

## RESEARCH ARTICLE

# Extensive Gray Matter Volume Reduction in Treatment-Resistant Schizophrenia

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## Abstract

**Background:** Approximately one-third of people with schizophrenia are treatment-resistant and some do not achieve remission with clozapine, the gold-standard antipsychotic medication for treatment-resistant schizophrenia. This study compared global and regional brain volumes between treatment-responsive and treatment-resistant patients with schizophrenia, including a group of patients who were clozapine-resistant.

**Methods:** T1-weighted brain MRIs were obtained on a 3T scanner in 20 controls and 52 people with schizophrenia who were selected based on their symptomatic responses to antipsychotic medication: 18 responded well to first-line atypical antipsychotics (FLR), 19 were treatment-resistant but responsive to clozapine monotherapy (TR), and 15 were ultra-treatment-resistant and did not respond to clozapine (UTR). Treatment groups were matched for disease duration and current psychopathology. SIENAX and FSL-VBM were used to investigate differences in the global brain, gray matter (GM), white matter, ventricular cerebrospinal fluid volumes, and regional GM volumes.

**Results:** GM volume was significantly reduced in the TR and UTR groups compared with controls and the FLR group ( $p < 0.05$ ). GM volume was significantly reduced in TR patients compared with FLRs in the superior, middle, and inferior temporal gyri, pre- and post-central gyri, middle and superior frontal gyri, right supramarginal gyrus, and right lateral occipital cortex. UTR patients showed reduced GM compared with FLRs in their right parietal operculum and left cerebellum. No significant volume differences were observed between TR and UTR groups.

**Conclusions:** These differences are unlikely to be solely due to medication effects, and reduced GM volume in treatment-resistant schizophrenia may represent an accelerated disease course or a different underlying pathology.

**Keywords:** clozapine, MRI, treatment-resistant schizophrenia, voxel-based morphometry

## Introduction

It is well established from post-mortem and *in vivo* neuroimaging studies that abnormalities in brain structure are a feature of schizophrenia. There is evidence of volume reduction compared with healthy subjects in both the gray matter (GM) and white matter (WM) of many brain regions, but in particular in

the temporal and frontal lobes (Highley et al., 1999; Selemon et al., 2002; Honea et al., 2005; Vita et al., 2012; Haijma et al., 2013). Reduced brain volumes are seen in people with first-episode schizophrenia, suggesting that the changes that lead to this observation may be neurodevelopmental in origin (Asami

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et al., 2012; Rais et al., 2012; Vita et al., 2012), but greater tissue loss over time has been observed in patients with established schizophrenia compared with healthy subjects, suggesting that there are also progressive brain changes throughout the disease (Andreasen et al., 2011; Olabi et al., 2011). These studies all demonstrate the structural heterogeneity between subjects and regions and over time, however, which may be related to some extent to the diversity of symptoms patients experience and their individual responses to antipsychotic medication.

Treatment-resistant schizophrenia is defined as either a poor or no symptomatic response to multiple (at least two) antipsychotic trials lasting an adequate duration (at least six weeks) and at a therapeutic dose (American Psychiatric Association, 2004). Approximately 30% of people with schizophrenia do not respond adequately to first-line antipsychotics and are considered treatment-resistant (American Psychiatric Association, 2004). Clozapine is the gold-standard antipsychotic for people with treatment-resistant schizophrenia due to its superior efficacy, although some people prescribed clozapine for treatment-resistant schizophrenia still remain symptomatic and are considered ultra-treatment-resistant (Mouaffak et al., 2006). Recently, Farooq et al. (2013) suggested using treatment response to classify subtypes of schizophrenia, which has several advantages over current classification systems and could establish a way to distinguish variations of the illness that may better represent differences in the underlying pathophysiology. Determining factors associated with treatment response may help to identify mechanisms responsible for treatment resistance and could aid in the early identification of people for whom clozapine or even combined antipsychotic treatment may be appropriate.

In first-episode schizophrenia, it has been shown that people who respond poorly to antipsychotic medication have greater structural brain abnormalities than those who respond well to treatment over periods of 12–18 weeks (Bodnar et al., 2010; Szeszko et al., 2012; Palaniyappan et al., 2013). Whilst it is valuable to investigate the treatment response and outcomes in first-episode patients, it is unclear whether patients who do not respond well to initial antipsