuroanatomy and the association between any progressive brain abnormalities and clinical outcome variables. Previously, Ahmed and colleagues (Ahmed et al., 2015) examined cortical changes in the cohort, demonstrating on-going volumetric reduction in the right and left medial prefrontal cortex and in the periventricular area and cortical thinning in patients compared to controls over time, despite significant symptomatic and functional improvement in patients.

Manuscripts 2 and 3 develop this prior work by examining in detail subcortical and white matter abnormalities over time and their clinical correlates (Figure 2).

Manuscript 2 entitled **"*Progressive subcortical volume loss in treatment-resistant schizophrenia patients after commencing clozapine treatment*"**, comprehensively investigates whether subcortical structures demonstrate progressive neuroanatomical changes after 6 months of clozapine treatment and whether any such changes are related to clinical variables, including treatment response and amount of clozapine taken.

Manuscript 3 entitled **"*White Matter Microstructure and Structural Networks in Treatment-Resistant Schizophrenia Patients After Commencing Clozapine Treatment: A Longitudinal Diffusion Imaging Study*"** combines different diffusion MRI techniques to investigate whether after 6 months of switching to clozapine, white matter microstructure and structural network organisation demonstrate any progressive changes compared to healthy controls. The study also assesses whether any white matter changes are related to clinical variables and serum level of clozapine at follow-up.

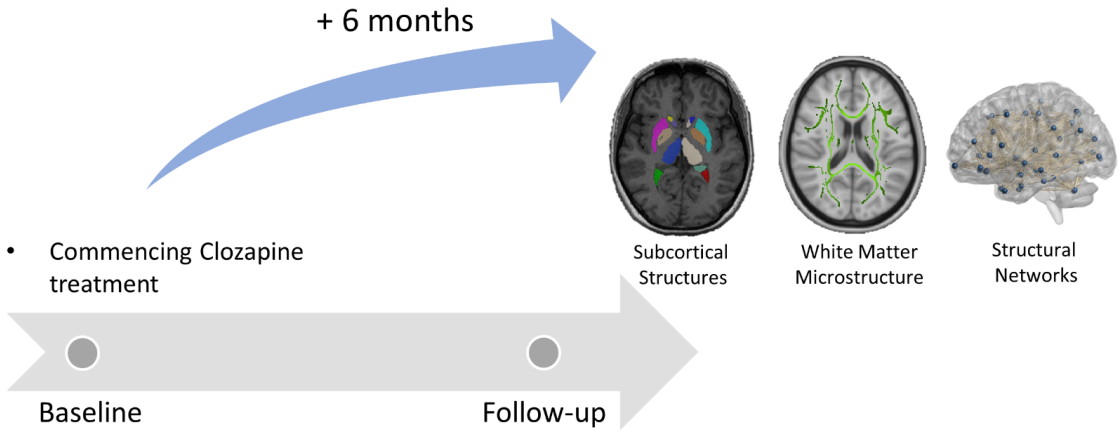

Figure 2. Visual representation of longitudinal study-design in Manuscript 2 & 3.

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Cognitive and Clinical Predictors of Prefrontal Cortical Thickness Change Following First-Episode of Psychosis

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Abstract

The association of neuroanatomical progression with cognitive and clinical deterioration after first-episode of psychosis remains uncertain. This longitudinal study aims to assess whether i) impaired executive functioning and emotional intelligence at first presentation are associated with progressive prefrontal and orbitofrontal cortical thinning ii) negative symptom severity is linked to progressive prefrontal cortical thinning. 1.5T MRI images were acquired at baseline and after 3.5 years for 20 individuals with first-episode psychosis and 18 controls. The longitudinal pipeline of Freesurfer was employed to parcellate prefrontal cortex at two time points. Baseline cognitive performance was compared between diagnostic groups using MANCOVA. Partial correlations investigated relationships between cognition and negative symptoms at baseline and cortical thickness change over time. Patients displayed poorer performance than controls at baseline in working memory, reasoning/problem solving and emotional intelligence. In patients, loss of prefrontal and orbitofrontal thickness over time was predicted by impaired working memory and emotional intelligence respectively at baseline. Moreover, exploratory analyses revealed that the worsening of negative symptoms over time was significantly related to prefrontal cortical thinning. Results indicate that specific cognitive deficits at the onset of psychotic illness are markers of progressive neuroanatomical deficits and that worsening of negative symptoms occurs with prefrontal thickness reduction as the illness progresses.

Keywords

First-episode psychosis, Cognitive impairment, Magnetic resonance imaging, Cortical thickness, Longitudinal study, Negative symptoms

1. Introduction

Patients experiencing their first-episode of psychosis display cognitive impairments compared with healthy controls across different domains, such as verbal learning, executive functions, general intelligence, social cognition, attention and working memory (Aas et al., 2014; Kenney et al., 2015). Recent findings have identified executive impairments as one of the most central deficits in patients with schizophrenia (Chan et al., 2006a, 2006b; Orellana and Slachevsky, 2013). In neuropsychology, the term executive function is used to indicate higher-order cognitive processes which enable people to control and plan their behaviours. This set of cognitive abilities includes planning, working memory, attention, problem solving, verbal reasoning, inhibition, mental flexibility and monitoring of actions (Chan et al., 2008). Neuroimaging, neuropsychological and lesion studies have shown that optimal executive functioning depends on healthy prefrontal cortex (Gazzaniga, 2004).

In this study, we focused our analyses on cortical thickness, given that it is highly heritable and driven by specific cellular mechanisms (Panizzon et al., 2009). It therefore represents an important measure for the identification of prognostically meaningful biological markers in patients experiencing their first-episode of psychosis. Longitudinal studies of patients following their first-episode of psychosis have reported progressive cortical changes, including cortical volume loss in frontal regions (Arango et al., 2012; Pina-Camacho et al., 2016; Roiz-Santiáñez et al., 2014), which may be attributable to reduced cortical thickness. Cortical thinning over time in frontal and prefrontal regions has been widely reported in patients with established schizophrenia and first-episode of psychosis compared with controls (Gutiérrez-Galve et al., 2015; Nesvåg et al., 2008). Janssen et al's study identified bilateral cortical thinning in the superior prefrontal cortex in patients with early-onset first-episode psychosis (Janssen et al., 2009).

The relationship between cortical thickness and cognition has been explored in some longitudinal studies. Specifically, reductions of cortical thickness in patients with schizophrenia have been shown to relate to executive functioning (Ehrlich et al., 2012; Geisler et al., 2015). Less improvement over time in general cognitive performance has been demonstrated to correlate with a greater longitudinal volume loss in frontal regions, particularly medial frontal gyrus and inferior frontal gyrus, in patients with first-episode of

schizophrenia (Asami et al., 2012). Another longitudinal study, based on 20 patients experiencing their first-episode of psychosis and 25 healthy controls, found that low working memory at baseline predicts frontal and parietal cortical thinning 2 years later (Gutiérrez-Galve et al., 2015).

Although executive dysfunction reflects a cognitive impairment and negative symptoms are a characteristic of schizophrenic illness, these two indices of dysfunction tend to overlap and are associated with similar behaviours, such as incongruous emotional responses, reductions in speech, impaired attention and loss of spontaneity (Orellana and Slachevsky, 2013). Cross-sectional studies tend to report a significant correlation between executive impairment and negative symptoms in schizophrenia (Bagney et al., 2013; Nieuwenstein et al., 2001) and first-episode of psychosis (Faerden et al., 2009). The large ENIGMA cross-sectional meta-analysis reported a significant association between prefrontal thinning and negative symptom severity in schizophrenia, specifically in the left medial orbitofrontal cortex, left lateral orbifrontal gyrus and left pars opercularis (Walton et al., 2018). This relationship has also been explored using a longitudinal design, with reports that less improvement in negative symptoms was significantly correlated with volume loss in middle and inferior frontal gyrus over time (Asami et al., 2012). These studies emphasise how both negative symptoms and executive dysfunction can occur with abnormalities in prefrontal regions.

Emotional intelligence, a domain of social cognition, has also been reported to be impaired in patients experiencing their first-episode of psychosis compared with healthy volunteers (Healey et al., 2016; Kenney et al., 2015). Emotional intelligence is a subset of social intelligence described by Salovey and Mayer (1990) as the ability to monitor one's own and others' feelings and emotions, to discriminate among them and to use this information to guide one's thinking and actions' (Salovey and Mayer, 1990). The prefrontal cortex plays an important role in the regulation of emotional processing (Forbes and Grafman, 2010); in particular the orbitofrontal cortex is implicated in emotional and social cognition (Beer et al., 2006; Nestor et al., 2013), while emotional intelligence is reduced in those with lesions in the right orbitofrontal cortex (Barbey et al., 2014).

Although several studies have investigated the cross-sectional relationship between cognition, cortical thickness and clinical symptoms in first-episode psychosis, few have carried out longitudinal analyses to clarify such associations over time. The present study, with its longitudinal design offers an excellent opportunity to explore whether impaired executive functioning and negative symptom severity at the onset of psychosis (markers of a potentially more severe illness process) are predictors of prefrontal cortex thinning in subsequent years; and also to clarify whether impaired emotional intelligence at onset of psychosis is associated with loss of orbitofrontal cortical thickness over time. We considered that more severe executive dysfunction and negative symptoms at baseline would be associated with more progressive neuroanatomical changes and hypothesize three associations: (1) that performance in those executive functions tests showing an impairment in patients at baseline compared to healthy controls will be significantly associated with loss of thickness in total prefrontal cortex in patients over time; (2) impaired emotional intelligence at baseline will be associated with orbitofrontal cortex thinning in patients as the illness progresses; and (3) severity of negative symptoms at baseline will be associated with prefrontal cortical thinning in patients over time. Additionally, through exploratory analyses we investigated the relationship of cognitive and clinical change over time with prefrontal cortical thickness change.

2. Method

2.1 Participants

As reported in our previous study (Kenney et al., 2015), 23 individuals in their first-episode of psychotic illness and 21 healthy controls participated at both baseline and follow-up in clinical and cognitive assessments. Of these, 20 patients and 18 healthy controls also participated in MRI scanning and were included in the present study. The recruitment and clinical assessment of participants was previously described in detail (Kenney et al., 2015; McFarland et al., 2013; Scanlon et al., 2014). Exclusion criteria for all participants included neurological disorders, learning disability, life-time substance dependence, a history of head injury resulting in loss of consciousness for over 5 minutes, oral steroid use in the previous 3 months and general MRI contraindications. Exclusion criteria for controls included also a personal or family history of psychosis or affective disorder. The study was approved by the Research Ethics Committees of the National University of Ireland Galway and Galway University Hospital. Fully informed written consent was obtained for all participants.

2.2 Neuropsychological assessment

The MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al., 2008) was used to assess patients and controls at baseline and follow-up. MCCB was chosen for its excellent test-retest reliability and minimal practice effects (Nuechterlein et al., 2008). Within the MCCB, only the tests assessing specific domains of executive functions were utilised in the analyses. Following the definition of executive functioning as set of abilities, presented by Chan et al., (2008), we were able to cover the following domains: working memory (Wechsler Memory Scale (WMS®-III): Spatial Span forward and backward and Letter Number Span); attention (Continuous Performance Test (CPT): Identical Pairs); fluency (Category fluency: Animal Fluency) and reasoning & problem solving (Neuropsychological Assessment Battery (NAB): Mazes). Emotional intelligence was measured using the Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT): Managing Emotions. A detailed description of the neuropsychological tests has been outlined previously (Kenney et al., 2015).

2.3 Clinical assessment

Patients were diagnosed using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders IV text revision version at both timepoints. The antipsychotic medication taken by patients was recorded and the total dose converted to chlorpromazine (CPZ) equivalents (Lehman and Steinwachs, 1998; Woods, 2003). The doses at baseline and follow-up were the daily doses at the time of scanning converted to CPZ equivalents. The cumulative dose was calculated assessing what antipsychotics were prescribed over the 3 years by chart review, converting daily amount to CPZ and multiplying by the number of days the patient was taking antipsychotics for each medication type. The severity of negative and positive symptoms was assessed at both timepoints using the 0-6 point Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Our interest was specifically directed to negative symptoms. The original PANSS negative symptom subscale contains two items better considered to be part of the cognitive domains: “Stereotyped Thinking” and “Difficulty in Abstract Thinking” (Daniel, 2013; Emsley et al., 2003). We therefore organised the Negative Subscale according to the Five-Factor solution where Blunted Affect, Emotional Withdrawal, Poor Rapport, Passive Withdrawal, Lack of Spontaneity, Motor Retardation and Active Social Avoidance were the included items (Lehoux et al., 2009). Social, occupational and psychological functioning of patients was assessed using a Global Assessment Functioning score (Hall, 1995) at both time points.

2.4 MRI data acquisition

MRI images were acquired for all participants at baseline and follow-up at University Hospital Galway in a 1.5 Tesla Siemens Magnetom Symphony scanner (Erlangen, Germany) equipped with a 4-channel head coil. A magnetisation prepared rapid gradient echo sequence was used to generate high-resolution volumetric T1-weighted images with the following parameters: repetition time=1140 ms, echo time=4.38 ms, inversion time=600 ms, flip angle=15°, matrix size=256 x 256: slice thickness=0.9 mm and in plane resolution=0.9 mm x 0.9 mm.

2.5 MRI processing

Volumetric T1-weighted images were intensity inhomogeneity corrected using non-parametric, non-uniform intensity normalisation (N3) (Sled et al., 1998) as previously described (Ahmed et al., 2015; Scanlon et al., 2014). The longitudinal stream (Reuter et al.,

2012) of Freesurfer v.5.3.0 (“FreeSurfer,” 2013) was employed to parcellate prefrontal cortical regions at two time points. This technique has sufficient sensitivity and reliability for small sample sizes and uses a robust and inverse consistent registration method to create an unbiased within-subject anatomical template, overcoming the risk of underestimating change and avoiding over-regularization or temporal smoothness constraints (Reuter et al., 2012, 2010). The processing pipeline included skull-stripping (Ségonne et al., 2004), Talairach transformation, subcortical gray/white matter segmentation according to the Desikan-Killiany atlas (Desikan et al., 2006; Fischl et al., 2002), intensity normalization (Sled et al., 1998), tessellation of the gray/white matter boundaries, automated topological correction (Fischl et al., 2001; Ségonne et al., 2007) and surface deformation following intensity gradients in the subject template (Dale et al., 1999). At each step, the output was visually inspected, and if necessary corrected according the protocol (“FreeSurfer Quality Control Guide,” 2013). The selection of subregions of the prefrontal cortex (Carlen, 2017) included the following bilaterally: superior frontal gyrus; middle frontal gyrus subdivided into rostral and caudal division; inferior frontal gyrus subdivided into pars opercularis, triangularis and orbitalis; orbito frontal subdivided into lateral and medial division and frontal pole. These regions were all added to create a total prefrontal cortical region of interest (ROI) (Figure 1A). The orbitofrontal ROI was created by adding the lateral and medial orbitofrontal subregions (Figure 1A). Lastly, the thickness, defined as the average distance between the gray-white boundary and the pial surface within each ROI, was extracted at baseline and follow-up for all the regions of interest for all the patients.

2.6 Statistical analysis

2.6.1 Clinical and demographics

All analyses were carried out with the Statistical Package for the Social Sciences version 23 for Windows. Shapiro-Wilk Test was used to test for normal distribution of each cognitive, clinical and neuroimaging variable. Outliers were defined as greater or less than 3 by standard deviation from the mean. Age, years of education, gender, time between scanning and cognitive testing were compared between groups using t-test, chi-square and Mann-Whitney Test. Differences between baseline and follow-up on clinical variables were tested using Wilcoxon Signed-ranks Test and Paired-Sample T-test. Raw scores of the cognitive tests for

both patients and controls were age and gender corrected using normative data previously collected (Kern et al., 2008).

2.6.2 Cognitive impairment at baseline & prefrontal cortical thickness change over time

One-way MANCOVA was used to compare executive functioning and emotional intelligence performance at baseline between patient and control groups, covarying for years of education. Partial correlation, covarying for age, gender and intracranial volume (ICV), was used to assess associations between the tests showing impairment in executive functioning and emotional intelligence in patients at baseline and total prefrontal and orbitofrontal thickness change respectively. Change in neuroanatomical measures was expressed using the following formula: $\frac{Follow-up\ -\ Baseline}{Baseline} \times 100$. In the case of statistically significant correlation with total prefrontal cortex, post-hoc analysis assessed whether cognitive impairment at baseline significantly correlated with specific subregions.

2.6.3 Negative symptoms severity at baseline & prefrontal cortical thickness change over time

Partial correlation was used to assess association between negative symptoms severity at baseline and total prefrontal cortical thickness in patients. Age, gender and ICV were added as confounding variables. Change in neuroanatomical measures was expressed using the following formula: $\frac{Follow-up\ -Baseline}{Baseline} \times 100$.

2.6.4 Cognitive and clinical change & prefrontal cortical thickness change

Partial correlation was used to explore the relationship between cognitive and clinical change (*Follow-up–Baseline)* with prefrontal cortical thickness progression over time ($\frac{Follow-up\ -\ Baseline}{Baseline} \times 100$) in patients. Post-hoc analysis was carried out to clarify which specific prefrontal cortical subregions were involved. Given the exploratory nature of the analyses, all the results were corrected for multiple comparisons, using the Benjamini-Hochberg procedure with α= 0.05, which decreases the false discovery rate (Benjamini and Hochberg, 1995; Chen et al., 2017).

3. Results

Patient and control groups were matched for gender, age, time between scans and predicted IQ, measured using the national adult reading test (Table 1). Years of education was significantly different between patients and controls and was included as a covariate in all analyses. Patients significantly improved over time in positive, general and total score in the PANSS scale (Table 2). Global assessment of functioning strongly improved 3.5 years following the first-episode of psychosis. At the follow-up time point, patients were diagnosed with schizophrenia (n=5), schizoaffective disorder (n=3), delusional disorder (n=1), psychotic disorder not otherwise specified (n=3), bipolar type I (n=6) and psychotic depression (n=2).

Table 1. Demographic characteristics of the participants.

	Patients (*n*=20)	Controls (*n*=18)	Test statistic / *p*-value
Gender N (m/f)	13/7	10/8	X^2 = 0.35 / 0.552
Age at onset (mean years ± SD)	24.9 ± 9.2		
Age Baseline (mean years ± SD)	28.1 ± 8.1	30.3 ± 7.6	*t*= 0.85 / 0.399
Age Follow-Up (mean years ± SD)	32.8 ± 8.0	33.7 ± 7.8	*t*= 0.36 / 0.724
Education (mean years ± SD)	15.7 ± 2.8	18.1 ± 2.9	*t*= 2.60 / **0.014**
Time between Scans (mean years ± SD)	3.6 ± 1.0	3.2 ± 1.2	**U*= 129.50 / 0.141
NART (Predicted IQ) (mean score ± SD)	112.9 ± 8.0	114.9 ± 7.2	*t*= 0.83 / 0.416

Note: *= variable non-normal distribuited; NART= National Adult Reading Test.

Table 2. Clinical features at baseline and follow-up of patient sample (*n*=20)

	Baseline	**Follow-up**	**Test statistic / *p*-value**
	Mean ± SD	Mean ± SD	
Duration of untreated psychosis (DUP)(months)	12.9 ± 15.2		
Positive and negative Syndrome scale			
PANSS positive score	17.2 ± 4.1	10.4 ± 3.7	*z= -3.41 / **0.001**
PANSS negative score	14.1 ± 4.8	12.0 ± 6.8	*z= -1.57 / 0.115
Negative factor according to Five Factor solution	6.7 ± 4.7	5.75 ± 6.6	*z= -0.78 / 0.438
PANSS general score	31.2 ± 4.3	23.3 ± 6.3	*z= -3.23 / **0.001**
PANSS total score	62.5 ± 8.1	45.6 ± 14.7	*z= -3.46 / **0.001**
Functionality			
Global assessment of functioning	52.0 ± 10.8	72.0 ± 15.5	*z= -3.83/ >**0.001**
Neuropsychological measures			
Category Fluency	48.9 ± 11.7	55.9 ± 9.9	*z=-0.15/0.879
CPT: Identical Pairs	42.0 ± 11.2	50.9 ± 8.3	t= -2.27/ **0.035**
WMS: Spatial Span	41.4 ± 10.0	49.9 ± 9.1	t=-1.12/0.275
Letter Number Span	42.2 ± 8.8	47.5 ± 4.8	t= 1.92/0.071
NAB: Mazes	39.3 ± 7.4	43.1 ± 9.4	t= -2.22/**0.039**
MSCEIT: Managing Emotions	45.5 ± 13.0	55.5 ± 9.7	t= -1.36/0.190
Medication (N)			
Antipsychotics	19	13	
Mood stabilisers	0	2	
Anti-depressants	6	4	
No medication	1	9	
Chlorpromazine equivalent daily dose	204.0 ± 226.3	175.0 ± 276.8	*z= -0.92 / 0.355
Chlorpromazine equivalent total amount of cumulative dose		266642.40 ± 63246.43	

Note: *= variable non-normal distribuited; Medication at baseline= 6 patients were taking antidepressant + antipsychotic medications; 9 patients were taking more than one antipsychotic medication. Medication at follow-up= 4 patients were taking more than one antipsychotic medication; 2 patients were taking antidepressant + antipsychotic medications. Chlorpromazine equivalent= antipsychotic medication was converted to chlorpromazine (CPZ) equivalents (Lehamn and Steinwaschs,1998; Woods, 2003).

3.1 Groups differences at baseline in cognition

There was a significant difference between patients and controls at baseline when considering jointly the six cognitive measures Wilk's Λ $F(6,30)= 4.823$, $p= <.001$. For executive functioning patients scored significantly worse than controls in Category Fluency: Animal fluency, CPT: Identical Pairs, WMS: Spatial Span and NAB: Mazes and not WMS: Letter Number Span. Patients' performance in MSCEIT: Managing Emotions was significantly worse compared with controls (Figure 1B, table 3).

3.2 Executive impairment & prefrontal cortical thickness

In the patient group, change in total prefrontal cortical thickness, specifically loss of thickness over time, was significantly associated with impaired working memory: spatial span at baseline (r=0.517; p=0.040, Figure 1C). Post-hoc analysis conducted to determine the prefrontal subregions involved in the patient group revealed a significant involvement of rostral middle frontal cortex (r=0.546; p=0.029) and the frontal pole (r=0.507; p=0.045). However, this association lost significance after correcting for multiple comparisons [rostral middle frontal & frontal pole (p=0.245; p=0.245)]. No significant correlation was found between total prefrontal cortical thickness change and the remaining impaired executive functioning tests (*r-range*=-0.064 – 0.458; *p-range*=0.075 – 0.814). In the control group, none of the executive function measures at baseline (working memory, attention, fluency and reasoning & problem solving) were significantly correlated with total prefrontal thickness change over time (*r-range*=-0.362–0.325; *p-range*=0.185–0.759). Additionally, the relationship between spatial working memory and total prefrontal cortical thickness change was significantly different (z=2.28; p=0.02) in patients compared to controls. Exploratory analyses investigating the relationship between change in cognitive performance and change in total prefrontal thickness did not reveal any significant associations (r=-0.273–0.327; p= 0.216–0.959).

Table 3. Difference between first-episode of psychosis group and healthy control group on cognition

		BASELINE				FOLLOW-UP				GROUP * TIME	
GLM				*F* (6,30)	*p*			*F* (6,30)	*p*	*F* (5,18)	*p*
				4.82	**0.001**			4.47	**0.002**	1.60	0.160
		FEP	**HC**			**FEP**	**HC**				
	TEST SCORES USED	Mean ± SD	Mean ± SD	*F* (1,34)	*p*	Mean ± SD	Mean ± SD	*F* (1,35)	*p*		
Category fluency	Total number of animals named	48.9 ± 11.7	48.9 ± 9.2	4.50	**0.041**	55.9 ± 9.9	59.9 ± 12.5	12.87	**0.001**		
CPT: identical pairs	Mean d' value across 4 conditions	42.0 ± 11.2	46.5 ± 12.1	6.03	**0.019**	50.9 ± 8.3	51.7 ± 4.7	3.40	0.089		
WMS: spatial span	Sum of raw scores	41.4 ± 10.0	44.4 ± 13.2	10.16	**0.003**	49.9 ± 9.1	54.7 ± 9.2	9.98	0.222		
Letter number span	Number of correct trials	42.2 ± 8.8	46.7 ± 12.2	3.45	0.072	47.5 ± 4.8	52.7 ± 6.9	4.53	0.115		
NAB: mazes	Total raw score	39.3 ± 7.4	43.1 ± 9.4	20.21	**<0.001**	51.5 ± 8.7	53.3 ± 10.0	8.83	0.201		
MSCEIT: managing emotions	Branch score using general consensus scoring	45.5 ± 13.0	48.1 ± 10.3	7.32	**0.010**	55.5 ± 9.7	52.8 ± 9.7	1.61	**0.044**		

Note: the table shows the difference between FEP group and HC group on tests assessing executive functioning and emotional intelligence at baseline (F(6,30)=4.82,p=0.001), at follow-up (F(6,30)=4.47;p=0.002) and over time (F(5,18)=1.60;p=0.160). Legend: FEP= first-episode of psychosis patients; HC= healthy controls; GLM= generalized linear model. CPT= Continuous performance Test; WMS= Wechsler Memory Scale; NAB= Neuropsychological Assessment Battery; MSCEIT= Mayer-Salovey-Caruso Emotional Intelligence Test; Test scores used = description of test scores used reported in Nuechterlein et al. (2008); d' value: ability of the participant to discriminate between signal and noise.

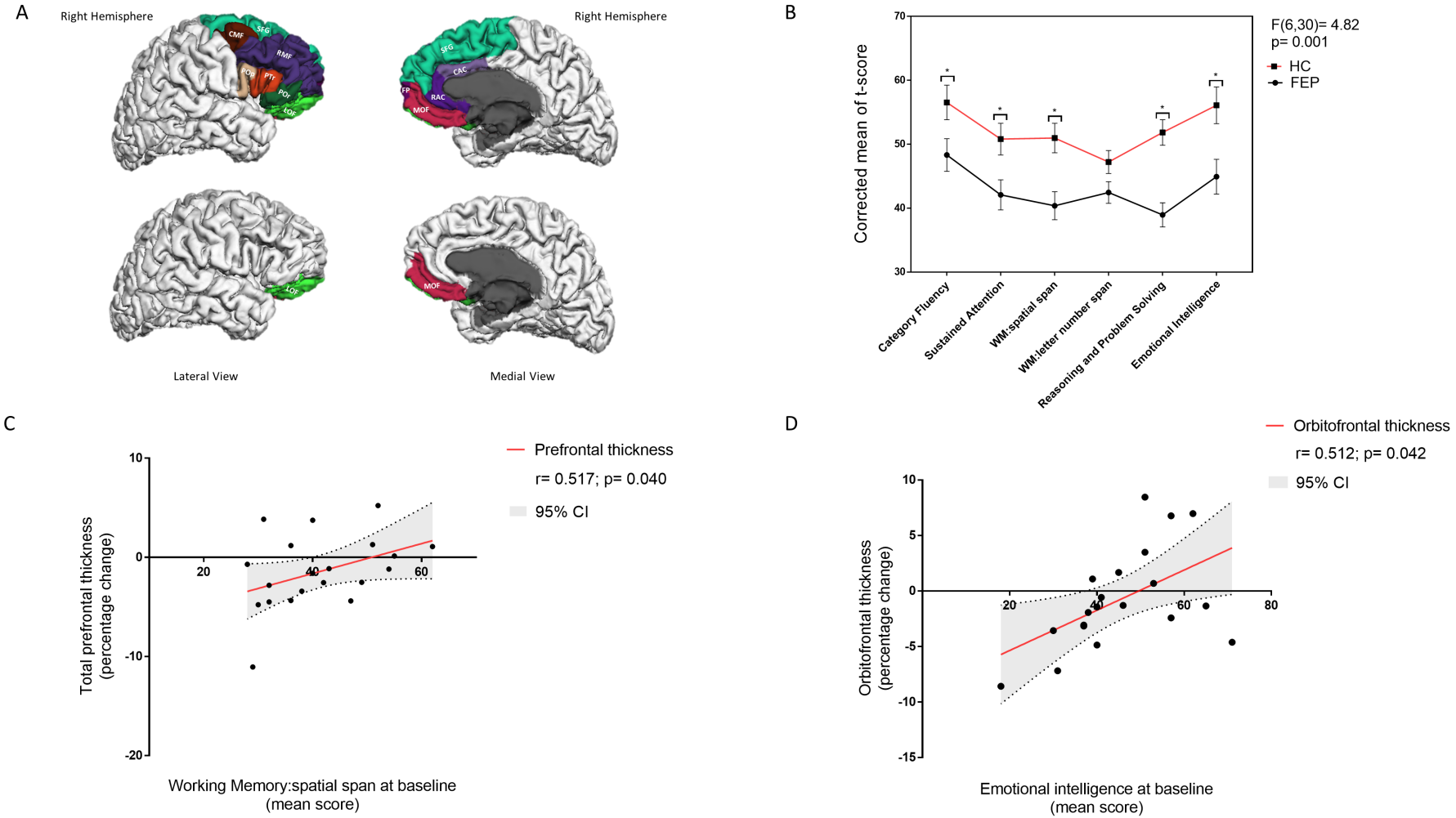

Figure 1. (A) Sub division of prefrontal cortex based on the Desikan-Killiany atlas. Schematic illustration of two regions of interest (the subregions were added bilaterally): above total prefrontal cortex, below orbitofrontal cortex. SFG = superior frontal gyrus; CMF= caudal middle frontal; RMF= rostral middle frontal; LOF= lateral orbitofrontal; POr= pars orbitalis; PTr= pars traingularis; POp= pars opercularis; CAC= caudal anterior cingulate; RAC= rostral anterior cingulate; FP= frontal pole; MOF= medial orbitofrontal. (B) Graphic representation of differences between groups on cognition at baseline. Legend: FEP= first-episode of psychosis patients; HC= healthy controls; WMS= Wechsler Memory Scale; NAB= Neuropsychological Assessment Battery; MSCEIT= Mayer-Salovey-Caruso Emotional Intelligence Test. Note: years of education included in the model as covariate; *=significant group difference. (C) Partial correlation between working memory: spatial span at baseline in patients and percentage of total prefrontal thickness change. (D) Partial correlation between emotional intelligence at baseline in patients and percentage of orbitofrontal thickness change. Note: years of education, age, gender and ICV included as covariates in all the correlations.

3.3 Emotional intelligence impairment & orbitofrontal thickness

When investigating the relationship between emotional intelligence at baseline and orbitofrontal thickness change, we found that impaired emotional intelligence in the patient group was a significant predictor of loss of orbitofrontal thickness 3.5 years following the first-episode of psychosis (r=0.512; p=0.042, Figure 1D). Although this significant relationship was not present in the control group (r=0.178; p=0.542), the difference between the relationships in patients and controls was not statistically significant (z=1.09; p=0.138).

3.4 Negative symptoms & prefrontal cortical thickness

The severity of negative symptoms at illness onset was not significantly related to total prefrontal thickness change over time (r= -0.095; p=0.717). However, our exploratory analysis after correcting for multiple comparison revealed that change in negative symptoms was strongly correlated with reduction of total prefrontal cortical thickness over time (r=-0.627; p=0.007, Figure 2A). This association remained significant after controlling for both positive symptoms change and total medication intake (r=-0.553; p=0.032). Specifically, we found the involvement of medial orbitofrontal (r=-0.721; p=0.01, Figure 2B), caudal (r=-0.659; p=0.01) and rostral anterior cingulate (r=-0.604; p=0.02), and rostral middle frontal cortex (r=-0.695; p=0.01); with the exception of pars triangularis (r=-0.519; p= 0.07) which did not survive multiple comparison correction. When using the standard negative subscale of the PANSS, our results were very similar: correlation between change in negative symptoms and change in total prefrontal cortical thickness was significant (r=-0.644; p=0.005), with the involvement of medial orbitofrontal (r=-0.721; p=0.002), caudal (r=-0.659; p=0.002) and rostral anterior cingulate (r=-0.604; p=0.002), and rostral middle frontal (r=-0.695; p=0.035). These correlations were present even though the difference between negative symptoms at baseline and follow-up was not statistically significant (Table 2).

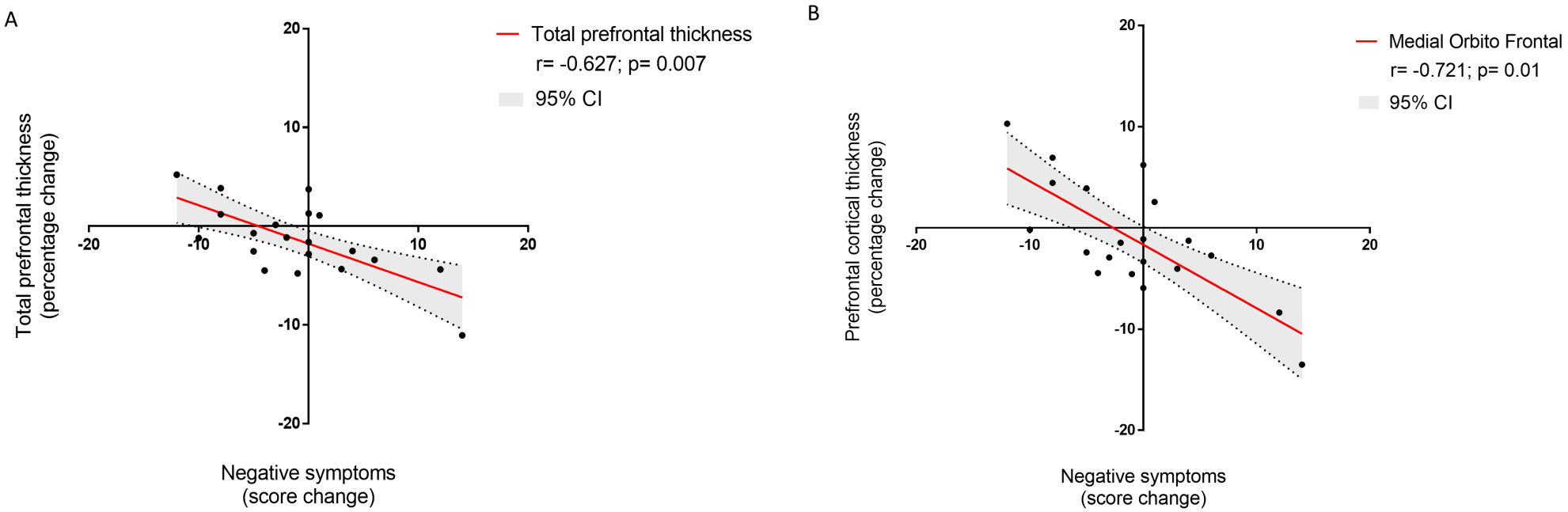

Figure 2. (A) Partial correlation between negative symptoms change and percentage of total prefrontal thickness change. (B) Partial correlation between negative symptoms change and percentage of thickness change in medial orbito frontal region, the strongest correlation among all the prefrontal subregions. Note: age, gender and ICV included as covariates in all correlations.

4. Discussion

In this study, consistent with the extant literature we found that patients in their first-episode of psychotic illness perform significantly worse on several tests assessing different aspects of executive functions compared to healthy controls, including category fluency, attention, working memory and reasoning & problem solving (Holmén et al., 2012; Leeson et al., 2010; Perez-Iglesias et al., 2010; Zabala et al., 2010). Of these executive functioning impairments and consistent with our first hypothesis, we found that poorer performance at baseline in spatial working memory was a significant predictor of loss of total prefrontal cortical thickness in the initial years after illness onset. Furthermore, this relationship between cognitive function and brain change was not found in the control group, consistent with a disorder-related pathological process marked by working memory dysfunction and underpinning cortical thinning over time. Working memory has been considered a central component of executive functioning since they share a large proportion of common variance (McCabe et al., 2010; Zillmer & Spiers, 2001) and the nature of executive functions in controlling and monitoring information is intertwined with the function expressed by working memory, understood as dynamic manipulation of contents (Miyake et al., 2000). Our finding is consistent with that of Gutierrez-Galve et al'(2015), which reported that poor working memory present at the time of the first assessment in first-episode of psychosis patients was associated with frontal cortical thinning after 2 years (Gutiérrez-Galve et al., 2015). In an 18 years longitudinal study on first-episode of schizophrenia patients, working memory was also found to be associated with frontal grey and white matter loss (Andreasen et al., 2011). In a healthy control study, low working memory performers showed significantly less surface area in the inferior, superior frontal gyrus and medial orbitofrontal gyrus compared to high working memory performers (Nissim et al., 2017).

Although post-hoc results did not survive multiple comparisons and require replication, we detected relationships between impaired working memory at baseline and thinning of the rostral middle frontal gyrus and frontal pole over time. The contribution of the dorsolateral prefrontal cortex to optimal functioning of spatial working memory has been extensively reported in both human and non-human primates (Goldman-rakic, 1996). The involvement of mid-dorsolateral frontal cortex has been demonstrated when the working memory task required active monitoring and manipulation of spatial information (Owen et al., 1996). In

patients with schizophrenia, greater dysfunction in the physiological activation of the dorsolateral prefrontal cortex has been linked to poorer working memory performance (Perlstein et al., 2001). Bertolino and colleagues reported that in schizophrenia the functional integrity of neurons within the dorsolateral prefrontal cortex has also predictable physiological impacts throughout the entire working memory cortical network (Bertolino et al., 2000). Our study additionally identified a significant relationship between impaired working memory at baseline and frontal pole thinning. The activation of the lateral frontopolar area during working memory tasks has been also reported in meta-analysis based on healthy controls (Bludau et al., 2014).

We also found impairment of emotional intelligence at baseline in individuals experiencing first-episode of psychosis compared to controls, as demonstrated by other studies focusing on schizophrenia (Dawson et al., 2012; Frajo-apor et al., 2017). Impaired emotional intelligence was significantly associated with a reduction of orbitofrontal thickness over time in patients after their first-episode of psychosis, supporting our second hypothesis. Orbitofrontal cortex is an area crucial for the generation of emotions that guide interpersonal behaviour (Beer et al., 2006) and critical for emotional processes, given its connection to the limbic system (Krueger et al., 2009; Nestor et al., 2013). Nestor and colleagues reported that subregions of orbitofrontal cortex were involved in performance on behavioural measures of various aspects of social cognition (Nestor et al., 2013). In schizophrenia middle prefrontal abnormality has been linked to emotional attribution deficit (Yamada et al., 2007).

The neurobiological mechanism that underlies the progressive loss of prefrontal thickness is still unknown, although some evidence suggests that neuropil pruning could be the cause of this progressive reduction of grey matter in schizophrenia (Selemon and Goldman-rakic, 1999). Reduced N-acetyl aspartate (NAA), which is an amino acid involved in the synthesis pathway of glutamate and used as a marker of neural viability, is reduced in prefrontal regions in schizophrenia (Abbott and Bustillo, 2006) and in the left frontal lobe of patients at risk of developing schizophrenia (Jessen et al., 2006). NAA reduction might be due to reduced neuropil, as indicated by post-mortem studies (Selemon and Goldman-rakic, 1999). Although the pathogenetic mechanisms underlying neuropil reduction requires further clarification, we speculate that cognitive deficits, such as spatial working memory and emotional intelligence impairments at presentation of psychotic illness, could represent biomarkers that signal a

neuroprogressive process culminating in loss of cortical thickness as the illness progresses. Spatial working memory impairment has been also presented as an effective endophenotypic marker for schizophrenia (Glahn et al., 2003) and significantly associated with a major candidate gene: Disrupted in Schizophrenia-1 (DISC-1) (Carless et al., 2011). The variation in DISC1 sequence seems to affect both neuroanatomy and cognition; Vázquez-bourgon et al.'s study showed the potential role of this gene in modulating longitudinal cortical thinning in patients suffering from a first-episode of non-affective psychosis, especially prominent in the frontal cortex (Vázquez-bourgon et al., 2016).

Whilst on the one hand, our findings show that cognitive deficits at the onset of psychotic illness are associated with progressive prefrontal cortical thickness reduction, our exploratory analysis failed to find any association between change in cognitive performance and change in total prefrontal thickness, as reported elsewhere (Gutiérrez-Galve et al., 2015). The executive functioning and emotional intelligence impairment remain stable in patients, without showing a significant worsening over time compared to controls (Table 2). These findings suggest that cognitive impairment at onset of psychosis represents a trait marker and that the progressive neuroanatomical thinning over time in the prefrontal cortex does not mediate cognitive deterioration.

Our study failed to find any significant association between severity of negative symptoms at illness onset and total prefrontal thickness change, thus rejecting our third hypothesis. In contrast, our exploratory analysis revealed that the clinical observation of worsening negative symptoms is indeed associated with total prefrontal thickness reduction over time. When exploring which prefrontal subregions were involved, we found thickness reduction in caudal and rostral anterior cingulate, medial orbitofrontal and rostral middle frontal cortex. A 4-year longitudinal study based on 24 patients with chronic schizophrenia and 25 controls found that greater negative symptoms severity was associated with faster rates of frontal and temporal brain volume changes, indicators of faster deterioration (Mathalon et al., 2001). In a voxel-based morphometry 1.5-year longitudinal study on first-episode schizophrenia, Asami et colleagues reported that less improvement in negative symptoms, assessed with Brief Psychiatric Rating Scale, was correlated with more longitudinal loss, in inferior and superior frontal gyrus (Asami et al., 2012). Negative symptom severity in a large ENIGMA study was found to be significant related to left lateral orbitofrontal cortical thickness (Walton et al.,

2018). Other longitudinal studies failed to find any association over time (Cobia et al., 2012; Gutiérrez-Galve et al., 2015). The observation from the current study that prefrontal neuroanatomical progression more closely aligned with progression of negative symptoms than of cognitive impairment suggests a progressive pathophysiological process plays an important role in the worsening of clinical symptoms.

Strengths and limitations

The main strength of this study is the longitudinal nature of the sample, which can capture the progression after the first-episode of psychosis of anatomical, cognitive and clinical variables and their intrinsic relationships. The careful parcellation of prefrontal cortex using the longitudinal stream of Freesurfer based on an unbiased within-subject anatomical template (Reuter et al., 2012) allowed us to increase the anatomical sensitivity and hence better detect anatomical changes over time.

The main weakness of the study is the relatively small sample size, which might have reduced the power to detect more subtle differences in cognitive, neuroanatomical and clinical variables. Furthermore, due to the available cognitive battery, we could not assess two important facets of executive functions, inhibition and switching. In addition, to reduce multiple analysis we assessed the prefrontal subregions summed bilaterally and did not explore any lateralised effects or other parts of the brain. We employed a measure of negative symptoms which excluded cognitive symptoms however alternative measurements of core negative symptoms incorporating a scale such as SANS (Andreasen, 1989) may have produced different results (Kirkpatrick et al., 2006).

Conclusion

This longitudinal study tracking the interplay between neuroanatomy, cognition and clinical presentation indicates that working memory and emotional intelligence impairment at the onset of psychotic illness are a trait marker of progressive prefrontal thinning, and that worsening of negative symptoms is associated with prefrontal thickness reduction as the illness progresses. These results suggest that there is already a cognitive signature at the onset of psychosis, which is associated with poorer outcome in terms of other neuroanatomical and clinical measures. Further longitudinal studies with larger sample size, multimodal assessments and repeated sampling will help to confirm and develop these findings.

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Progressive Subcortical Volume Loss in Treatment-Resistant Schizophrenia Patients After Commencing Clozapine Treatment

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Abstract

The association of antipsychotic medication with abnormal brain morphometry in schizophrenia remains uncertain. This study investigated subcortical morphometric changes 6 months after switching treatment to clozapine in patients with treatment-resistant schizophrenia compared with healthy volunteers, and the relationships between longitudinal volume changes and clinical variables.

1.5T MRI images were acquired at baseline before commencing clozapine and again after 6 months of treatment for 33 patients with treatment resistant schizophrenia and 31 controls, and processed using the longitudinal pipeline of Freesurfer v.5.3.0. Two-way repeated MANCOVA was used to assess group differences in subcortical volumes over time and partial correlations to determine association with clinical variables.

Whereas no significant subcortical volume differences were found between patients and controls at baseline($F(8,52)=1.79$; $p= 0.101$), there was a significant interaction between time, group and structure($F(7,143)=52.54$, $p<0.001$). Corrected *post-hoc* analyses demonstrated that patients had significant enlargement of lateral ventricles ($F(1,59)=48.89$; $p<0.001$) and reduction of thalamus ($F(1,59)=34.85$; $p<0.001$), caudate ($F(1,59)=59.35$; $p<0.001$), putamen ($F(1,59)=87.20$; $p<0.001$) and hippocampus ($F(1,59)=14.49$; $p<0.001$) volumes. Thalamus and putamen volume reduction was associated with improvement in Total PANSS ($r=0.42$; $p=0.021$, $r=0.39$; $p=0.033$), SANS ($r=0.36$; $p=0.049$, $r=0.40$; $p=0.027$) and GAF ($r=-0.39$; $p=0.038$, $r=-0.42$; $p=0.024$) scores. Reduced thalamic volume over time was associated with increased serum clozapine level at follow-up ($r=-0.44$; $p=0.010$).

Patients with treatment-resistant schizophrenia display progressive subcortical volume deficits after switching to clozapine despite experiencing symptomatic improvement. Thalamo-striatal progressive volumetric deficit associated with symptomatic improvement after clozapine exposure may reflect an adaptive response related to improved outcome rather than a harmful process.

1. Introduction

Approximately 30% of patients with schizophrenia meet criteria to be considered treatment-resistant (Lieberman et al., 1994; Meltzer, 1997), usually defined as the failure to respond to at least two adequate trials of antipsychotic medication (Suzuki et al., 2012). Clozapine has an established superior clinical effect to control symptoms in treatment-resistant patients, with 60-70% having a positive response(Chakos et al., 2001; Stroup et al., 2003). Patients treated with clozapine also often experience troublesome side effects including significant weight gain and lipid abnormalities (Henderson et al., 2000), which notably have been associated with improvement in symptomatology (Lally et al., 2013; Meltzer et al., 2003). Cross-sectional MRI studies of patients with treatment resistant schizophrenia (TRS) receiving clozapine and other antipsychotic medications have reported a range of brain abnormalities compared with controls, including reduced global grey matter (Anderson et al., 2015; Molina et al., 2008), predominantly in frontal and temporal regions (Kubera et al., 2014; Quarantelli et al., 2014; Zugman et al., 2013), and volumetric reduction of the amygdala and hippocampus (Quarantelli et al., 2014; Zugman et al., 2013).

The association of antipsychotic medication use with progressive brain deficits has been explored in longitudinal studies of schizophrenia (Ho et al., 2011; Vita et al., 2015). These studies mostly use an observational rather than randomised design approach and thus cannot fully account for illness or service-related factors which influence clinician and patient medication choice. In a meta-analysis of longitudinal MRI studies based on 1155 patients with schizophrenia and 911 healthy controls, Vita and colleagues (Vita et al., 2015) reported reduced cortical grey matter volume over time in patients which was related to cumulative exposure and mean daily dose of antipsychotic medications. Patients treated with typical antipsychotic medications compared to atypical antipsychotics displayed more progressive grey matter loss, which correlated with higher mean daily antipsychotic dose. Likewise, van Haren and colleagues' (van Haren et al., 2011) 5-year longitudinal study reported an association between higher cumulative dose of typical antipsychotics over time and more marked cortical thinning, while higher dose of atypical antipsychotics in contrast was associated with less cortical thinning. However, patients who received clozapine treatment during the interscan interval showed more pronounced superior temporal cortical thinning compared with those not treated with clozapine. In contrast, in another analysis of this

cohort, higher cumulative dose of clozapine during the interscan interval was related to attenuated loss of grey matter in the left superior frontal gyrus (van Haren et al., 2007).

Longitudinal subcortical neuroimaging studies specifically of treatment-resistant clozapine-naïve patients are sparse, with small numbers of participants or without a matched control group. An early study of subcortical structures by Chakos and colleagues (Chakos et al., 1995) based on 15 patients, and without a control group, reported a 10% decrease in caudate volume after 55 weeks, when switched from treatment with typical antipsychotic medications to clozapine. In contrast, patients who stayed on typical antipsychotic medications displayed an 8% enlargement in the caudate. In another study of 26 patients by Scheepers and colleagues (Scheepers et al., 2001b) volume reduction of caudate nucleus was identified after 24 weeks of treatment with clozapine. There was no neuroanatomical correlation with clinical response. In the same cohort, after 52 weeks of treatment, reduced volume of the left caudate was greater in patients who responded to treatment compared to non-responders (Scheepers et al., 2001a). Another small study with 8 patients and 8 controls reported reduced caudate volume after 2 years of treatment with clozapine, with analogous results for the putamen, which was not statistically significant (Frazier et al., 1996). Thus, these early studies consistently indicate that switching patients from typical antipsychotics to clozapine is associated with a decrease of caudate volume over time, and has generally been interpreted as a correction by clozapine of caudate hypertrophy induced by typical antipsychotic medications due to their potent dopamine blockade and the high concentration of dopamine receptors in the caudate (Seeman et al., 2006). However, nowadays most patients are already taking atypical antipsychotic medications prior to clozapine commencement and it remains unclear whether switching to clozapine in such circumstances would have a similar effect on the basal ganglia. Furthermore, other subcortical structures such as the hippocampus and thalamus have not been investigated in longitudinal studies of switching to clozapine.

Given the importance of identifying factors predicting response to clozapine, the association of clinical response with baseline alterations in subcortical structures has also been studied, with conflicting results. In a randomised controlled trial by Arango and colleagues (Arango et al., 2003), whereas larger right prefrontal cortex predicted improvement in SANS scores compared with haloperidol treated patients, there was no such association between clinical symptom change and caudate or hippocampal volume at

baseline. Smaller hippocampal volume compared to healthy controls at baseline predicted improvement in disorganised symptoms over time in a longitudinal study by Molina and colleagues (Molina et al., 2003). In another longitudinal study, decreased left caudate volume over time was related to a significant improvements in positive and general symptoms, but not negative symptoms (Scheepers et al., 2001a).

We have previously investigated cortical anatomy in a sample of patients before and after switching to clozapine in comparison to healthy volunteers (Ahmed et al., 2015), and demonstrated on-going cortical thinning in TRS patients over a 6 month period, in particular for younger patients. The present study, using a unique sample of treatment-resistant clozapine-naïve schizophrenia patients, offers a novel opportunity to comprehensively investigate whether subcortical structures demonstrate progressive neuroanatomical changes after 6 months of clozapine treatment and whether any such changes are related to clinical variables including treatment response and amount of clozapine taken.

2. Method

2.1 Participants

As previously reported (Ahmed et al., 2015) 39 patients with treatment-resistant schizophrenia (TRS) prior to clozapine initiation and 40 healthy volunteers (HC) were initially recruited for the baseline assessment. At the follow-up, 33 patients, after 6 months of treatment with clozapine and a total of 31 healthy controls, matched for sex and age, were successfully re-recruited, scanned and assessed (Table 1). Patients were included if aged 18-60 years and clinically due to switch to clozapine because of treatment resistance. Patients and controls were excluded from the study if they had a previous trial of clozapine treatment, a learning disability, history of neurological illness, history of head injury which resulted in loss of consciousness for over 5 minutes, treatment with oral steroid in the three months prior to participation, history of comorbid alcohol/ substance dependency as defined by the DSM-IV criteria or any contraindication to MRI scanning. Exclusion criteria for controls also included a current or past axis I mental disorder or any psychotic disorder in a first-degree relative. The study was approved by the Clinical Research Ethics Committee, Galway University Hospitals. Fully informed written consent was obtained for all participants.

2.2 Clinical assessment

All patients were diagnosed using the Diagnostic and Statistical Manual for Mental Disorders 4th Edition text revision (DSM-IV-TR) (American Psychiatric Association, 2000). Treatment resistance was defined as the failure to respond to at least two adequate trials of antipsychotic medications, including at least one atypical antipsychotic drug, with a prolonged period of moderate to severe positive and/or negative symptoms (National Institute of Health and Clinical Excellence, 2014). The severity of positive and negative symptoms was assessed at both time points using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1982a) and the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982b). Social, occupational and psychological functioning was assessed using a Global Assessment of Functioning Score(Hall, 1995). We used the symptomatic remission criteria of Andreasen(Andreasen et al., 2005) with the exclusion of the maintenance over 6-month observation period(Egerton et al., 2018). Remission at the 6 month follow-up assessment was therefore defined as having

scores of mild or less (item scores of ≤2 using the 0-6 range) on all eight of the following PANSS items: delusions (P1), conceptual disorganisation (P2), hallucinatory behaviour (P3), blunted affect (N1), social withdrawal (N4), lack of spontaneity (N6), mannerisms / posturing (G5), unusual thought content (G9).

2.3 MRI data acquisition

MRI images were acquired for all participants at baseline and after 6 months at University Hospital Galway in a 1.5 Tesla Siemens Magnetom Symphony scanner (Erlangen, Germany) equipped with a 4-channel head coil. A magnetisation prepared rapid gradient echo (MPRAGE) sequence was acquired to generate high resolution volumetric T1-weighted images, with the following parameters: repetition time (TR): 1140 ms, echo time (TE): 4.38 ms, inversion time (TI): 600 ms, flip angle: 15°, matrix size: 256x256, interpolated to 512 x 512, slice thickness: 0.9 mm and in-pixel resolution: 0.45 mm^2

2.4 MRI processing

Volumetric T1-weighted images used in the analyses were intensity inhomogeneity corrected using non-parametric, non-uniform intensity normalisation (N3) (Sled et al., 1998) as previously reported(Ahmed et al., 2015; Scanlon et al., 2014). Eight subcortical regions-of-interest: lateral ventricle, thalamus, hippocampus, caudate, putamen, globus pallidus, amygdala and nucleus accumbens, were bilaterally segmented using the longitudinal pipeline of Freesurfer v.5.3.0 ("FreeSurfer," 2013; Reuter et al., 2012). Specifically, this technique is based on an unbiased within-subject anatomical template (Reuter et al., 2012), created using a robust and inverse consistent registration method (Reuter et al., 2010), is able to overcome the limitations of longitudinal processing methods. It reduces the risk of underestimating change, giving an unbiased estimation of the neuroanatomical structure volume over time, removing asymmetry-induced processing bias and avoiding over-regularization or temporal smoothness constraints (Reuter et al., 2012). This technique has also sufficient sensitivity and reliability for small sample sizes (Reuter et al., 2012). The several steps of the processing pipeline to obtain the output have previously been described in detail(Tronchin et al., 2020). Intracranial volume (ICV), is computed by dividing a predetermined constant with the factor by which the input magnetic resonance (MR) images are scaled in size to align to the MNI305 head atlas("eTIV - Estimated Total Intracranial Volume"; "talairach_avi,"; Evans AC, Collins DL,

Mills SR, Brown ED, Kelly RL, 193AD). At each time point, quality check of the segmentation output was performed, which involves a visual inspection at each of the analysis stages, to verify that the segmentation was anatomically accurate and computationally successful ("FreeSurfer Quality Control Guide," 2013). Six images failed the quality check and required manual editing using control points to fix intensity normalization ("ControlPoints - freeview"). Following quality check and manual editing, no images were excluded. Subsequently subcortical volumes were bilaterally extracted and summed together to obtain one measure for each ROI.

2.5 Statistical analysis

Statistical Package for the Social Sciences version (SPSS Inc., v23, IBM, New York, USA) was used to carry out all analyses. The Shapiro-Wilks Test was used to test for normal distribution of demographics, clinical, neuroanatomical and anthropomorphic variables, with outliers defined as greater or less than 3 standard deviations from the mean. Age, gender and time between scanning were compared between groups using either a T-test, Chi-square or Mann-Whitney U Test. Differences between baseline and follow-up on clinical variables and anthropomorphic measurements were tested using the Wilcoxon Signed Ranks and Paired-Sample T-test. An initial one-way Multivariate analysis of covariance (MANCOVA) was performed to evaluate differences between groups at baseline on the eight subcortical structures, covarying for age, sex and ICV. *Post-hoc* analyses were performed to assess differences at baseline on the 8 subcortical structures between controls and patients previously treated with atypical and/or typical medications. Thereafter two-way repeated MANCOVA was used to assess the course of changes in volume of subcortical structures over time between groups, covarying for age, sex and ICV. The group-by-age interaction was used to determine the effect of age on anatomical change between groups over time. *Post-hoc* analysis, corrected for multiple comparison (Bonferroni, $\alpha = 0.006$) was carried out to clarify which regions were significantly changing over time. An additional one-way MANCOVA and subsequently a two-way repeated MANCOVA was performed to assess differences between clozapine responders and non-responders at baseline and over time on subcortical structures, covarying for age, sex and ICV. Partial correlations were carried out controlling for the potential influence of age, sex and ICV on the relationship between the subcortical brain regions which showed a significant change over time ($\frac{Follow-up - Baseline}{Baseline} \times 100$) and

change in PANSS, SANS, SAPS and GAF (*Follow-up–Baseline*)(Molina et al., 2008). These correlations were hypothesis driven and not corrected for multiple comparison. Pearson correlation analyses were performed to explore the relationship between subcortical structures showing a significant change over time in TRS patients and the variables age, duration of illness, body mass index (BMI), daily dose and serum level of clozapine at follow-up.

3. Results

3.1 Clinical characteristics

Patient and control groups did not differ across age, sex, and time between scans (Table 1). Patients after treatment with clozapine displayed a substantial and statistically significant improvement in each symptom and function rating scale. At follow-up, patients also displayed a significant increase of weight, waist, body mass index, total cholesterol and triglycerides compared to baseline (Table 2). Twelve patients had previously been prescribed typical antipsychotic drugs and 5 were still taking typical antipsychotic medications at the point of the baseline scan. At baseline before switching to clozapine, 21 patients were on monotherapy with one atypical antipsychotic medication (olanzapine=7, quetiapine=4, aripiprazole=4, amisulpiride=1, paliperidone=1, risperidone long acting injection=1), 10 patients were treated with two antipsychotic medications (olanzapine + another antipsychotic=7), with one patient treated with three and another patient treated with four antipsychotic medications. At follow-up 16 patients (48%) were in remission.

Table 1. Characteristics of patients with treatment resistant-schizophrenia and controls.

	Patient group (n=33)	Control group (n=31)	Test statistic/p-value
Sex (m/f)	23/10	20/11	X^2= 0.19; 0.660
Age at onset (years)	22.8 ± 0.8		
Age at baseline (years)	36.4 ± 10.7	39.3 ± 10.6	t= 1.10; 0.274
Age range	(22-61)	(23-59)	
Time between baseline and follow-up MRI scans (months)	6.6 ± 1.7	7.4 ±3.2	t= 1.21; 0.230
Illness duration before commencing clozapine (years)	13.6 ± 8.8		
Intracranial volume (mm^3)	1610322.58 ± 29886.83	1591515.15 ± 27500.42	t= 0.46; 0.644

Table 2. Clinical features of patient group at baseline and follow-up (n=33)

	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	Test statistic/ p-value
Clinical scales			
PANSS positive score	14.1 ± 5.7	6.1 ± 5.0	*z= -4.98; < 0.001
PANSS negative score	16.2 ± 7.0	9.1 ± 7.1	*z= -4.51; < 0.001
PANSS general score	24.1 ± 8.9	11.7 ± 8.3	*z= -4.90; < 0.001
PANSS Total Score	54.3 ± 17.8	26.9 ± 17.6	t= 10.04; < 0.001
SANS	42.5 ± 20.7	27.8 ± 22.9	*z=-3.78; < 0.001
SAPS	28.0 ± 16.3	13.2 ± 11.0	*z= -4.45; < 0.001
Global assessment of functioning	46.8 ± 10.8	64.9 ± 14.1	t= 13.12; < 0.001
Medications			
Typical antipsychotics (n)	5	0	
Atypical antipsychotics (n)	33	2	
Clozapine (n)	0	33	
Serum level of clozapine at follow-up (ng/ml)		0.5 ± 0.1	
Daily dose of clozapine at follow-up (mg)		349.2 ± 17.8	
Daily dose of clozapine range (mg)		(200-625)	
Anthropomorphic measurements			
Weight (kg)	85.9 ± 15.4	90.1 ± 16.6	t=-3.31; 0.002
Waist circumference (cm)	97.8 ± 12.1	103.1 ± 13.4	t=-4.94; < 0.001
Body Mass Index	28.0 ± 4.9	29.3 ± 5.0	*z= -2.78; 0.005
Total Cholesterol (mmol/L)	4.8 ± 1.1	5.5 ± 0.8	t=-3.38; 0.003
Triglycerides (mmol/L)	1.8 ± 1.0	2.5 ± 1.4	*z= -2.62; 0.009

Note: *= variable non-normal distributed; PANSS: Positive and negative Syndrome scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms. PANSS 0-6 scale was used. Twelve patients were prescribed typical antipsychotic drugs at some stage of their illness

3.2 *Differences between groups on subcortical regions at baseline and over time*

There was no significant difference between TRS patients and controls at baseline (n=33 TRS; n=31 HC) when considering jointly the 8 subcortical structures and taking account of multiple comparisons ($F(8,52)= 1.79$; $p= 0.101$, Table 3). We also assessed for differences in subcortical structures at baseline in the larger initially recruited sample (n=39 TRS; n=40 HC). Volumetric changes in structures such as hippocampus and lateral ventricles did not survive overall multiple comparison correction ($F(8,66)=1.82$; $p= 0.088$, Suppl. Table 1), but were in keeping with the effects sizes (circa 0.5) identified for such structures in larger case control samples of patients with schizophrenia (Van Erp et al., 2016). However, a strong significant overall interaction between time, group and brain structure was demonstrated ($F(7,143)= 52.54$; $p<0.001$, Table 3). *Post-hoc* analyses, robustly corrected for multiple comparison (Bonferroni, $\alpha=0.006$), revealed a significant volumetric increase in lateral ventricle ($F(1,59)=48.89$; $p<0.001$, Figure 1A) and decrease in thalamus ($F(1,59)=34.85$; $p<0.001$, Figure 1B), caudate ($F(1,59)=59.35$; $p<0.001$, Figure 1C), putamen ($F(1,59)=87.20$; $p<0.001$, Figure 1D) and hippocampus ($F(1,59)=14.49$; $p<0.001$, Figure 1E) volumes for patients compared to healthy controls (Table 3). The relative consistency of the progressive volumetric changes in the patient cohort is apparent from the individual level data points displayed in Supplementary Figure 1. There was no significant group-by-age interaction on the progression of the subcortical structures between patients and controls ($F(84,112)= 1.13$; $p=0.272$). *Post-hoc* analysis revealed no significant differences at baseline between controls and patients previously treated with atypical and/or typical medications when considering the 8 subcortical structures ($F(8,16)=1.49$; $p= 0.117$).

Table 3. Uncorrected means (SD) in mm^3 for each subcortical structure at baseline and follow-up, and results of statistical comparisons.

	SCHIZOPHRENIA (n=33)	HEALTHY CONTROL (n =31)	GLM Baseline	SCHIZOPHRENIA (N=33)	HEALTHY CONTROL (N=31)	GLM Follow-Up	GLM Group*Time* Structure	Mean Vol. Diff. Over time (mm^3) [95% C.I]	% Volume Difference Over Time (SD)		
STRUCTURES	BASELINE	BASELINE	F (8,52) = 1.79, p= 0.101	FOLLOW-UP	FOLLOW-UP	F (8,52) = 3.11; p= **0.006**	F (7,41) = 52.54; p< **0.001**		TRS	HC	TRS compared to HC
			p			*p*	*p*				
	Means ± SD	Means ± SD		Means ± SD	Means ± SD						
Lateral Ventricle	16647.05 ± 9189.84	15272.53 ± 8836.20	0.128	18750.23 ± 9524.54	15413.12 ± 8927.10	**0.013**	**< 0.001**	1962.58 [1351.80, 2573.36]	14.96 (11.63)	1.01 (3.58)	13.95 (11.34)
Thalamus	17443.74 ± 2078.97	17995.40 ± 2234.72	0.111	16883.71 ± 2065.36	18023.49 ± 2262.84	**0.002**	**< 0.001**	-588.13 [-774.19, -402.06]	-3.21 (2.63)	0.15 (0.15)	-3.36 (3.22)
Hippocampus	8832.06 ± 773.80	9226.80 ± 830.35	0.027	8596.37 ± 773.36	9226.94 ± 846.99	**0.001**	**< 0.001**	-235.83 [-359.41, -112.26]	-2.63 (3.51)	0.00 (1.60)	-2.63 (3.63)
Caudate	8456.97 ± 1194.26	8193.08 ± 1204.06	0.189	8052.13 ± 1174.16	8190.45 ± 1200.68	0.597	**< 0.001**	-402.21 [-501.83, -302.59]	-4.83 (2.49)	-0.03 (2.38)	-4.80 (3.63)
Putamen	12781.62 ± 1790.28	12459.92 ± 1761.53	0.388	11993.99 ± 1610.45	12457.48 ± 1798.72	0.073	**< 0.001**	-785.20 [-947.18, -623.22]	-6.07 (2.50)	-0.03 (2.18)	-6.04 (3.78)
Pallidus	4200.93 ± 669.20	3964.46 ± 706.62	0.088	4116.39 ± 612.12	3951.75 ± 704.01	0.243	0.282	-71.83 [-166.04, 22.39]	-1.74 (4.94)	-0.27 (2.98)	-1.47 (5.34)
Amygdala	3210.78 ± 339.72	3295.14 ± 426.97	0.428	3141.08 ± 364.80	3265.88 ± 410.05	0.213	0.364	-40.45 [-114.05, 33.15]	-2.16 (4.88)	-0.77 (4.03)	-1.39 (5.28)
Nucleus Accumbens	1188.58 ± 199.04	1198.76 ± 213.56	0.518	1145.21 ± 198.17	1194.95 ± 220.84	0.169	0.060	-39.57 [-74.71, -4.43]	-3.50 (6.24)	-0.32 (5.02)	-3.18 (7.60)

Note: the table shows the difference between treatment-resistant schizophrenia patient and healthy control groups on subcortical structures at baseline, follow-up and over time. *Post-hoc* analyses corrected for multiple comparisons (Bonferroni, α= 0.006). Legend: GLM= generalized linear model. C.I = confidence interval; % Vol. Diff. = percentage volume difference; calculated as follows: 100 × [(volume at follow-up − volume at baseline)/ volume at baseline], Negative value indicates a % volume decrease over time. TRS= treatment resistant-schizophrenia patients; HC= healthy controls; **Bold** = significant values.

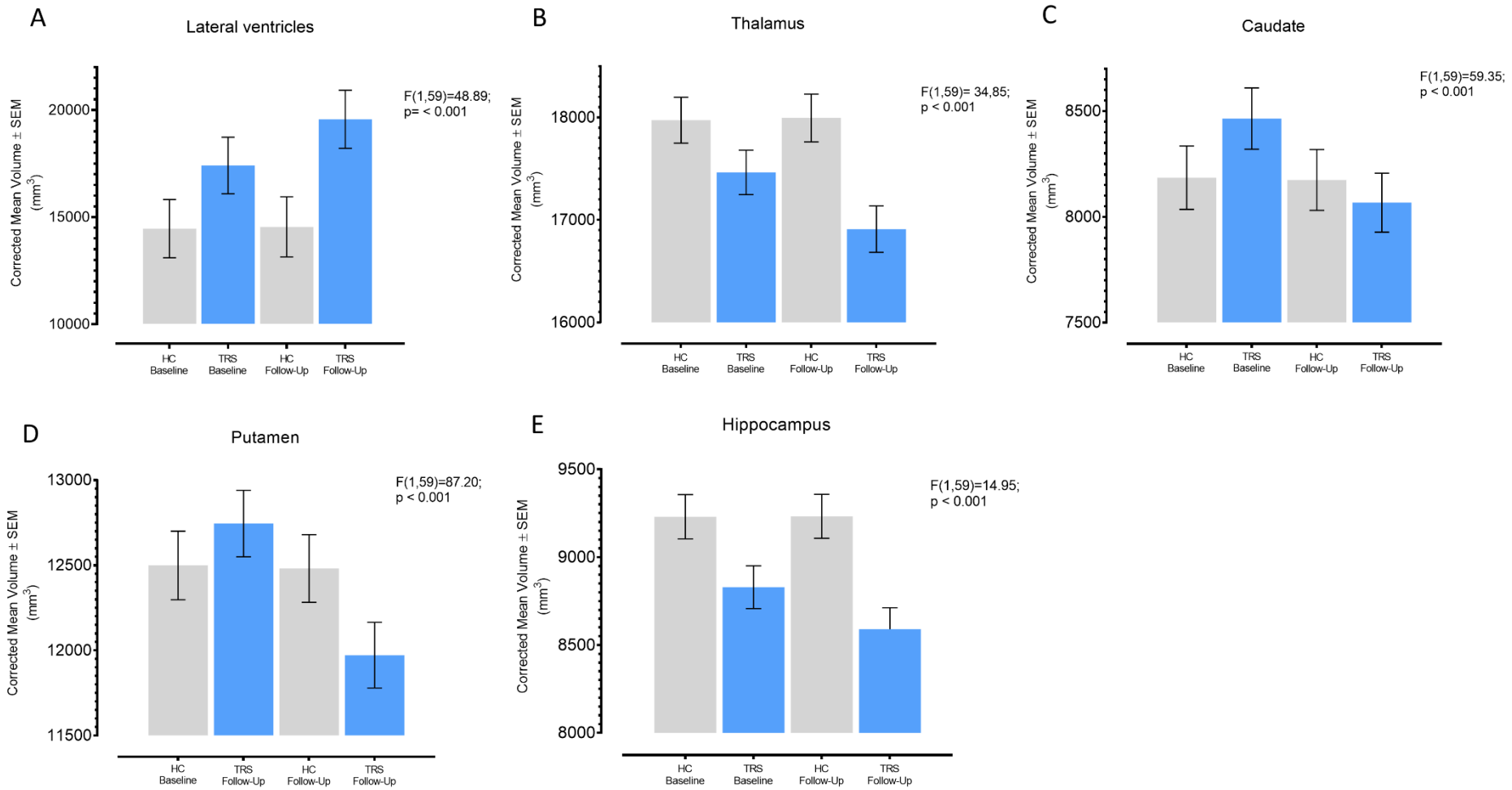

Figure 1. A, B, C, D, E Plots of subcortical structures that presented significant changes over time in treatment-resistant schizophrenia patients compared with healthy controls. Note: all values corrected for age, sex and ICV.

3.3 *Response to clozapine and subcortical changes at baseline and over time*

When investigating the baseline differences between those who remitted on clozapine treatment (n=16) and non-responders (n=17) for the 8 subcortical structures, no significant differences were revealed ($F(8,21)=1.32$; $p=0.286$). Likewise, there was no significant overall effect of time on subcortical brain structures between patients responding to clozapine compared to patients non-responders ($F(7,20)=0.50$; $p=0.834$).

3.4 Correlation between neuroanatomy and clinical variables in treatment-resistant patients

In TRS patients, when covarying for age, sex and ICV, volumetric reduction of thalamus and putamen over time were significantly associated with improvement in PANSS Total score ($r=0.42$, $p=0.021$; $r=0.39$, $p=0.033$, respectively Figure 2A) and improvement in negative symptoms assessed with the SANS scale ($r=0.36$, $p=0.049$; $r=0.40$, $p=0.027$, respectively Figure 2B). Similarly, improvement in PANSS General score was significantly related to decreased volume in thalamus over time ($r=0.39$; $p=0.034$). Controlling for serum clozapine level at follow-up and duration of illness did not impact on the above findings, however improvement of GAF was additionally found to relate to reduced thalamic ($r=-0.39$; $p=0.038$) and putaminal ($r=-0.42$; $p=0.024$) volume (Figure 2C). Improvement in SAPS was associated with reduced putaminal volume ($r=0.39$; $p=0.035$), but this association weakened slightly and lost significance ($r=0.31$; $p= 0.102$) when removing one outlier who demonstrated a 76% improvement in positive symptoms. No other associations were found between change in other subcortical brain structures and clinical variables (Suppl. Table 2).

3.5 Exploratory analyses between structures showing significant change over time in patients and treatment-related factors.

When exploring the association between changes over time in subcortical structures and treatment related factors in patients, including BMI change, and serum clozapine at follow-up, a significant association was identified between reduced volume of the thalamus over time and increased clozapine serum level at follow-up ($r=-0.44$; $p=0.010$, Figure 2D), with this correlation strengthening ($r=-0.49$; $p=0.010$) when controlling for change in clinical symptoms (PANSS, SAPS, SANS) and functioning (GAF).

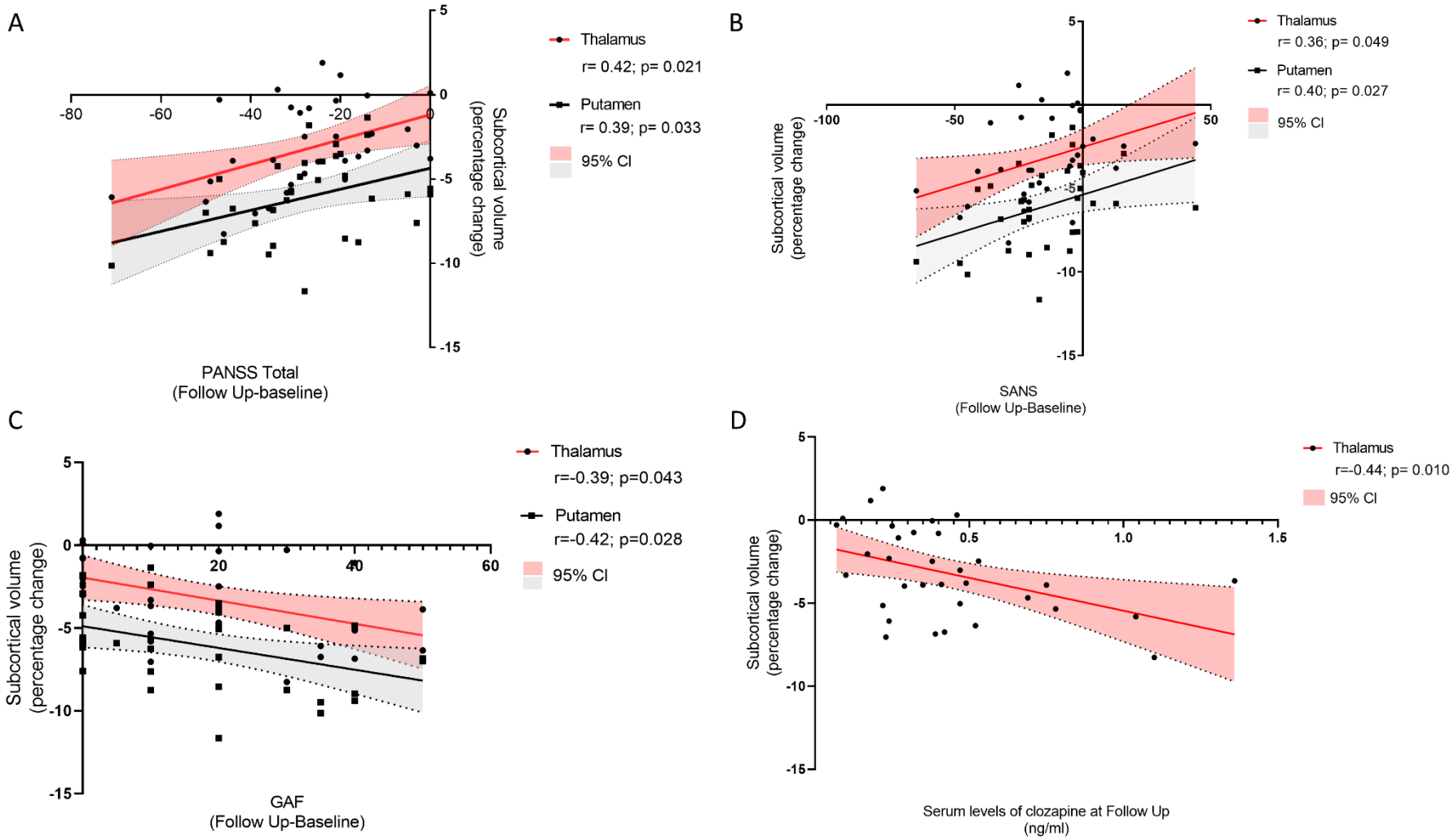

Figure 2. Association between percentage of volume change in thalamus and putamen and change in (A) PANSS total score (B) SANS (C) GAF and (D) association between percentage of volume change in thalamus and level of serum of clozapine at follow-up

4. Discussion

To the best of our knowledge, this is the largest sample to date to examine the effects of switching to clozapine on subcortical regions in a relatively clinically homogenous sample of TRS patients using a longitudinal semi-automated subject-specific approach (Freesurfer v.5.3.0) ("FreeSurfer," 2013). In this longitudinal study, minor subcortical differences were detected between patients and controls at baseline, which failed to survive multiple comparisons correction. However, we identified substantial progressive volumetric reduction of the thalamus, hippocampus, caudate, putamen and enlargement of lateral ventricles over a 6-month period in patients compared to controls. Reduced caudate volume over time has been consistently reported in the majority of studies of patients switched from typical antipsychotic medications to clozapine (Chakos et al., 1995; Frazier et al., 1996; Scheepers et al., 2001a, 2001b) and has been interpreted as reversal of previous enlargement due to excessive dopamine blockade. Consistent with this, longitudinal studies demonstrate basal ganglia enlargement when taking typical medications was reversed by switching to atypical antipsychotic medications (Lang et al., 2004; Westmoreland Corson et al., 1999). Reduction of thalamic volume over time was also reported in a 5-year longitudinal voxel-based morphometry study(van Haren et al., 2007). However, no association has previously been reported between cumulative doses of clozapine and subcortical deficits. The hippocampal progressive reduction identified in this cohort on switching to clozapine has not been previously reported, but notably the direction of change is in keeping with the other subcortical structures. The lateral ventricle enlargement over time could be interpreted as ventricular expansion as a result of the significant reduction of surrounding subcortical regions (Crespo-Facorro et al., 2008). The degree of volumetric change in this cohort after 6 months in regions such as the hippocampus and lateral ventricles is comparable to the rate of change detected in previous longitudinal studies over longer time periods (Ho et al., 2017; Kempton et al., 2010). The high density of dopamine D2 receptors (Howes and Kapur, 2009) in basal ganglia and other structures such as thalamus and hippocampus, renders them major targets to which dopaminergic pathways project (Money and Stanwood, 2013). In a preclinical study, Guma and colleagues (Guma et al., 2018), presented evidence that D2 receptors play a significant role in mediating antipsychotic induced structural changes, whereby volumetric

reduction in cortical areas, hippocampus and thalamus, was induced by genetic deletion of D2 receptors.

Our study did not detect any difference in subcortical structures between those who achieved clinical remission with clozapine treatment and non-responders, either at baseline or over time, consistent with some previous studies (Anderson et al., 2015; Scheepers et al., 2001b). In one longitudinal study of patients (which did not include a control sample), responders showed a significant reduction in left caudate volume after 24 weeks of clozapine treatment (Scheepers et al., 2001a).

These results lead us to speculate on three reasons for the lack of significant baseline subcortical volume deficits in patients compared with controls in this cohort and the subsequent marked progressive volume loss over time after commencing clozapine. (i) Direct effects of clozapine treatment, (ii) withdrawal of prior treatment with other medications, or (iii) illness progression independent of medication use.

(i) This cohort of TRS patients may be a categorically different illness subtype with different underlying mechanisms and pathophysiology compared with D2 receptor antagonist responsive schizophrenia (Gillespie et al., 2017; Lally et al., 2016). Lack of the striatal dopaminergic elevation in TRS, typical in schizophrenia could explain why treatment with dopamine antagonists are ineffective as they target the wrong processes (Howes and Kapur, 2014). Abnormal glutamatergic function, with higher glutamate + glutamine level concentrations have been reported in TRS compared to first-line responders (Gillespie et al., 2017; Goldstein et al., 2015). Indeed it has been suggested that clozapine's efficacy might relate to its ability to attenuate glutamate release, as demonstrated in preclinical studies (Amitai et al., 2012). In our cohort the previous lack of symptomatic response to typical and atypical antipsychotic medications may have related to relative subcortical volume preservation compared with healthy controls. Hence, the subcortical volume loss after commencing clozapine treatment may directly have been related to clozapine efficacy (Scheepers et al., 2001b). Indeed, cross-sectional studies on neuroanatomy of TRS patients are usually on patients already receiving clozapine, and demonstrate reduction of cortical and subcortical volumes (Anderson et al., 2015; Quarantelli et al., 2014; Zugman et al., 2013), as we see at the follow-up point in our study when patients are on clozapine treatment. It may also be that acutely symptomatic phase of illness is linked to increased neuroinflammation

which has been associated with increases in local blood flow, vascular permeability, microglia activation and extracellular volume (Graeber et al., 2011) . In this scenario, successful treatment with clozapine might have resulted in an anti-inflammatory process (Al-amin et al., 2013) that reversed these inflammatory changes, resulting in subcortical volume reduction.

(ii) Prior exposure of this cohort to antipsychotic medications over the years might have ameliorated or corrected disease-related volume loss (Lieberman et al., 2005; Van Erp et al., 2016; van Haren et al., 2011; Vita et al., 2015), which may explain our finding of only minor baseline volume differences. Interestingly unmedicated patients have been reported to display greater subcortical deficits, especially of the caudate and thalamus, compared to medicated patients (Haijma et al., 2013; Van Erp et al., 2016). On this interpretation, the progressive brain volume change of subcortical structures on switching to clozapine treatment might have been related to the withdrawal of other atypical antipsychotic medications. The neurobiological mechanism that underlies the progressive volumetric loss of subcortical structures is still unknown, however neural apoptosis, necrosis, synaptic pruning might play a role in producing volume deficits (Ho et al., 2003).

(iii) The progressive volume loss of subcortical structures in patients revealed by scanning over two time-points was not associated with pharmacotherapy, but rather to the underlying pathophysiology of this malignant form of schizophrenia illness and/or other illness-related factors which were not present in controls. However, this explanation seems unlikely since patients in our cohort have a mean illness duration of 13 years and only some were in the early stages of illness.

The progressive loss of volume in subcortical structures despite symptomatic and functional improvement suggests that volume loss as detected by neuroimaging in vivo in our cohort should not be necessarily interpreted as harmful to patients. Although cognitive impairment has been related to cortical thinning or volume reduction in schizophrenia (Antonova et al., 2005; Fan et al., 2019; Koshiyama et al., 2018), grey matter loss has been associated with greater response to atypical antipsychotics (Gur et al., 1998; Sporn et al., 2003). Moreover, cortical thinning in first-episode schizophrenia patients on pharmacotherapy has been associated with physiological and cognitive improvement (Lesh et al., 2015). Consistent with this, progressive volumetric reduction of putamen and thalamus was significantly associated with better response to clozapine. This result was unaltered after controlling for the serum

level of clozapine and duration of illness. Interestingly Scheepers and colleagues, reported an association between clinical improvement in positive and general symptoms and reduction of left caudate volume, in TRS patients (Scheepers et al., 2001a). Molina and colleagues, in a 2 years randomised clinical trial of clozapine on 17 neuroleptic-naïve patients with schizophrenia and 11 controls, have shown that inferior frontal thinning, specifically, pars orbitalis, opercularis and triangularis, was positively associated with better clinical and cognitive response to clozapine (Molina et al., 2014).

We also found that patients who were exposed to higher amounts of clozapine displayed a greater reduction of thalamus volume, this association was further reinforced when controlling for clinical symptoms and functioning, suggesting a direct effect of clozapine on the volumetric change of the thalamus. Vita and colleagues' meta-analysis described a consistent finding where the greater the exposure to antipsychotics the greater the reduction in grey matter volume (Vita et al., 2015). Two longitudinal studies have shown that the amount of exposure to antipsychotics predicted reduction of caudate and grey matter volumes(Ho et al., 2011) and the greater progressive brain reduction and ventricular enlargement were predicted by greater exposure to antipsychotic medication (Guo et al., 2015). Although these studies have been interpreted as consistent with a toxic effect of antipsychotic medication on grey matter, generally patients were not randomised in these longitudinal studies and it is likely that patients with more severe illness were given larger amounts of medication. In our study other variables, such as age, duration of illness and daily dose of clozapine were not significant moderators of subcortical volume change over time, as previously reported (Vita et al., 2015).

A recent systematic review concludes that after 25 years of research it remains unclear which are the biological predictors of symptomatic response to clozapine (Samanaite et al., 2018). Greater integrity and activity in prefrontal cortical areas associated with a good response to clozapine is the most consistent finding, however, studies have failed to find any accurate and reproducible neuroanatomical biomarker to inform clinical decision-making. Although our study identified a relationship between thalamo-striatal progression and clinical and functional improvement, we did not identify any baseline subcortical predictor of remission on clozapine.

Strengths and limitations

The main strength of this study is the longitudinal nature of a relatively large and homogenous sample of TRS patients. The careful segmentation of the subcortical structures using the longitudinal stream of Freesurfer based on an unbiased within-subject anatomical template (Reuter et al., 2012) enabled increased anatomical sensitivity to better detect anatomical changes and relationships to clinical symptoms and functioning. A potential limitation of this study is the lack of a comparative group of schizophrenia patients treated with other antipsychotic medications, in order to disentangle disease effects from treatment effects. However, such a comparative group may represent a less malign subgroup of patients with schizophrenia who are not treatment resistant and consequently may have a different underlying pathophysiology/impact of antipsychotic medication on their neuroanatomy. Ultimately including MR imaging in longitudinal studies of schizophrenia where patients are randomised to different antipsychotic medications would be necessary to tease apart illness from treatment effects but only three such studies have been conducted to our knowledge (Lieberman et al., 2005; Molina et al., 2014; Roiz-santiáñez et al., 2012) and none on patients with treatment resistance. In addition, to reduce multiple analyses we assessed only subcortical structures summed bilaterally and did not explore any lateralised effects.

Conclusion

This study demonstrates that, despite the clinical and functional improvement of most patients with schizophrenia who are switched to clozapine, there is a counterintuitive progressive volume reduction in several subcortical structures over time. Furthermore, patients who have the greatest symptomatic improvement display the largest thalamo-striatal reductions, suggesting that volume reduction reflects an adaptive response associated with symptom improvement rather than a harmful process in these treatment resistant patients. Further longitudinal studies with larger sample size, randomised designs and multimodal imaging will be necessary to disentangle the potentially dynamic effects of neuroprogression and antipsychotic treatment on different brain structures in schizophrenia.

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Supplementary Data.

Supplementary Table 1. Uncorrected means (SD) in mm3 for each subcortical structure at baseline and results of statistical comparisons

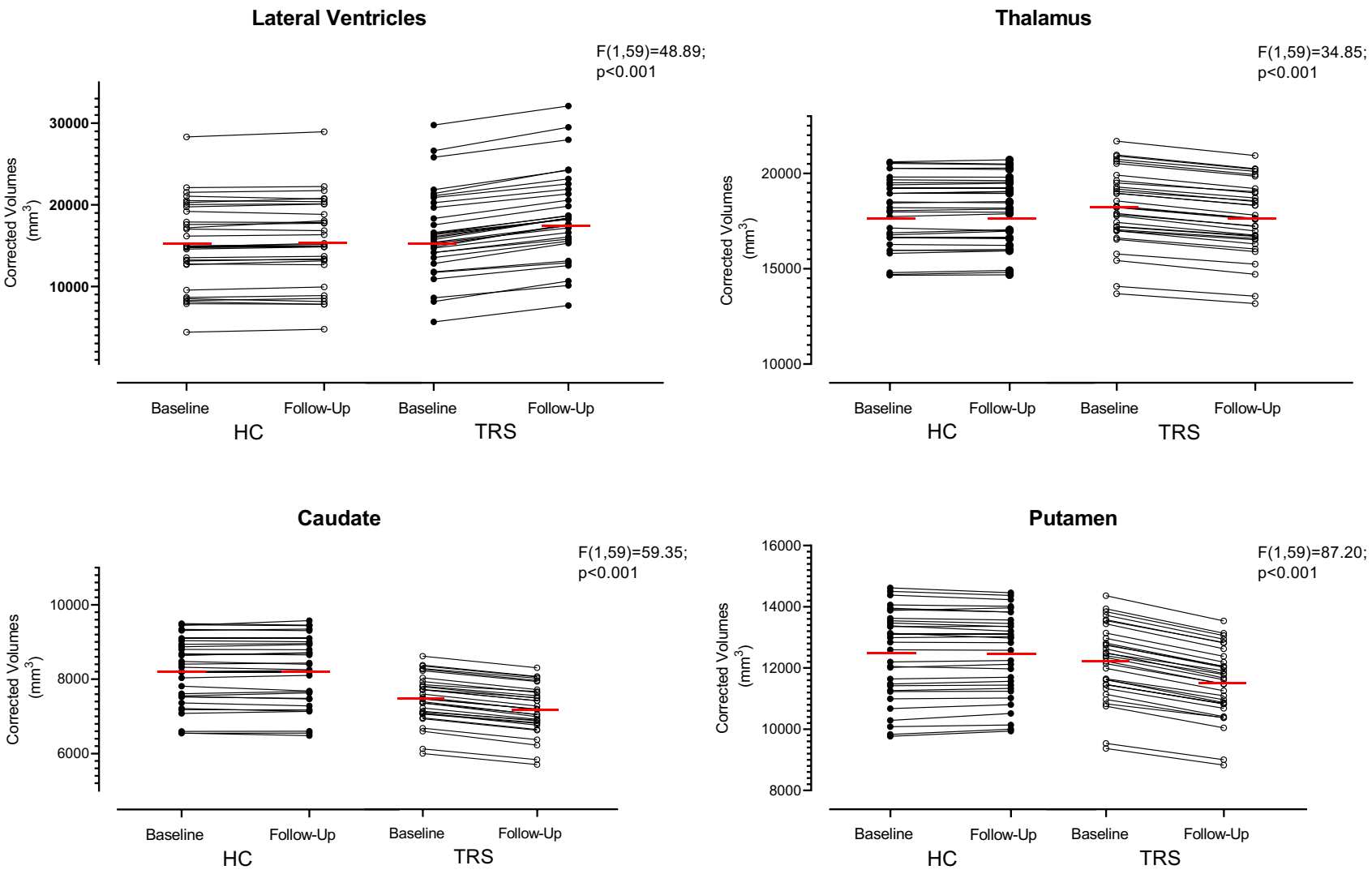
	SCHIZOPHRENIA (n=39)	HEALTHY CONTROL (n=40)	GLM Baseline	Effect size *Cohen's d*
STRUCTURES	BASELINE	BASELINE	F (8,66) = 1.83, p= 0.088	
			p	
	Means ± SD	Means ± SD		
Lateral Ventricle	16645.83 ± 9038.13	14397.65 ± 8612.52	0.023	0.5
Thalamus	17375.83 ± 2130.64	17553.04 ± 2238.17	0.112	0.3
Hippocampus	8852.54 ± 829.88	9134.66 ± 760.41	0.022	0.5
Caudate	8409.65 ± 1151.67	8044.83 ± 1177.15	0.188	0.3
Putamen	12630.16 ± 1746.00	12044.24 ± 1801.58	0.350	0.2
Pallidus	4138.89 ± 659.62	3817.94 ± 708.69	0.119	0.1
Amygdala	3202.95 ± 360.02	3252.12 ± 393.17	0.399	0.1
Nucleus Accumbens	1189.09 ± 186.98	1182.88 ± 219.69	0.456	0.1

Note: the table shows the difference between treatment-resistant schizophrenia patient and healthy control groups (Whole initial recruited sample) on subcortical structures at baseline. Interpretation of Cohen's d effect size: 0.0-0.1 No Effect/ 0.2-0.4 Small Effect/ 0.5-0.7 intermediate Effect/ 0.8- ≥1.0 Large Effect.

Supplementary Table 2. Correlations between neuroanatomical change and clinical variables change in patients

	PANSS Negative		PANSS Positive		PANSS General		PANSS Total		SAPS		SANS		GAF	
STRUCTURES	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Lateral Ventricle	-0.08	0.692	0.22	0.243	0.08	0.692	0.08	0.686	-0.03	0.879	-0.03	0.891	0.03	0.895
Thalamus	0.32	0.086	0.34	0.063	**0.39**	**0.034**	**0.42**	**0.021**	0.19	0.320	**0.36**	**0.049**	-0.32	0.088
Caudate	0.19	0.328	0.21	0.275	-0.00	0.996	0.13	0.491	0.04	0.823	0.23	0.218	-0.14	0.467
Putamen	0.31	0.097	0.34	0.067	0.34	0.066	**0.39**	**0.033**	***0.39**	**0.035**	**0.40**	**0.027**	-0.36	0.052
Hippocampus	-0.16	0.411	-0.02	0.902	0.03	0.874	-0.05	0.795	-0.08	0.676	0.03	0.877	0.00	0.988

Note: PANSS: Positive and Negative Syndrome Scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms. In the correlations (Follow-up - Baseline)/Baseline×100) was used to express the volumetric change in subcortical structures and (Follow-up - Baseline) was used to express change in the clinical variables. Correlations controlled for age, sex and ICV. * After removing 1 outlier this correlation lost significance (r=0.31; p= 0.102).



Supplementary Figure 1. Illustration of the change in lateral ventricles, thalamus, caudate and putamen volume from baseline to follow-up for each patient and healthy control. Red bars represent the mean.

White Matter Microstructure and Structural Networks in Treatment-Resistant Schizophrenia Patients After Commencing Clozapine Treatment: A Longitudinal Diffusion Imaging Study

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Abstract

Clozapine has a superior clinical effect in refractory schizophrenia, however its potential impact on white matter microstructure and neural networks is unclear. This study investigates such changes after 6 months of switching to clozapine in schizophrenia patients compared to controls, and whether any changes are related to clinical variables.

T1 and diffusion-weighted MRI images were acquired at baseline before commencing clozapine and after 6 months of treatment for 22 patients with treatment-resistant schizophrenia and 23 controls. The Tract-based spatial statistics approach was used to compare changes over time between groups in fractional anisotropy (FA). Changes in structural network organisation and subnetwork connectivity weighted by FA and number of streamlines were assessed using graph theory and network-based statistics.

Patients displayed a significant reduction of FA over time (p<0.05) compared to controls in the genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata. There was no correlation between FA change in patients and changes in clinical variables or serum level of clozapine. There was no significant overall interaction between time, group and structural network global (F(7,280)= 2.80;p=0.187) or local (F(15,600)= 0.747, p=0.737) measures. No subnetwork was identified when testing for time by group interaction.

This longitudinal study demonstrated progressive focal FA abnormalities in key anterior tracts, such as corpus callosum and corona radiata, but preserved brain structural network organisation in patients. The FA reduction was independent of any clinical measures and may reflect progression of the underlying pathophysiology of this malignant form of schizophrenia illness.

1. Introduction

Schizophrenia is a complex and heterogenous mental illness, defined as conduction deficit by Eugen Bleuler during the 20th century (Bleuler, 1911), and through the development of advanced neuroimaging techniques is conceived as a dysconnectivity disorder characterized by disturbed integration of functions between different brain areas (Friston and Frith, 1995). Diffusion Magnetic Resonance imaging (dMRI), which provides an indirect measure of the microstructural organization of white matter (WM) (Jones, 2008), has found evidence of white matter dysconnectivity in schizophrenia. Fractional anisotropy (FA), the most reported dMRI outcome measure, reflects the heterogeneous water diffusion in WM, which is sensitive to fibre orientation, coherence, density and degree of myelination of neurons within fibre bundles (Beaulieu, 2002; Pierpaoli et al., 1996; Tournier et al., 2011).

dMRI meta-analyses have reported significant FA reductions in schizophrenia patients compared to controls in WM tracts interconnecting the frontal lobe, thalamus, cingulate and tracts traversing left temporal WM regions (Bora et al., 2011; Ellison-wright and Bullmore, 2009). A large-scale international collaborative meta-nalysis (ENIGMA) based on 2359 healthy controls and 1963 schizophrenia patients reported significant widespread FA reductions in schizophrenia patients, most prominent in the anterior corona radiata and the body and genu of the corpus callosum (Kelly et al., 2017). Another recent meta-analysis by Vitolo and colleagues (2017) of voxel-based morphometry and diffusion tensor imaging (DTI) studies focusing on WM alterations between schizophrenia patients and controls, based on 59 studies, reported widespread alteration of white matter bundles, including frontal, temporal, and limbic pathways. Other circuits such as callosal, commissural, and cortico-cerebellar-thalamic-cortical were also altered in schizophrenia patients compared to controls (Vitolo et al., 2017).

Although treatment resistance, usually defined as failure to respond to at least two adequate trials of classic antipsychotic medications (Suzuki et al., 2012), affects approximately 30% of patients with schizophrenia (Lieberman et al., 1994; Meltzer, 1997), few diffusion weighted studies have specifically investigated this illness subtype. Cross-sectional voxel based-morphometry studies of treatment-resistant schizophrenia patients (TRS) have reported decreased whole brain white matter compared with controls (Anderson

et al., 2015; Maller et al., 2012). In contrast, Molina et al., (Molina et al., 2008) found significant white matter volume increase in the frontal, parietal and occipital lobe in treatment-resistant patients compared to controls. Previously, a case control DTI study from our group examining white matter volume in chronic severe treatment resistant schizophrenia, who had not yet commenced clozapine treatment, reported reduced FA with corresponding increased radial diffusivity in the genu, body, and splenium of the corpus callosum, the right posterior limb of the internal capsule, right external capsule, and the right temporal inferior longitudinal fasciculus in patients compared to controls (Holleran et al., 2013).

However, none of these cross-sectional studies were able to assess whether introducing clozapine treatment may affect measures of structural connectivity in schizophrenia. Clozapine has an established superior clinical effect in refractory schizophrenia, with 60-70% of patients showing a positive response (Chakos et al., 2001; Kane et al., 1988; Stroup et al., 2003), however, its effect on brain structures and neural networks is still unclear. In previous works from our group, we demonstrated on-going cortical thinning of the left medial frontal cortex, right middle temporal cortex and a progressive subcortical volumetric reduction in patients with treatment-resistant schizophrenia after 6 months of switching to clozapine treatment (Ahmed et al., 2015; Tronchin et al., 2020a), despite symptomatic improvement. Molina et al., in a small 6-month longitudinal volumetric study of T1 weighted images found that 13 TRS patients who had started clozapine treatment presented a marked decrease in frontal, parietal and occipital white matter compared to 11 controls (Molina et al., 2008). In contrast, a longitudinal DTI study by Ozcelik-Eroglu et al., (2014) based on 16 schizophrenia patients and 8 controls and investigating the effect of 12 weeks of clozapine treatment on white matter, reported in the patient group increased FA values in 31 regions at follow-up compared to baseline. When comparing these changes with the control group the study reported significant FA increases in patients in the left superior parietal lobule and the left inferior fronto-occipital fasciculus (Ozcelik-Eroglu et al., 2014).

Structural Networks

In recent years focus has shifted to study the entire structural connectivity of the brain, through network analysis, which has offered a complementary approach to explore the concept of dysconnectivity (Fornito et al., 2015, 2012, Sporns, 2013a, 2013b). Employing

graph theory, structural networks can be modelled using metrics derived from structural magnetic resonance imaging and dMRI, where cortical and subcortical structures are defined as “nodes” and white matter connections as “edges” (Bullmore and Sporns, 2009). When nodes and edges are mapped, the brain, conceived as a graph, can be assessed for its topological properties, such as efficiency and pattern of connections. Such techniques enable the investigation of connectivity features that are not otherwise measurable by focusing on information from single brain regions (Van Den Heuvel and Fornito, 2014).

Network studies have reported impaired structural connectivity in schizophrenia (Fornito et al., 2012; Pettersson-Yeo et al., 2011), including reductions in global communication efficiency (Zalesky et al., 2011) and related longer average path length (Ottet et al., 2012; Van Den Heuvel et al., 2010; Zhang et al., 2012). In particular, Van den Heuvel and colleagues, in a cross-sectional study based on 40 schizophrenia patients and 40 healthy controls reported reduced global efficiency of the frontal, temporal, and occipital brain regions in schizophrenia (Van de heuvel 2010). A recent cross sectional study (Luo et al., 2020) explored the characteristics of structural network in chronic schizophrenia patients (never treated n=17, treated with clozapine (n=17) and risperidone (n=17) monotherapy for over 5 years). The study suggested a general disruption in the organization of white matter structural networks as well as decreased nodal and connectivity characteristics across all the schizophrenia groups, specifically the alteration was more prominent in never treated and clozapine treated patients. Kraguljac and colleagues (Kraguljac et al., 2019) investigated in 42 patients with schizophrenia, who were medication naïve or off antipsychotic medications for at least 2 weeks, the effect of a trial of risperidone on white matter diffusion indices. The study observed no changes in micro- or macrostructural white matter after 6 weeks of treatment in patients with schizophrenia.

Recently, Mackay and colleagues (2018) (Mackay et al., 2018), published a review analysing current findings of system-level brain dysconnectivity in treatment-resistant schizophrenia patients, suggesting that this group compared to chronic schizophrenia present unique biological circuitry, characterized by a pronounced and widespread dysfunction throughout the entire brain, involving both cortical and subcortical regions. A cross-sectional functional network-based study reported widespread reductions in functional connectivity at the whole brain level in treatment-resistant schizophrenia patients compared to controls, specifically involving temporal, occipital and frontal regions. They also reported reduced global efficiency

and increased local efficiency in patients compared to controls (Ganella et al., 2017). To our knowledge, there are no studies investigating structural network changes longitudinally in treatment-resistant schizophrenia patients exposed to clozapine treatment.

The present study sought to comprehensively investigate whether, after 6 months of switching to clozapine, white matter microstructure and structural network organisation demonstrate any progressive changes in a unique sample of treatment-resistant clozapine-naïve schizophrenia patients compared to healthy controls. We also sought to assess whether any connectivity changes were related to clinical variables and serum level of clozapine at follow-up.

2. Method

2.1 Participants

As previously reported (Ahmed et al., 2015; Tronchin et al., 2020a) 33 patients with treatment-resistant schizophrenia (TRS) and 31 healthy volunteers (HC) matched for sex and age successfully participated at both baseline, prior to clozapine initiation in patients, and 6 months follow-up clinical assessments and MRI scanning. Of these 64 participants, diffusion MRI data were available at both time points for 22 patients and 23 healthy controls (Table 1). Patients and controls were excluded from the study if they had a history of a previous trial of clozapine treatment, a learning disability, history of neurological illness, history of head injury which resulted in loss of consciousness for over 5 minutes, use of oral steroids in the three months prior to participation, history of comorbid alcohol/ substance dependency as defined by the DSM-IV or any contraindication to MRI scanning. Exclusion criteria for controls also included having a current or past axis I disorder (DSM-IV-TR) or any psychotic disorder in a first-degree relative. The study was approved by the Galway University Hospital Clinical Research Ethics Committee. Fully informed written consent was obtained for all participants.

2.2 Clinical assessment

All patients were diagnosed using the Diagnostic and Statistical Manual for Mental Disorders 4th Edition text revision (DSM-IV-TR) (American Psychiatric Association, 2000). Treatment resistance was defined as the failure to respond to at least two adequate trials of antipsychotic medications (normally 6 months), including at least one atypical antipsychotic drug, with a prolonged period of moderate to severe positive and/or negative symptoms (National Institute of Health and Clinical Excellence, 2014). The severity of positive and negative symptoms was assessed at both time points using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), The Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1982a) and The Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982b). Social, occupational and psychological functioning was assessed using a Global Assessment Functioning Score (Hall, 1995). As previously described (Tronchin et al., 2020a) we used the symptomatic remission criteria of Andreasen et al., (Andreasen et al., 2005) with the exclusion of the maintenance over 6-month observation period (Egerton et al., 2018) in order to categorically determine treatment response.

2.3 *MRI data acquisition*

MRI images were acquired for all participants at baseline and after 6 months at University Hospital Galway in a 1.5 Tesla Siemens Magnetom Symphony scanner (Erlangen, Germany) equipped with a 4-channel head coil. Magnetisation prepared rapid gradient-echo (MPRAGE) sequence was employed to acquired high resolution volumetric T1-weighted images, with the following parameters: repetition time (TR): 1140 ms, echo time (TE): 4.38 ms, inversion time (TI): 600 ms, flip angle: 15°, matrix size: 256x256, interpolated to 512 x 512, slice thickness: 0.9 mm and in plane resolution: 0.45 mm x 0.45 mm. Diffusion-weighted MRI data were collected with a standard 8-channel head coil using an echo planar imaging (EPI) diffusion sequence acquired with parallel imaging. The acquisition parameters were as follows; 64 independent diffusion gradient directions, comprising 2 separate multi-direction diffusion-weighted sequences that are subsequently concatenated in ExploreDTI (Leemans et al., 2009), including 7 reference images with no diffusion gradient (B_0) i.e. (34 directions + $3B_0$) & (37 directions +$4B_0$), *b* = 1300s/m. m^2, TR = 8100ms, TE = 95ms, FOV = 240 x 240mm^2, matrix size 96 x 96, 60 axial slices and total imaging time of 10.24 minutes generating an in plane resolution of 2.5mm x 2.5mm with slice thickness of 2.5 mm.

2.4 MRI processing

T1-weighted images were processed through the longitudinal stream (Reuter et al., 2012) of Freesurfer v.5.3.0 (“FreeSurfer,” 2013) to parcellate cortical and subcortical regions at two time points. The several steps of the processing pipeline to obtain the output have previously been described in detail (Tronchin et al., 2020b). At each step, the output was visually inspected, to verify that the parcellation was anatomically accurate and computationally successful (“FreeSurfer Quality Control Guide,” 2013). At each time point, diffusion-weighted images were corrected for motion artefacts, eddy -current distortions and rotation of the b-matrix using ExploreDTI v4.8.6. The data quality was defined after visually inspecting for geometric distortion, subject head motion, signal dropout, abnormal model residuals and registration accuracy (Tournier et al., 2011). Following quality check, no images were excluded.

2.5 Voxel-based Statistical Analysis and Statistic

Voxel-by-voxel based statistical analysis was performed using the tract-based spatial statistic (TBSS) approach (Smith and Nichols, 2009) for baseline analysis and adapted for longitudinal analysis ("FSL Support"). The Fractional Anisotropy images were exported from the diffusion motion corrected native images, using ExploreDTI v4.8.6 (Leemans et al., 2009). Subject-specific registration was employed, all FA images were non-linearly aligned to the target subject specific image and subsequently the affine transformation to standard MNI152 $1mm^3$ space was applied (FSL v. 6.0.1). All the individual FA images were merged and averaged to get a mean FA image. Then, a study-specific thinned mean FA skeleton was produced (0.2 FA threshold was applied to exclude non-WM voxels). Each individual subject's FA image was projected onto the skeleton by searching perpendicular to the skeleton to find the maximum FA for use in the statistical analysis. At each of the previous steps a qualitative analysis was carried out. Only for the longitudinal analysis FA difference maps were then produced for each subject (Baseline – Follow-up) (De Groot et al., 2016; Minett et al., 2018; Reis Marques et al., 2014). Randomise voxel-wise statistics analysis was performed and threshold-free cluster enhancement (TFCE) applied to correct for multiple comparisons (p<0.05)(Nichols and Holmes, 2003; Smith and Nichols, 2009). Age and sex were included as covariates in the model. The John Hopkins University (JHU) ICBM_DTI_81 white-matter labels atlas (Wakana et al., 2007) was used to identify clusters that showed significant statistical group differences. Significant clusters were masked and FA at baseline and FA change over time was extracted ("fslwiki Cluster," 2013). For the patient group FA at baseline and FA change over time was correlated with change in clinical and anthropomorphic variables and serum level of clozapine at follow-up using Partial correlation covarying for age and sex and Spearman correlation where appropriate (SPSS v23). To estimate longitudinally the values of other white matter difusion tensor imaging parameters, such as mean diffusivity (MD) (The level of water diffusion in any direction at each point of the tract, which increases with loss of structural barriers that normally restrict water diffusion) and radial diffusivity (RD) (The degree of diffusion perpendicular to the primary tract axis; where a marked increase may be associated with demyelination) (Song et al., 2003, 2002; Van Den Heuvel and Fornito, 2014), the original FA nonlinear registration was applied to the non-FA data, and subsequently projected into the original mean FA skeleton. Randomise voxel-wise statistical analysis was performed as

described above. An additional one-way MANCOVA was performed to assess differences between clozapine responders and non-responders at baseline on FA at baseline, covarying for age and sex. A visual description of this pipeline is outlined in Figure 1.

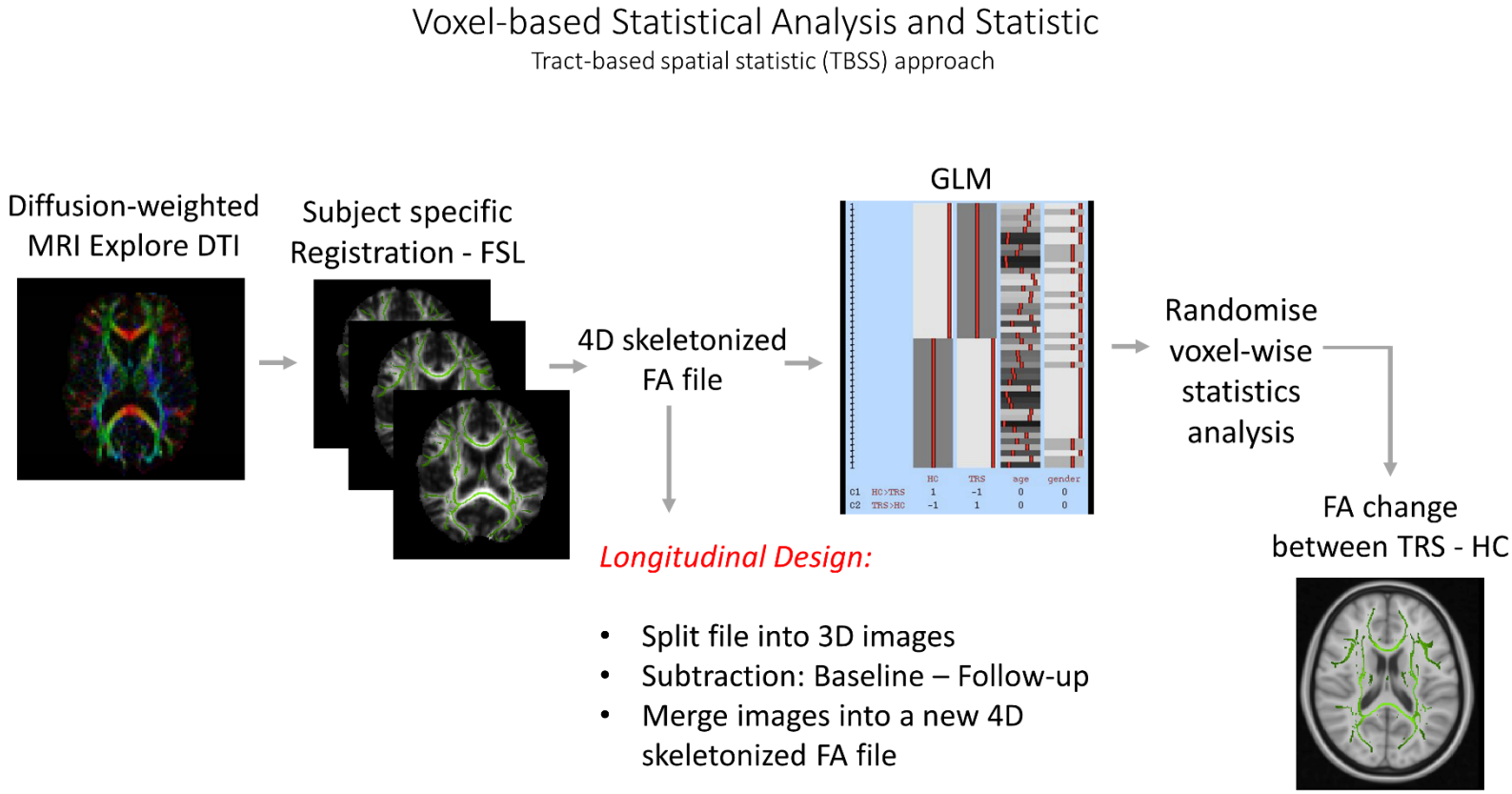


Figure 1. Voxel-based Statistical Analysis Method

2.6 Network Reconstruction and Statistic

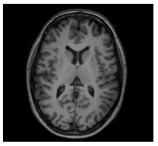
A deterministic non-tensor-based constrained spherical deconvolution (CSD) algorithm was applied to the motion corrected diffusion-weighted data and included recursive calibration of the response function (ExploreDTI v4.8.6) (Jeurissen et al. 2011; Tournier et al. 2008). Fiber tracking commenced in each voxel, continued with 1 mm step size, 2 mm^3 seed point resolution, and terminated at >30° angle curvature, <20 or >300 mm in length, and <0.2 FA. Eighty-six regions, including 34 cortical, 8 subcortical and cerebellar hemispheres bilaterally were defined using the longitudinal pipeline of Freesurfer v5.3.0, based on the Desikan-Killiany atlas (Desikan et al., 2006; Fischl., 2002). For each participant and at each time point, unweighted connectivity matrices (86x86) were generated (ExploreDTI v4.8.6) and connections that were present in each subject at both baseline and follow-up were retained to increase sensitivity in changes over time. The identified connections were used as definition of which weights to keep at baseline and follow-up for matrices weighted by fractional anisotropy (FA) and number of reconstructed streamlines (NOS). Quality check involved ensuring that post-thresholded networks remained fully connected which resulted in the removal of 1 healthy control. Global measures of whole-brain connectivity including global efficiency, characteristic path length, average clustering coefficient and average strength were derived from weighted matrices. Local measures of clustering coefficient and strength were obtained from weighted matrices (Brain Connectivity Toolbox v1.52)(Rubinov and Sporns, 2010) for thalamus, putamen, caudate and hippocampus, whose volume was found decreased over time in patients compared to controls in our previous work (Tronchin et al., 2020a). An initial one-way multivariate analysis of covariance (MANCOVA) was performed to evaluate differences between groups at baseline on global and subsequently local network measures, covarying for age and sex. Thereafter two-way repeated MANCOVA was used to assess the course of changes in global and local network measures over time between patients and controls, covarying for age and sex. An additional one-way MANCOVA was performed to assess differences between clozapine responders and non-responders at baseline on subcortical structures, covarying for age, sex. A visual description of this methodological pipeline is outlined in Figure 2.

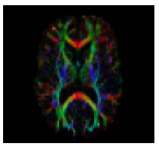
Structural Connectivity

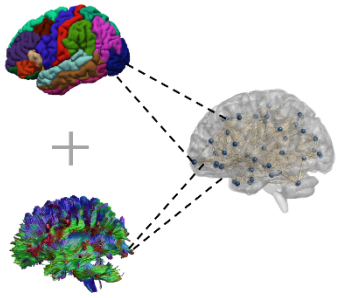
A

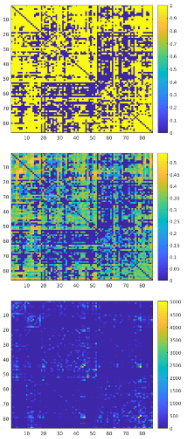
T1-weighted MRI, Freesurfer v5.3.0

Diffusion-weighted MRI, CSD tractography, ExploreDTI v4.8.6







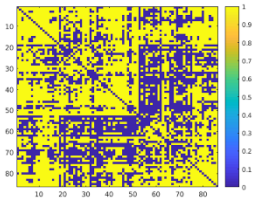


B

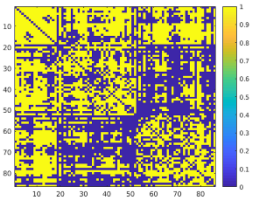
Longitudinal Design

i

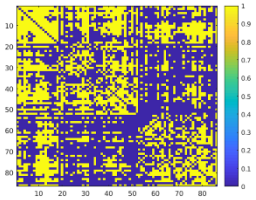
Baseline - Unweighted



Follow-up - Unweighted

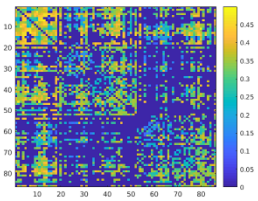


Mask - Binary

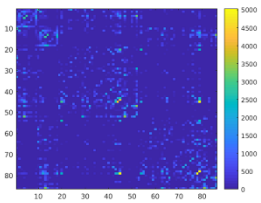


ii

Matrices weighted by Fractional Anisotropy (FA)



Matrices weighted by Number of Streamlines (NOS)



iii

Global measures

Two-way repeated ANCOVA

- Global Efficiency
- Characteristic Path Length
- Clustering Coefficient
- Strength

Local measures

Two-way repeated ANCOVA

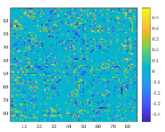
- Clustering Coefficient
- Strength

Subnetwork measures

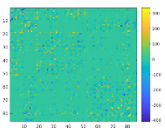
nbs CONNECTOME

iv

Subtraction Matrices: Baseline – Follow Up



Fractional Anisotropy (FA)



Number of Streamlines (NOS)

Statistic:

- F-statistic threshold of 1.75, 2, 2.5
- Contrast: 1, -1, 0, 0 / -1, 1, 0, 0

Figure 2. Network Reconstruction.

A) The image represents an overview of the steps to obtain structural connectivity matrices. Processing of T1-weighted images was performed through the longitudinal stream of Freesurfer v5.3.0 based on the Desikan-Killiany atlas (Desikan et al., 2006; Fischl., 2002) to parcellate 86 regions, including 34 cortical, 8 subcortical and cerebellar hemispheres bilaterally to create the nodes of the network. Processing of diffusion-weighted images was done using ExploreDTI v4.8.6, including motion correction, quality assessment and non-tensor-based constrained spherical deconvolution (CSD) tractography to obtain edges of the network (Jeurissen et al. 2011; Tournier et al. 2008). Unweighted and fractional anisotropy (FA)- and number of streamlines (NOS)-weighted structural matrices (86x86) were obtained for each participant and at each time point using ExploreDTI v4.8.6 by combining parcellated cortical and subcortical regions with CSD tractography-defined white matter. Fiber tracking commenced in each voxel, continued with 1 mm step size, 2 mm^3 seed point resolution, and terminated at >30° angle curvature, <20 or >300 mm in length, and <0.2 FA. B) The image represents the longitudinal design applied to the structural connectivity metrices. i) For each participant, a mask was created using unweighted connectivity matrices to include only the connections present at both baseline and follow-up. ii) The mask was used to threshold FA and NOS-weighted matrices. Quality check involved ensuring that post-thresholded networks remained fully connected which resulted in the removal of 1 healthy control iii) Global measures of whole-brain connectivity including global efficiency, characteristic path length, average clustering coefficient and average strength were derived from weighted matrices (Brain Connectivity Toolbox v1.52) (Rubinov and Sporns, 2010). Local measures of clustering coefficient and average strength were obtained for thalamus, putamen, caudate and hippocampus, whose volume was found decreased over time in patients compared to controls in our previous work (Tronchin et al., 2020a). Two-way repeated ANCOVA, covarying for age and sex was used for both local and global measures to assess differences over time on the connectivity measures between patients and controls. iv) We investigated the interaction between time and group on anatomical subnetwork connectivity using cluster-based statistical methods that control for the family-wise error rate (FWER) (network-based statistic, NBS v1.2). Using the thresholded matrices weighted by FA and NOS, we created a new matrix for the analysis, obtained by subtracting baseline from follow-up for each participant. A F-statistic representing the interaction between time and group for each connection was calculated using a general linear model (Pearson's correlation equivalent). A F-statistic threshold of 1.75, 2 and 2.5 corresponding to $p<0.043$, 0.02, 0.008 was applied and 5000 permutations used to calculate FWER-corrected p-values (pFWE) at 0.05 for every remaining connected component against a null distribution of maximum component size (Zalesky et al., 2010).

2.7 Anatomical Subnetwork Connectivity

We investigated the interaction between time and group on anatomical subnetwork connectivity using cluster-based statistical methods that control for the family-wise error rate (FWER) (network-based statistic, NBS v1.2). To do so, subtraction matrices were formed by subtracting the weighted and thresholded matrices at the follow-up from those at the baseline. A F-statistic representing the interaction between time and group for each connection was calculated using a general linear model (Pearson's correlation equivalent). A F-statistic threshold of 1.75, 2 and 2.5 corresponding to $p<0.043$, 0.02, 0.008 respectively was applied and 5000 permutations were used to calculate FWER-corrected p-values (pFWE) at 0.05 for every remaining connected component against a null distribution of maximum component size (Zalesky et al., 2010). A visual description of the methodological pipeline is outlined in Figure 2.

3. Results

3.1 Clinical characteristics

The patient and control groups did not differ in age, sex, or time between scans (Table 1). After treatment with clozapine, patients displayed a statistically significant improvement in PANSS, SANS, SAPS and GAF as previously reported (Ahmed et al., 2015; Tronchin et al., 2020a). At follow-up, patients also displayed a significant increase of weight, waist circumference, body mass index, and total cholesterol compared to baseline (Table 2). Ten patients were prescribed typical antipsychotic drugs at some stage of their illness and 2 were still taking typical antipsychotic medications at the timepoint of the baseline scan. At baseline before switching to clozapine, 15 patients were on monotherapy with one atypical antipsychotic medication (olanzapine=5, quetiapine=1, aripiprazole=5, amisulpiride=1, paliperidone=1, risperidone long acting injection=1), 7 patients were treated with two antipsychotic medications (olanzapine + another antipsychotic=5), with one patient treated with four antipsychotic medications. At follow-up 11 patients (50%) had achieved remission.

Table 1. Characteristics of patients with treatment resistant-schizophrenia and controls

	Patient group (n=22)	**Control group (n=23)**	**Test statistic/p-value**
Sex (m/f)	16/6	14/9	X^2= 0.71; 0.399
Age at onset (years)	23.3 ± 4.9		
Age at baseline (years)	37.1 ± 9.5	41.3 ± 10.3	t= 1.43; 0.161
Age range	(22-51)	(23-55)	
Time between baseline and follow-up MRI scans (months)	6.5 ± 1.6	7.2 ± 1.8	t= 1.42; 0.163
Illness duration before commencing clozapine (years)	13.8 ± 7.9		

Table 2. Clinical features of patient group at baseline and follow-up (n=22)

	Baseline (Mean ± SD)	**Follow-up (Mean ± SD)**	**Test statistic/ p-value**
Clinical scales			
PANSS positive score	12.2 ± 5.1	5.8 ± 4.5	*z=-4.05; < 0.001
PANSS negative score	15.8 ± 7.2	9.0 ± 6.6	t=5.32; < 0.001
PANSS general score	21.5 ± 7.5	11.3 ± 8.1	t=7.77; < 0.001
PANSS total score	49.5 ± 16.7	26.1 ± 16.9	t=7.76; < 0.001
SANS	42.9 ± 20.6	28.9 ± 22.2	*z=-3.02; 0.003
SAPS	25.7 ± 10.2	13.2 ± 11.1	*z=-3.62; < 0.001
Global assessment of functioning	47.1 ± 9.5	63.9 ± 14.0	t = -5.15; < 0.001
Medications			
Typical antipsychotics (n)	3	0	
Atypical antipsychotics (n)	22	2	
Clozapine (n)	0	22	
Serum level of clozapine at follow-up (ng/ml)		0.4 ± 0.3	
Daily dose of clozapine at follow-up (mg)		336.4 ± 85.1	
Daily dose of clozapine range (mg)		(200-550)	
Anthropomorphic measurements			
Weight (kg)	85.0 ± 14.7	87.1 ± 15.0	t=-2.91; 0.008
Waist circumference (cm)	98.1 ± 13.0	103.3 ± 15.0	t=-4.22; <0.001
Body Mass Index	27.5 ± 5.1	28.2 ± 5.0	t=-2.90; 0.009
Total Cholesterol (mmol/L)	5.0 ± 0.8	5.5 ± 0.7	t=-2.16; 0.049
Triglycerides (mmol/L)	1.8 ± 1.2	2.0 ± 1.1	*z=-0.77; 0.443

Note: *= variable non-normal distributed; PANSS: Positive and negative Syndrome scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms. PANSS 0-6 scale was used. Ten patients were prescribed typical antipsychotic drugs at some stage of their illness

3.2 Voxel-based Statistical Analysis

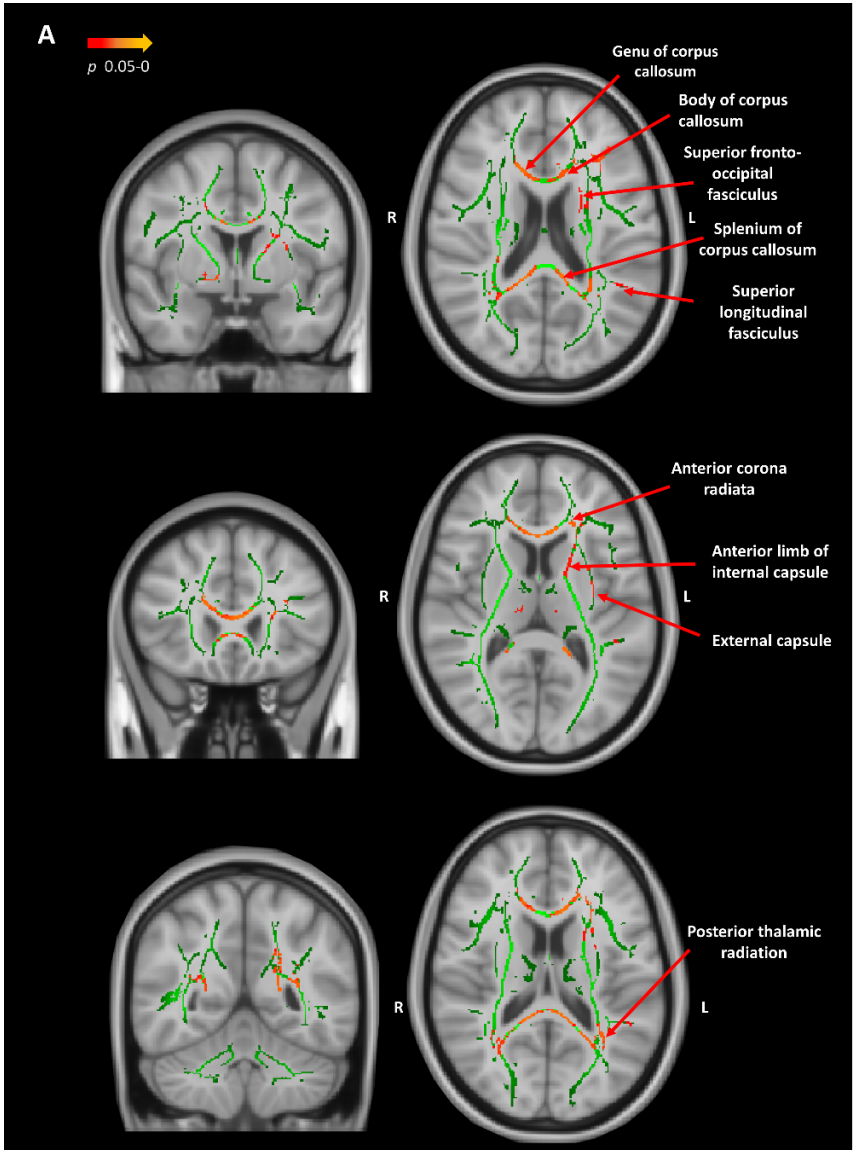
3.2.1 *Baseline comparison*

When investigating differences between groups at baseline, 7 significant clusters (Table 3) were found where the highest statistical group difference was identified using a p-value threshold of $p<0.05$, showing lower FA in treatment-resistant schizophrenia patients compared to controls, consistent with the baseline comparison reported in a smaller sample (Holleran et al., 2013). The clusters included genu, body and splenium of the corpus callosum, bilaterally anterior, superior and posterior corona radiata, posterior thalamic radiation, external capsule, bilaterally anterior and posterior limb of intern capsule, right retrolenticular part of internal capsule, left superior longitudinal fasciculus, left superior fronto-occipital fasciculus, cingulum, bilaterally tapetum; fornix, bilaterally medial lemniscus, cerebellar peduncles and bilaterally cortico-spinal tract (Figure 3A). In terms of baseline neuroimaging metrics predicting clinical change, there was a significant moderate correlation between mean FA at baseline extracted from the largest cluster (Cluster n.7) in patients and change in SANS ($r=0.532$, $p=0.016$) (Figure 3B), but no significant correlations between mean FA at baseline and change in other clinical and anthropomorphic variables or serum level of clozapine at follow-up (Suppl. Table 1). When investigating the baseline differences between those who remitted on clozapine treatment (n=11) and non-responders (n=11) for the largest cluster (Cluster n.7), no significant differences were revealed ($F(1, 18=3.179$, $p=0.091$).

Table 3. Significant white matter clusters of lower fractional anisotropy at baseline and reduce over time between patients and controls

CLUSTER INDEX	REGIONS	VOXELS	MAX	MAX x (vox)	MAX y (vox)	MAX z (vox)	COG x (vox)	COG y (vox)	COG z (vox)
Baseline									
7	- Genu, body, splenium of corpus callosum - Anterior, posterior, superior corona radiata R/L - Anterior limb of internal capsule L - Posterior thalamic radiation R/L - External capsule L - Cingulum (cingulate gyrus) L - Superior longitudinal fasciculus L - Superior fronto-occipital fasciculus L	7249	0.974	95	110	98	94.5	115	92.3
6	- Pontine crossing tract - Corticospinal tract R/L - Medial lemniscus R/L	2493	0.967	72	110	65	84.1	108	66.1
5	- Corticospinal tract R/L - Cerebral peduncle R/L - Anterior limb of internal capsule R - Posterior limb of internal capsule R/L - Retrolenticular part of internal capsule R - Fornix (cres) R	341	0.952	90	89	29	90.5	91.2	37.7
4	- Middle cerebellar peduncle	60	0.952	74	100	44	75.2	102	42.6
3	- Corticospinal tract L	24	0.951	94	100	37	94.9	102	38.1
2	- Superior longitudinal fasciculus	11	0.95	133	81	91	132	79.8	94.2
1	- Inferior cerebellar peduncle L	10	0.95	95	85	30	96.4	85.5	30.3
Over time									
1	- Genu and body of corpus callosum - Anterior and superior corona radiata R/L	1181	0.966	83	150	82	94.2	147	89.9

Note: Table reporting the different significant clusters, their size, and information about their location and contents. Cluster Index: a number for each cluster from 1 to N (larger clusters have bigger numbers); Regions: list of regions included in each cluster, identified using the John Hopkins University (JHU) ICBM_DTI_81 White-Matter Labels atlas (Wakana et al., 2007); Voxels: number of voxels in the cluster; MAX: the value of the z-statistic, 0.95 corresponds to a p-value of 0.05 ; MAX x/y/z: the location of the maximum intensity voxel, given as X/Y/Z coordinate values in voxel coordinates (vox); COG X/Y/Z (vox): the location of the Centre Of Gravity for the cluster (“fslwiki Cluster,” 2013)



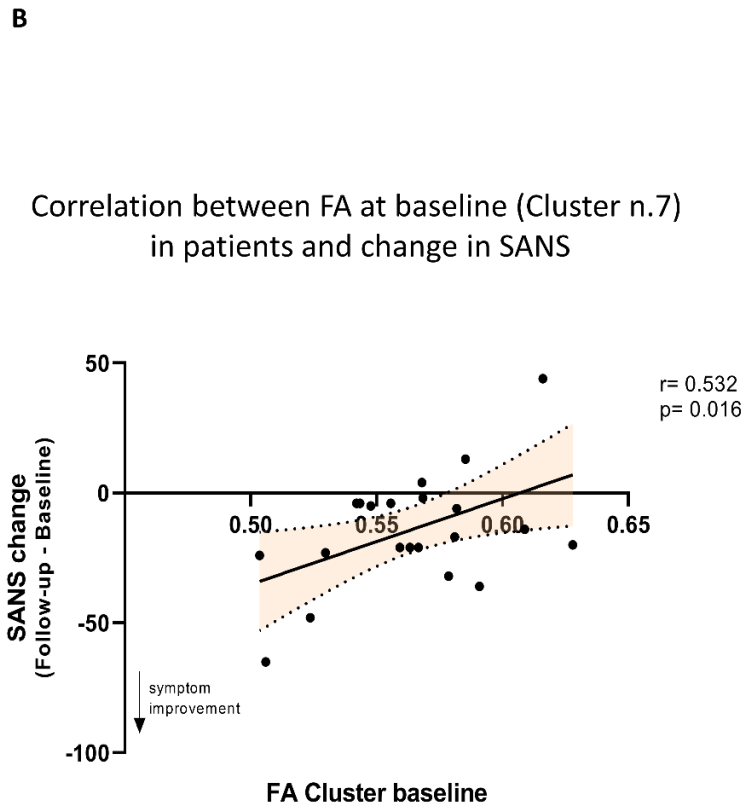


Figure 3. A) Fractional anisotropy (FA) differences at baseline between patients and controls. In the image the green represents the mean skeleton mask, in red (*p=0.05*) to yellow (lowest *p-value*) significant voxels for the biggest cluster (Cluster n.7) where FA was significantly lower in patients compared to controls (p<0.05, threshold-free cluster enhancement, TFCE). In red some of the main regions of lower FA (Fully listed in Table 3) in treatment-resistant schizophrenia patients compared to healthy controls: genu, body and splenium of corpus callosum, left superior longitudinal fasciculus, left superior fronto-occipital fasciculus, bilaterally anterior corona radiata, left interior limb of internal capsule, left external capsule and bilaterally posterior thalamic radiation. B) Correlation between FA at baseline (Cluster n.7) in patients and change in The Scale for the Assessment of Negative Symptoms (SANS). Negative values in SANS change reflect symptoms improvement over time.

3.2.2 *Longitudinal analysis*

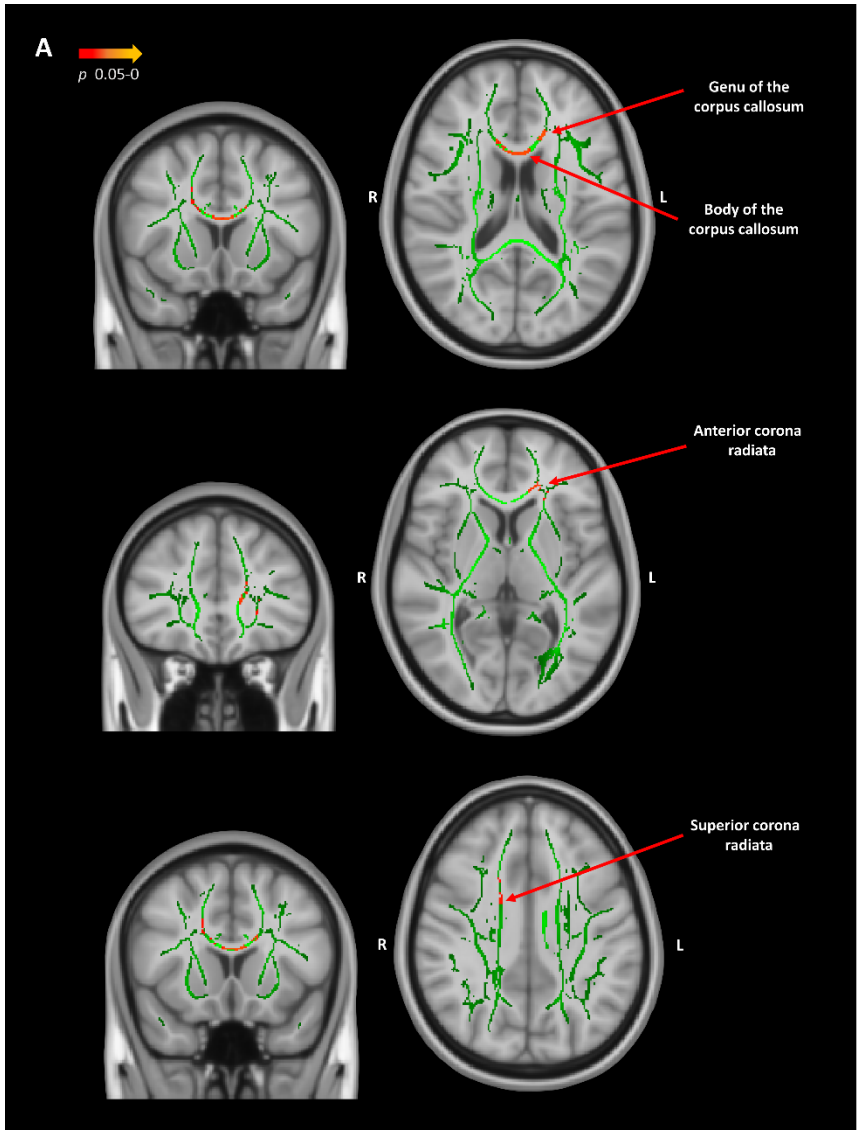
When looking at differences in FA between treatment-resistant schizophrenia patients and controls over time, 1 significant cluster of voxels was found where the highest statistical group difference was identified using a p-value threshold of $p< 0.05$. The cluster showed greater change in FA in the patient group compared to controls over time, reflecting a FA reduction in the genu and body of the corpus callosum and bilaterally in the anterior corona radiata (ACR) and superior corona radiata (SCR) (Figures 4A,4B). There was no correlation between FA change in patients and any change in clinical and anthropomorphic variables or serum level of clozapine at follow-up (Suppl. Table 1). MD and RD were not significantly different between groups over time.

3.3 Network Connectivity

There was no significant difference between patients and controls at baseline ($F(8,33)= 1.66$; $p=0.146$, Table 4) when examining global measures of global efficiency, characteristic path length, clustering coefficient and strength weighted by FA and NOS and correcting for multiple comparisons. Likewise, when investigating the baseline differences between those who remitted on clozapine treatment (n=11) and non-responders (n=11) for global measures, no significant differences were found ($F8,11) = 2.123$, $p=0.123$).

There was no significant overall interaction between time, group and structural global measures ($F(7,280)=2.80$; $p= 0.187$, Table 4). Local measures of clustering coefficient and strength weighted by FA and NOS for thalamus, putamen, caudate and hippocampus (regions demonstrated in our previous study to reduce in volume over time (Tronchin et al., 2020a) did not show a significant overall difference between patients and controls at baseline ($F(15,26)=0.930$;$p=0.546$, Suppl. Table 2) or over time ($F(15,600)=0.747$, $p= 0.737$, Suppl. Table 2). The local measure of strength FA-weighted for caudate and weighted by NOS for thalamus showed a significant difference between patients and controls at the baseline and follow-up, although did not survive multiple comparison correction (Suppl. Table 2).

No subnetwork (weighted by FA and NOS) was identified when examining the time by group interaction.



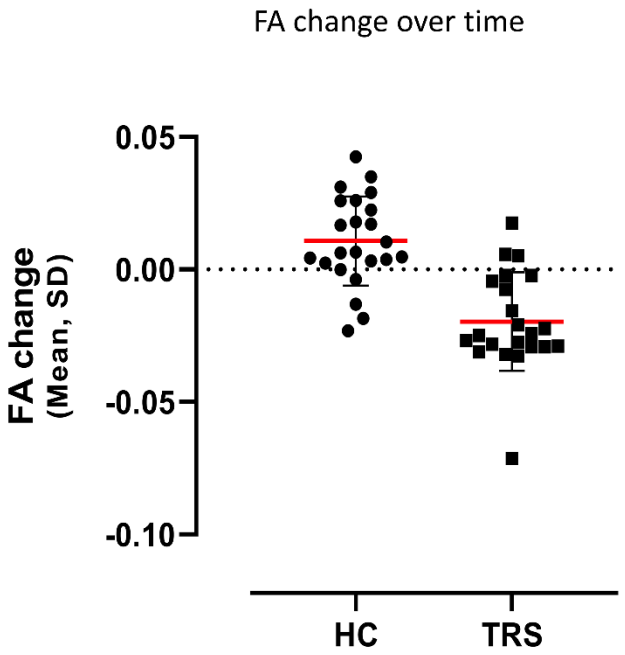


Figure 4. A) Fractional anisotropy (FA) differences over time between patients and controls. In the image the green represents the mean skeleton mask, in red (*p=0.05*) to yellow (lowest *p-value*) significant voxels where the change in FA from baseline to follow-up is significantly different between patients and controls (p< 0.05, threshold-free cluster enhancement, TFCE). In red regions of reduced FA over time in treatment-resistant schizophrenia patients compared to healthy controls in the body and genu of the corpus callosum, anterior corona radiata and bilaterally superior corona radiata. Radiological format is utilised.
B) Fractional anisotropy (FA) change over time. The graph represents the mean FA change from baseline to follow-up in the control and treatment-resistant schizophrenia groups within the significant cluster. The negative values reflect a reduction over time while positive values an increase.

Table 4. Uncorrected means (SD) for each global measure at baseline, follow-up, over time and results of statistical comparisons.

	SCHIZOPHRENIA (n=22)	HEALTHY CONTROL (n =22)	GLM Baseline	SCHIZOPHRENIA (N=22)	HEALTHY CONTROL (N=22)	GLM Follow-Up	GLM Group*Time* Structure
STRUCTURES	**BASELINE**	**BASELINE**	F (8,33) = 1.66, p= 0.146	**FOLLOW-UP**	**FOLLOW-UP**	F (8,33) = 1.32; p= 0.267	F (7,280) = 1.80; p=0.187
GLOBAL MEASURES			*p*			*p*	*p*
Global Efficiency	Means ± SD	Means ± SD		Means ± SD	Means ± SD		
FA	0.216 ± 0.013	0.220 ± 0.009	0.121	0.214 ± 0.014	0.218 ± 0.008	0.189	0.754
NOS	197.492 ± 25.403	213.240 ± 53.485	0.196	204.659 ± 41.149	195.907 ± 28.866	0.890	0.215
Characteristic Path Length							
FA	5.199 ± 0.330	5.072 ± 0.222	0.100	5.252 ± 0.384	5.131 ± 0.194	0.152	0.931
NOS	0.007 ± 0.002	0.007 ± 0.003	0.851	0.007 ± 0.002	0.007 ± 0.003	0.823	0.988
Global Clustering coefficient							
FA	0.5867 ± 0.030	0.594 ± 0.027	0.405	0.586 ± 0.029	0.592 ± 0.028	0.527	0.491
NOS	0.054 ± 0.011	0.050 ± 0.013	0.250	0.056 ± 0.010	0.048 ± 0.011	0.030	0.260
Global Strength							
FA	9.103 ±1.286	9.325 ± 1.110	0.509	9.004 ± 1.278	9.205 ± 1.120	0.562	0.786
NOS	4214.874 ± 700.274	4616.381 ± 1275.146	0.194	4363.440 ± 1060.191	4161.839 ± 709.172	0.858	0.187

Note: FA: fractional anisotropy; NOS: number of streamlines

4. Discussion

The longitudinal voxel-by-voxel based statistical analysis revealed a significant fractional anisotropy reduction over time in white matter microstructure in patients compared to controls, specifically in the genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata. Lower FA in the anterior corona radiata (Kelly et al., 2017) and body of the corpus callosum (Ellison-wright and Bullmore, 2009; Kelly et al., 2017; Vitolo et al., 2017) is the most prominent and reported finding from large cross-sectional studies and meta-analyses in schizophrenia and was again identified amongst the baseline group comparison findings in the current cohort. Our longitudinal findings suggest a progressive focal microstructural white matter abnormality in these critical regions in treatment-resistant schizophrenia patients.

4.1 Fractional anisotropy reduction

The progressive reduction of fractional anisotropy in the white matter of corpus callosum and corona radiata might be due to several factors. FA has been shown to be sensitive to the presence and microstructural organization of white matter fibers (Assaf and Pasternak, 2008). Change in this metric might be attributable for example to demyelination, oedema, inflammation or reduced axonal numbers. Anisotropy measures significantly change when myelin is damaged or when there is an elevated content of water in a tissue (Assaf and Pasternak, 2008; Beaulieu, 2002). Flynn and colleagues (2003) implementing MRI and T2 relaxation, which enables detection of signal related to water distribution in tissue (Whittall et al., 1997), showed reduced myelin water fraction in schizophrenia compared to healthy controls, with the left genu of the corpus callosum displaying the more prominent effect. The reduction was found to be greater in the chronic schizophrenia group compared to first-episode patients suggesting more pronounced abnormalities as the disease progresses (Flynn et al., 2003). A qualitative electron microscopy post-mortem study in patients with schizophrenia, reported signs of apoptosis and necrosis only in the oligodendroglia cells, which are responsible for the production of myelin sheath, that surrounds cell axons (Uranova et al., 2001). The fractional anisotropy reduction we observed in our study may reflect the impact of switching to clozapine on white matter (despite no association with serum level) or

may simply be completely independent of medication and related to the progression on white matter of the underlying pathophysiology of this malignant form of schizophrenia illness.

Corpus Callosum

The corpus callosum is the largest white matter interhemispheric pathway that links regions of the two hemispheres and is responsible for the integration and transfer of inter-hemispheric information to process sensory, motor, and high-level cognitive signals (Goldstein et al., 2020). Its disruption assumes an important role within the dysconnectivity hypothesis in schizophrenia (Friston and Frith, 1995) and is repeatedly reported in the literature using different methodological approaches (Pettersson-Yeo et al., 2011). Callosal white matter alteration has been proposed as a biomarker for chronic schizophrenia (Zhao et al., 2018). A recent cross-sectional DTI study reported low FA in white matter of body and splenium of the corpus callosum in treatment-resistant schizophrenia patients taking clozapine monotherapy compared to healthy controls (McNabb et al., 2019). In contrast to our study, a longitudinal DTI study by Ozcelik-Eroglu et al., (2014) based on 16 schizophrenia patients and 8 controls, reported in the patient group increased FA values in the corpus callosum at follow-up compared to baseline after 12 weeks of clozapine treatment (Ozcelik-Eroglu et al., 2014). There were several methodological differences between this and the current study which may account for these conflicting findings, including the statistical design implemented in the neuroimaging pipeline.

Corona Radiata

The corona radiata consists of a combination of association, projection and callosal fibers (Wakana et al., 2004) and interconnects the cerebral cortex with the thalamus and brainstem and is believed to be involved in information processing. Low FA values in anterior corona radiata have been associated with auditory verbal hallucination severity in schizophrenia (Ćurčić-Blake et al., 2013). McNabb and colleagues, investigating cross-sectional white matter aberrations in treatment-resistant subtypes of schizophrenia using DTI, reported low FA values in the superior and posterior corona radiata in patients on clozapine monotherapy compared to healthy controls (McNabb et al., 2019). Interestingly, consistent with our findings, a 6 weeks longitudinal study by Wang et al., (2013) on drug-naïve patients with schizophrenia reported a significant FA decrease in the white matter of the right corona

radiata in patients treated with antipsychotic medications compared to healthy controls (Wang et al., 2013).

In our previous work (Tronchin et al., 2020a) we found that progressive volumetric reduction of putamen and thalamus in patients after 6 months of clozapine treatment was significantly associated with symptom improvement, and patients who were exposed to higher amounts of clozapine displayed a greater reduction of thalamus volume. In this current study we did not find any correlation between FA reduction over time and change in clinical variables or serum level of clozapine at follow-up. Although the small sample size may have lacked statistical power to detect an association between FA change and clinical variables, any such association is likely to be very weak on the basis of the correlation coefficients detected, in contrast to the moderate associations detected for subcortical volume reduction that we reported in a similar sample (Tronchin et al., 2020a).

This result might suggest that in contrast to our previous study, the progressive fractional anisotropy reduction is not happening in the same individuals as the grey matter reduction and therefore not linked to symptomatic improvement, which could indicate an indiscriminate effect of switching to clozapine treatment without functional implications.

Consistent with this, longitudinal studies of first-episode antipsychotic drug-naive patients with schizophrenia reported FA change in the white matter of the corpus callosum and corona radiata which was not associated with symptom improvement or the dose of antipsychotic medication after 4 weeks or 6 weeks of antipsychotic treatment (Pan et al., 2016; Wang et al., 2013). While we found a reduction of FA over time in the patient group, we did not find a significant change over time between patient and controls in MD and RD. To the best of our knowledge there are no previous studies that have investigated longitudinal changes in MD or RD in schizophrenia patients.

At baseline, we found a widespread lower FA in the white matter of the patient group compared to healthy controls. The 7 significant clusters showed alteration in commissural and temporal lobe cortico-cortical fibres, confirming the findings reported in a previous work from our group on a smaller sample (Holleran et al., 2013). We found a significant correlation between lower FA values at baseline in the largest cluster in patients and improvement in negative symptoms over time. The lower the FA values in this cluster the greater the

improvement in negative symptoms over time in patients. The cluster included callosal, subcortical and fronto-temporal white matter regions. A similar counterintuitive relationship was reported in a longitudinal study by Molina and colleagues (2014) on first-episode schizophrenia, where the clinical improvement after 24 months of treatment was predicted by the baseline inferior frontal cortical thinning (Molina et al., 2014). Our finding warrants replication, but the lack of association between FA change in this region and negative symptom improvement indicates that any mechanism whereby clozapine improved negative symptoms in these patients is not mediated by change in this area.

4.2 Structural Network Connectivity

In our cohort, the structural network connectivity analysis found no difference between patients and controls at baseline or over time when examining global measures. We detected differences between patients and controls at baseline and follow-up on the local measure of strength weighted by FA for caudate and NOS for thalamus, however this did not survive multiple comparisons correction and requires investigation in a larger sample. We additionally did not find evidence that subnetwork connectivity changed differently over the 6-month period in patients compared to controls. A recent cross-sectional structural connectivity study did not find any difference between first episode schizophrenia and healthy controls on measures of clustering, path length or strength, while differences between chronic schizophrenia patients and healthy controls were found (Cea-Cañas et al., 2019). This suggests progression of dysconnectivity over time however the study lacks longitudinal arm. Our results are in contrast to several cross-sectional structural connectivity studies in chronic schizophrenia where altered global and local connectivity has been identified (Van Den Heuvel et al., 2010), as well as altered sub-networks compared to healthy controls (Collin et al., 2013; Zalesky et al., 2011). To date, there are no longitudinal studies that examine structural network connectivity over time in schizophrenia patients compared to healthy controls.

The preserved structural network findings in our study are in contrast to the focal areas of decreased fractional anisotropy at baseline and over time in this cohort of treatment-resistant schizophrenia patients. The lack of network level abnormalities suggests that despite evidence of focal progressive white matter microstructure abnormalities, the wider structural

brain network is not substantially impaired or significantly changed over a period of 6 months after commencement of clozapine treatment.

Strengths and Limitations

The main strength of the study is the longitudinal design combining different neuroimaging techniques to investigate the effect of 6 months of clozapine treatment on white matter microstructure and structural network organisation in a homogenous sample of treatment-resistant clozapine-naïve schizophrenia patients. The Freesurfer longitudinal pipeline used to define cortical and subcortical regions for the reconstruction of the structural network provided a subjects-specific parcellation (Desikan et al., 2006; Reuter et al., 2012; Reuter and Fischl, 2011) to increase the anatomical sensitivity. The reconstruction of estimated white matter trajectories was performed using a deterministic non-tensor-based constrained spherical deconvolution (CSD) algorithm, capable of deconvolving multiple fiber populations within a single voxel rather than the voxel-wise averages obtained using diffusion tensor tractography (Tournier et al., 2008).The CSD-deterministic approach has also been shown to have a lower number of false positives compared to CSD-probabilistic methods (Sarwar et al., 2019). A potential limitation of this study is the sample size, which may have provided limited sensitivity to detect more subtle effects. Additionally, our study did not have a comparative group of schizophrenia patients treated with other antipsychotic medications, in order to tease apart disease progression from treatment effects. However, this comparative group may represent a form of illness that is less malignant than treatment-resistant schizophrenia and subsequently show a different pattern of disease progression.

Conclusion

This longitudinal study demonstrated progressive fractional anisotropy abnormalities in the white matter microstructure of key anterior tracts, such as corpus callosum and corona radiata, and preserved brain structural network in a cohort of refractory schizophrenia. The FA reduction we observed was not associated with clinical improvement or clozapine serum level at follow-up, and may be associated with switching antipsychotic treatment to clozapine or with progression of the underlying pathophysiology of this malignant form of schizophrenia illness. Further longitudinal studies with larger sample size and randomised controlled designs will be necessary to better disentangle such disease related from medication related effects.

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Supplementary Data.

Supplementary Table 1. Correlations between FA change and change in clinical variables in patients

	PANSS Total (Change)		SAPS (Change)		SANS (Change)		GAF (Change)		BMI (Change)		Total Cholesterol (Change)		Serum level of clozapine		Duration of illness	
CLUSTER n.7	r	p	r	p	r	p	r	p	r	P	r	P	r	p	r	p
FA baseline	0.29	0.214	0.39	0.086	0.53	0.016	-0.21	0.371	-0.13	0.658	-0.05	0.857	0.051	0.829	-0.12	0.628
CLUSTER n.1	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
FA change	0.13	0.557	-0.13	0.573	-0.01	0.976	0.04	0.846	-0.03	0.891	-0.16	0.548	-0.23	0.312	-0.17	0.439

Note: PANSS: Positive and Negative Syndrome Scale; SANS: The Scale for the Assessment of Negative Symptoms; SAPS: The Scale for the Assessment of Positive Symptoms.

Supplementary Table 2. Uncorrected means (SD) for each local measure at baseline, follow-up, over time and results of statistical comparisons.

	SCHIZOPHRENIA (n=22)	HEALTHY CONTROL (n =22)	GLM Baseline	SCHIZOPHRENIA (N=22)	HEALTHY CONTROL (N=22)	GLM Follow-up	GLM Group*Time* Structure
STRUCTURES	**BASELINE**	**BASELINE**	F (15,26) = 0.930, p= 0.546	**FOLLOW-UP**	**FOLLOW-UP**	F (16,25) = 1.45, p= 0.195	F (15,600) = 0.747 p= 0.737
LOCAL MEASURES	Means ± SD	Means ± SD		Means ± SD	Means ± SD		*p*
Clustering coefficient FA							
Thalamus	0.998 ± 0.059	1.001 ± 0.088	0.983	0.996 ± 0.0625	0.999 ± 0.0913	0.951	0.790
Caudate	1.120 ± 0.070	1.115 ± 0.089	0.796	1.116 ± 0.069	1.112 ± 0.092	0.852	0.705
Putamen	0.946 ± 0.087	0.969 ± 0.094	0.418	0.946 ± 0.088	0.969 ± 0.094	0.437	0.778
Hippocampus	1.101 ± 0.090	1.119 ± 0.093	0.463	1.098 ± 0.091	1.117 ± 0.100	0.491	0.949
Clustering coefficient NOS							
Thalamus	0.091 ± 0.017	0.090 ± 0.025	0.756	0.094 ± 0.017	0.084 ± 0.017	0.062	0.124
Caudate	0.084 ± 0.016	0.089 ± 0.038	0.665	0.082 ± 0.015	0.075 ± 0.015	0.113	0.211
Putamen	0.096 ± 0.019	0.092 ± 0.030	0.670	0.100 ± 0.019	0.086 ± 0.027	0.079	0.086
Hippocampus	0.087 ± 0.017	0.085 ± 0.026	0.718	0.092 ± 0.020	0.082 ± 0.021	0.132	0.203
Strength FA							
Thalamus	32.869 ± 4.337	33.518 ± 4.514	0.567	32.629 ± 4.355	33.136 ± 4.080	0.595	0.807
Caudate	23.278 ± 3.138	26.714 ± 5.413	**0.013**	22.590 ± 2.883	26.231 ± 5.029	**0.005**	0.620
Putamen	37.755 ± 4.365	36.948 ± 5.798	0.803	37.555 ± 4.6139	36.967 ± 5.797	0.889	0.635
Hippocampus	24.978 ± 3.696	27.029 ± 5.218	0.202	24.585 ± 3.486	26.555 ± 5.153	0.184	0.941
Strength NOS							
Thalamus	18915.272 ± 2920.514	23586.864 ± 7774.532	**0.008**	18478.000 ± 2631.897	21362.136 ± 5992.642	**0.010**	0.343
Caudate	7459.272 ± 1324.049	11873.409 ± 8912.924	0.051	7183.455 ± 2108.761	9096.364 ± 4432.507	0.055	0.329
Putamen	19959.591 ± 3218.098	21333.727 ± 11084.638	0.537	20383.182 ± 3891.720	17864.227 ± 5240.139	0.249	0.193
Hippocampus	27418.864 ± 4247.196	33207.136 ± 17872.649	0.183	9711.727 ± 1784.240	11982.364 ± 8031.365	0.199	0.490

Note: FA: fractional anisotropy; NOS: number of streamlines

Thesis Discussion

This thesis, by combining different neuroimaging techniques explored the interplay between neuroanatomical progression and its cognitive or clinical correlates in samples of patients across different phases of psychosis spanning first episode of illness and treatment-resistant schizophrenia. This is a thesis by publication and incorporates 3 studies that are published/in press. This chapter will provide a summary of each of the three manuscript findings, elaborate further on the implications of the findings in the light of recent research, discuss cognitive, clinical and neuroimaging biomarkers during the course of illness, integrate the results obtained in manuscript 2 and 3, and finally, propose future research directions.

1. Summary of main findings

Manuscript 1. *Cognitive and Clinical Predictors of Prefrontal Cortical Thickness Change Following First-Episode of Psychosis.*
Psychiatry Research: Neuroimaging, https://doi.org/10.1016/j.pscychresns.2020.111100

In this study, based on a cohort of first-episode of psychosis patients, the longitudinal pipeline of Freesurfer v.5.3.0 was employed on 1.5T sMRI to bilaterally parcellate prefrontal cortex at two time points to explore whether impaired executive functioning and negative symptom severity at the onset of psychosis are predictors of prefrontal cortex thinning in subsequent years; and also to clarify whether impaired emotional intelligence at onset of psychosis is associated with loss of orbitofrontal cortical thickness over time. We demonstrated that patients in their first-episode of psychotic illness perform significantly worse on several tests assessing different aspects of executive functions compared to healthy controls, including category fluency, attention, working memory and reasoning & problem solving. The poorer performance at baseline in spatial working memory was a significant predictor of loss of total prefrontal cortical thickness in the initial years after illness onset (Figure 1, page 42). We also found that impairment of emotional intelligence at illness onset was significantly associated with a progressive reduction of orbitofrontal thickness in patients after their first-episode of psychosis (Figure 1, page 42). Finally, we demonstrated a correlation between neuroanatomical progression and clinical variables, specifically, worsening of negative symptoms was associated with prefrontal thickness reduction as the illness progresses (Figure

2, page 44). These results suggest that there is already a cognitive signature at the onset of psychosis, which is associated with poorer outcome in terms of other neuroanatomical and clinical measures.

Manuscript 2. *Progressive subcortical volume loss in treatment-resistant schizophrenia patients after commencing clozapine treatment.*
Neuropsychopharmacology, https://www.nature.com/articles/s41386-020- 0665-4

In this study, based on a cohort of treatment-resistant schizophrenia patients, the longitudinal pipeline of Freesurfer v.5.3.0 was employed with sMRI to bilaterally segment at two time points eight subcortical regions-of-interest: lateral ventricle, thalamus, hippocampus, caudate, putamen, globus pallidus, amygdala and nucleus accumbens. The study investigated whether subcortical structures demonstrate progressive neuroanatomical changes after 6 months of switching to clozapine treatment and whether any such changes were related to clinical variables including treatment response and amount of clozapine taken. We demonstrated a substantial progressive volumetric reduction of the thalamus, hippocampus, caudate, putamen and enlargement of lateral ventricles over a 6-month period in patients compared to controls (Figure 1, page 70). Furthermore, patients who had the greatest symptomatic and functional improvement displayed the largest thalamo-striatal reductions (Figure 2, page 72). We also showed that patients who were exposed to higher amounts of clozapine displayed a greater reduction of thalamus volume (Figure 2, page 72).

Manuscript 3. *White Matter Microstructure and Structural Networks in Treatment-Resistant Schizophrenia Patients After Commencing Clozapine Treatment: A Longitudinal Diffusion Imaging Study.*
Psychiatry Research, https://doi.org/10.1016/j.psychres.2021.113772

In this study, based on a similar cohort as manuscript 2, we further explored progressive neuroanatomical changes, focusing on white matter microstructure and neural networks. We also investigated the potential impact of clozapine and whether any progressive white matter changes were related to clinical variables. The tract-based spatial statistics approach was employed with dMRI to compare changes over time between patients and controls in fractional anisotropy (FA). Changes in structural network organisation and subnetwork

connectivity weighted by FA and number of streamlines were assessed using graph theory and network-based statistics. We demonstrated progressive focal FA abnormalities in key anterior tracts, such as genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata in patients compared to controls (Figure 4, page 109). The brain structural network organisation was preserved in patients compared to controls. The FA reduction was independent of any clinical measures and may reflect progression of the underlying pathophysiology of this malignant form of schizophrenia illness.

2. Cognitive, clinical and neuroimaging biomarkers during the course of the illness

To date, there are no objective biological markers able to inform clinical decision-making in the treatment of schizophrenia (Kraguljac et al., 2021; Samanaite et al., 2018).
Over the years neuroimaging, blood, cognitive and genetic markers have been investigated to enhance the understanding of course of schizophrenia illness and possibly validate new treatment targets, predict response or help in selection of patients for therapy.

Cognitive and clinical predictors

In Manuscript 1 we investigated possible cognitive and clinical predictors at onset of psychosis of later neuroanatomical abnormalities as the illness progresses. Cognitive impairment has been widely reported at onset of illness and has been identified as a risk factor for developing schizophrenia (Fusar-Poli et al., 2013). We identified different aspects of executive functions and emotional intelligence to be impaired at the time of first episode of psychosis, including category fluency, attention, working memory, reasoning & problem solving. Specifically, working memory impairment, was a predictor of loss of total prefrontal cortical thickness as the illness progresses and impaired emotional intelligence predicted reduction of orbitofrontal thickness over time. However, we failed to find clinical predictors at onset of psychosis of latter neuroanatomical abnormalities.

Deficits in neurocognition and social cognition are considered candidates to understand function outcome in schizophrenia (Green et al., 2000; Harvey et al., 2019).

Previously, in our group, cognitive impairment at illness onset has been explored as a potential predictor of clinical and functional outcome. In our cohort of first-episode of psychosis patients cognitive impairment at onset of the illness was not correlated to quality of life at follow-up (Kenney et al., 2015). Nevertheless, impairment in reasoning and problem solving and social cognition at onset of psychosis was a predictor of poor clinical outcome after 3.5 years, specifically worsening of negative symptoms (Kenney et al., 2015). A meta-analysis of 52 studies, including 2692 subjects, reported social cognition as a strong predictor of every day functioning (Fett et al., 2011). Verbal fluency, memory and social cognition may predict remission and relapses of first-episode of psychosis patients, within the first 2 years of the illness, while verbal memory seems to be a strong predictor of persistent negative symptoms and functional outcome (Schubert et al., 2015).

Neuroimaging predictors

Neuroimaging has been suggested as a potential direct measure of the pathophysiological underpinnings of the disease process, hence capable of capturing phenotypic variations in molecular and cellular disease targets, or brain circuits (Kraguljac et al., 2021). Moving from recent onset psychosis further along the illness trajectory to severe schizophrenia for the remainder of the thesis, in our sample of treatment-resistant schizophrenia patients (Manuscript 2 & 3) we explored neuroanatomical biomarkers before the exposure to antipsychotic treatment in order to predict response to clozapine. Using a binary responder variable, no differences were found between who remitted on clozapine treatment and non-responders when examining subcortical structure differences (Manuscript 2). Likewise, we failed to find white matter microstructure and structural brain network differences between patients who responded to 6 months of clozapine treatment and non-responders (Manuscript 3). A recent systematic review on biological predictors of clozapine response (Samanaite et al., 2018), suggested that greater volumes, particularly in frontal cortical regions, are associated with a better response to clozapine treatment, however, further studies have reported conflicting results (Mouchlianitis et al., 2018). Molina et al., reported that in a cohort of antipsychotic-naïve first-episode of psychosis patients, thinner baseline in the right pars opercularis cortex was a predictor of a greater improvement in clinical symptoms after 1 year of clozapine treatment. Although to date, the lack of consistent and reliable results does not

provide strong enough neuroimaging markers to be useful in clinical practice, more longitudinal observational studies are needed in order to track intra-individual variation over time and address the accuracy of prediction at the individual patient level.

3. Neuroanatomical grey and white matter progression in treatment-resistant schizophrenia patients

In the chronic treatment-resistant stage of the illness (Manuscript 2 and 3), when investigating grey and white matter neuroanatomical abnormalities after 6 months of clozapine treatment, there was a consistent progressive volumetric reduction in several subcortical structures (thalamus, hippocampus, caudate and putamen) as well as progressive focal abnormalities in white matter microstructure of key anterior tracts, such as genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata, however, structural network organization was preserved. Although, patients who were exposed to higher amounts of clozapine displayed a greater reduction of grey matter volumes over time, there was no association between medication treatment and change in white matter microstructure.

Results coming from different study designs on the effect of antipsychotic medications mostly suggest volumetric reduction and cortical thinning (Haijma et al., 2013; van Haren et al., 2011; Vita et al., 2015) while the effect on white matter is still not fully understood. One of the interpretations of the marked progressive subcortical volume loss over time (manuscript 2) after commencing clozapine treatment is that there might be a direct link with medication exposure, suggested by the significant relationship we found with serum level of clozapine and subcortical volume loss. The action of clozapine treatment might be related to its ability to attenuate the abnormal glutamatergic function that seems to characterize treatment-resistant schizophrenia patients (Amitai et al., 2012; A. L. Gillespie et al., 2017; Goldstein et al., 2015; Mouchlianitis et al., 2018).

A recent meta-analysis (Merritt et al., 2016) on fifty proton magnetic resonance spectroscopy studies in schizophrenia reported significant elevations in glutamate in the basal ganglia, glutamine in the thalamus and Glx (glutamine and glutamate) in the basal ganglia and medial temporal lobe. The findings of the study suggested the hypothesis that schizophrenia might be associated with excess glutamatergic neurotransmission in these striatal areas. Higher

levels of Glx in the putamen in treatment-resistant schizophrenia patients compared to first-line responders was also reported, suggesting that high level of Glx could represent a trait mark of treatment-resistant schizophrenia and a biomarker of response to clozapine (Goldstein et al., 2015). Goldstein and colleagues (2015) suggested that the difference between treatment-resistant and first-line responders could be related to differences in the activity of the enzyme that converts Glx to glutamate or to a problem with Glx reuptake, resulting in excess Glx (Goldstein et al., 2015). A longitudinal proton magnetic resonance spectroscopy study (de la Fuente-Sandoval et al., 2013) looked at the glutamate levels before and after 4 weeks of atypical antipsychotics treatment in 28 first-episode of psychosis compared to controls and showed that patients had higher levels of glutamate in the associative striatum and cerebellum compared to controls at baseline and a significant decreased and normalization was detected in the associative striatum after 4 weeks of effective treatment with atypical antipsychotics. A longitudinal study looked specifically at the effect of clozapine on brain glutamate in 37 treatment-resistant schizophrenia patients over 12 weeks of treatment. The study reported that after 12 weeks of treatment there was a reduction of glutamate in caudate nucleus. In the study the percentage of glutamate reduction was associated with symptoms improvement (McQueen et al., 2020). The relationship between volumetric loss and glutamate abnormalities has not been fully investigated. Théberge and colleagues (2007), in a longitudinal study on first-episode of psychosis patients explored whether glutamatergic changes in patients with schizophrenia correlate with grey matter losses after 10 and 30 months of treatment with antipsychotic medications (Théberge et al., 2007). The study found that the higher level of glutamate in the thalamus were significantly reduced after 30 months of treatment as well as a widespread grey-matter loss. Parietal and temporal volumetric reduction was corrected with thalamic glutamine loss.

In our study (Manuscript 2) there was counterintuitive progressive volume reduction with clinical and functional improvement: patients who had the greatest symptomatic improvement over time displayed the largest volumetric reduction of thalamus and putamen. What clinical-anatomic associations would be found if this patient cohort had only been assessed when already on clozapine treatment? We decided to further investigate this association cross-sectionally at follow-up, when the cohort has been exposed for 6 months to

clozapine treatment. As already reported in Manuscript 2, the thalamus volume at the follow-up was significantly smaller in the patient group compared to healthy controls, while the putamen, although in the same direction as the thalamus, did not reach statistical significance. When exploring cross-sectionally the relationships between thalamus volume at follow-up and clinical variables at follow-up, a similar association of greater volume reduction with improved symptoms emerged: patients who has lower total PANSS score and SANS score had smaller thalamus volume (Figure 1).

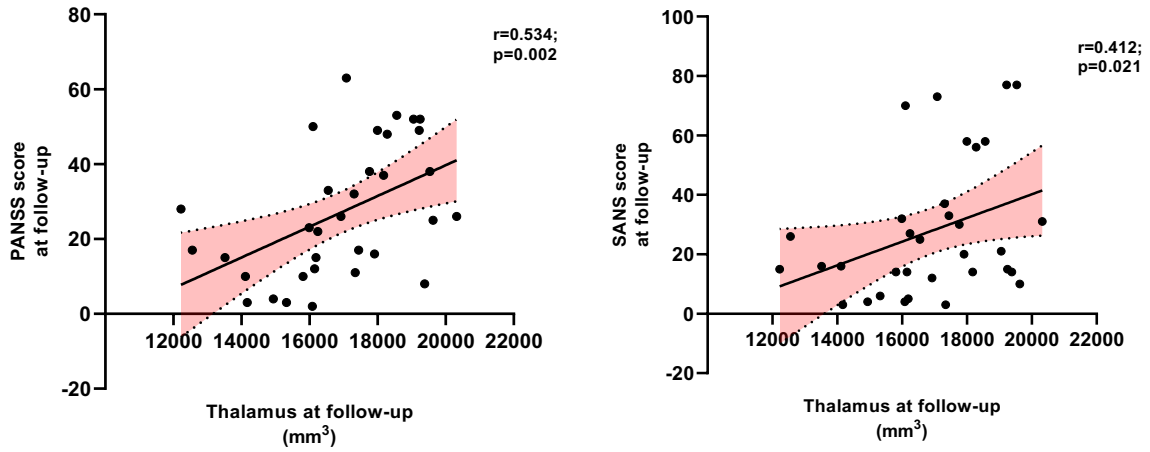

Figure 1. Correlation between thalamus at follow-up and clinical variable at follow-up. Age and sex included as covariates.

Apparent neuroanatomical progression associated with clinical improvement has been reported previously. Scheepers et al., reported in treatment-resistant schizophrenia patients a significant association between reduction of caudate volume and improvement in clinical symptoms (Scheepers et al., 2001). In a 2-years randomised clinical trial on neuroleptic-naïve patients with schizophrenia, the inferior frontal thinning was significantly associated with better clinical and cognitive response to clozapine (Molina et al., 2014).

While we did not find a correlation between clinical variables or serum level of clozapine with changes in white matter microstructure in our sample, we did detect progressive focal abnormalities in the white matter of genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata. Although the small sample size (n=22) may have lacked statistical power to detect an association between FA change and serum level of

clozapine and clinical variables any such association is likely to be very weak on the basis of the correlation coefficients detected [FA change and PANSS Total (r=0.13,p=0.557), SANS change (r=-0.13,p=0.976), serum level of clozapine (r=-0.23, p=0.312)], in contrast to the moderately sized correlations detected for subcortical volume reduction [Thalamus change and PANSS Total change (r=0.42, p=0.021), SANS change (r=0.36, p=0.049), serum level of clozapine (r=-0.44, p=0.010)] that we reported in a similar sample (n=33, Manuscript 2).

In order to further explore whether there was a correlation between grey and white matter progressive abnormalities in patients, a correlation was preformed between the subcortical brain regions which showed a significant volumetric reduction over time and change in the FA cluster reflecting a FA reduction in the genu and body of the corpus callosum and bilaterally in the anterior and superior corona radiata. However, we did not detect any significant correlation between the progressive volumetric reduction in subcortical structures and progressive focal abnormalities in white matter microstructure (Table 1).

Table 1. Correlation between percentage of volumetric change in subcortical brain regions which showed a significant change over time and change in white matter microstructure.

	FA change over time	
Percentage of volumetric change	r_s	p
Lateral Ventricle	0.027	0.907
Thalamus	-0.093	0.680
Hippocampus	-0.154	0.493
Caudate	0.067	0.766
Putamen	-0.211	0.347

Percentage of volumetric change was calculated as follows: 100 × [(volume at follow-up − volume at baseline)/ volume at baseline].

As mentioned above, the neuroanatomical changes of grey matter in schizophrenia reported by longitudinal neuroimaging studies have been mostly explained by the loss of neuropil and positively correlated with cumulative antipsychotic exposure without being functionally disabling (Fusar-Poli et al., 2013; Ho et al., 2011). A recent editorial (Kubicki and Lyall, 2018) highlighted how the trajectory for white matter is still under debate and it is unclear whether white matter changes are progressive in nature and whether this is modulated by the exposure to antipsychotic medications. A pre-clinical study (Konopaske et al., 2008) investigating the effect of antipsychotic exposure on astrocyte and oligodendrocyte numbers in macaque monkey, demonstrated that the lower grey matter glial cell number associated with typical and atypical antipsychotics exposure was due to a lower astrocyte number, whereas the oligodendrocyte number was affected to a smaller degree. A cross-sectional study (Xiao et al., 2018) looked at white matter abnormalities in never treated and treated patients with long-term schizophrenia. The study revealed more widespread alteration of white matter microstructure in never-treated schizophrenia patients than in those receiving long-term treatment with antipsychotics, moreover the degree of FA reduction in the never-treated group showed a faster decline with age when compared to treated-patients and healthy controls, particularly in the genu of the corpus callosum.

Taken together the combined results (Manuscript 2 & 3) bring us to speculate about the diverse nature of the progressive grey and white matter abnormalities we observed in this sample of treatment-resistant schizophrenia patients. We cannot conclude with certainty that the neuroanatomical progression is mediated by the medication exposure since to tease this apart a randomised clinical trial of treatment-resistant schizophrenia patients on or off clozapine with repeated scans would be necessary. However, we can observe that the progressive subcortical and focal white matter changes are not in the identical patients and thus these studies (Manuscript 2 & 3) have identified a divergence of neuroanatomical progression, where progressive atrophy in the thalamo-striatal circuits are linked to clinical and functional improvement, whereas progression in lateral ventricles, hippocampus and white matter are not, as demonstrated in the graphs below (Figure 2). As discussed above, glutamate reduction in caudate nucleus after clozapine exposure has been shown to be associated with symptom improvement (McQueen et al., 2020), while higher levels of glutamate in the thalamus were significantly reduced after 30 months of treatment with antipsychotic medications (Théberge et al., 2007). These findings suggest that the thalamo-

striatal circuits might be a key hub for clinical improvement in treatment-resistant schizophrenia patients and highlight the importance of focusing on the interaction between glutamatergic system, thalamo-striatal circuits and clozapine treatment using large scale longitudinal design studies

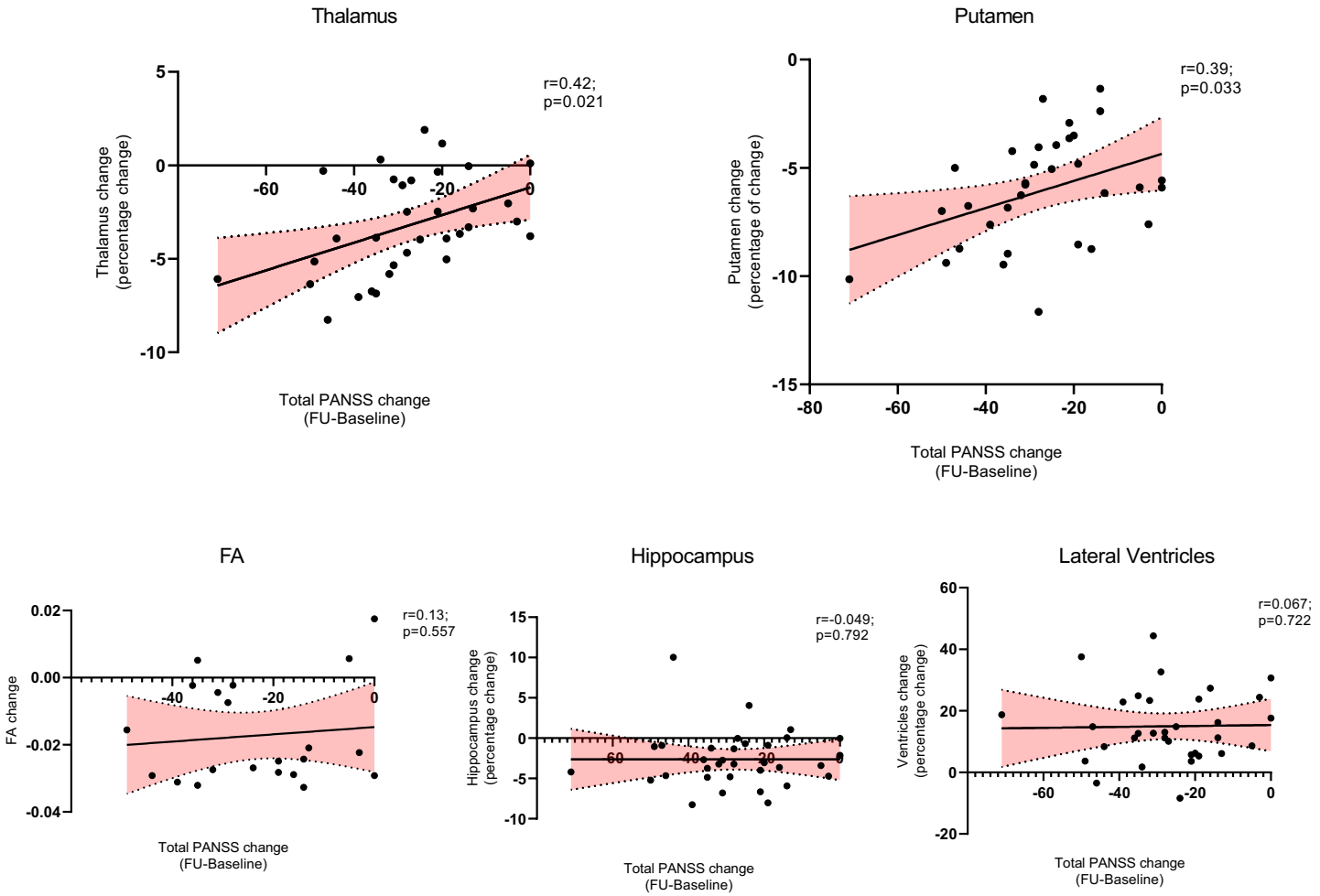

Figure 2. Contrasting correlations between neuroanatomical changes over time and change in total PANSS for different brain regions. Age and sex included as covariates.

4. Future directions

Future research likely to illuminate this area of neuroanatomical progression in psychosis should include large-scale longitudinal studies, randomised designs with repeated scanning cognitive assessment and rich phenotyping, tracking clinically homogenous subgroups, incorporating multimodal imaging (to include physiological or molecular level metrics to better understand the functional consequences of deviant neuroanatomy) linking genotypic variation with neuroimaging metrics, and including neuroimaging assessments as a potential biomarker in clinical trials. For example, emerging neuroimaging techniques and approaches could be very informative and concretely shed a light on the complexity of schizophrenia.

Magnetic Resonance Spectroscopy (MRS)

Magnetic Resonance Spectroscopy is the main technique used to assess the glutamatergic system. As described throughout this thesis, abnormal glutamatergic function seems to characterize treatment-resistant schizophrenia patients (A. Gillespie et al., 2017; Goldstein et al., 2015; Mouchlianitis et al., 2018). The hypothesis that antipsychotics that reduce or control the glutamatergic transmission may be beneficial to schizophrenia treatment (Merritt et al., 2016) brings attention on the importance of investigating this further. Future research on the glutamatergic system should focus on large longitudinal design to enable accuracy of prediction at the individual patient level.

Machine Learning

The application of multivariate data-driven approaches, such as machine learning to structural MR images suggests the possibility of use neuroanatomical biomarkers for direct clinical benefits. Machine learning uses statistical methods to find patterns in large amounts of data (Orrù et al., 2012), for example morphological alterations distributed throughout the brain, which may classify specific psychiatric disorders. Recently studies employed machine learning to classify schizophrenia using MR images and obtained an accuracy rate of 68.1%-85.0% (Pinaya et al., 2016; Xiao et al., 2019). Vieira and colleagues assessed the reliability of detecting neuroanatomical changes in the early stages of first-episode of psychosis using machine learning in 5 independent datasets (514 FEP and 444 matched controls). The study

reported lower classification accuracies than previous studies (50%-70%) (Vieira et al., 2020). Sample size directly affects the reliability of machine learning and accuracy is seen to decrease with sample size (Arbabshirani et al., 2017), therefore future studies should apply this technique to large multi-site datasets. Machine learning has been also used to make predictions on diagnosis, prognosis and response to treatment in psychosis using structural imaging data (Koutsouleris et al., 2009) as well as non-imaging data (Mechelli et al., 2017).
Reliable data from machine learning could help to stratify patients in clinical trials for new treatments (Dazzan, 2014) and identify particular structural characteristic of the brain in patients with schizophrenia.

Free-water imaging

Free-water imaging, a novel non-invasive in vivo diffusion-weighted magnetic resonance imaging (dMRI) technique can differentiate between neuroinflammation that is expected to be expressed in the water content in the extracellular space in the brain, and white matter deterioration that is expected to be expressed in the tissue itself (Pasternak et al., 2009), in order to identify neuroinflammatory biomarkers for targeting early therapeutic innervations. This technique could be beneficial not only applied to first-episode of psychosis patients, but also to more chronic stages of the illness, when resistant to treatment. It has been suggested that some inflammatory biomarkers might be a reliable predictor of treatment resistance (Noto et al., 2015), hence the application of free-water imaging could shed a light on the effect of clozapine treatment on refractory schizophrenia patients and clarify its anti-inflammatory action during the acute symptomatic phase of the illness.

5. Conclusion

This thesis by tracking the longitudinal interplay between neuroanatomy, cognition, clinical presentation and medication treatment in cohorts of first-episode psychosis and treatment-resistant schizophrenia patients contributes to the understanding of biomarker progression in schizophrenia and paves the way for further investigations.
Taken together our results indicate that at onset of psychosis working memory and emotional intelligence impairment represents a trait marker of progressive prefrontal thinning as the illness progresses. We also demonstrated that, in patients who experience first episode of

psychosis, worsening of negative symptoms is associated with prefrontal thickness reduction over time, indicating a functional consequence of anatomical progression in psychosis.

In those with the chronic treatment resistant stage of the illness, there is a consistent progressive volume reduction in several subcortical structures (thalamus, caudate, hippocampus, putamen) as well as progressive focal abnormalities in the white matter microstructure of key anterior tracts, including corpus callosum and corona radiata, but a preserved brain structural network. However, our findings suggest a divergence of neuroanatomical progression, where progressive atrophy in the thalamo-striatal circuits are linked to clinical and functional improvement, whereas no such association is found with longitudinal progression in lateral ventricles, hippocampus and white matter.

This thesis confirms the importance of investigating the neurocognitive dimension at illness onset in order to enhance understanding of the functional consequences of illness progression as well as identifying potential markers at illness onset. It also highlights the potential role of the thalamo-striatal circuits in tracking recovery in treatment-resistant schizophrenia patients, suggesting that the investigation of the interaction between these circuits, glutamatergic system, and clozapine treatment using large scale longitudinal design studies could significantly contribute to the identification of biomarkers in refractory schizophrenia.

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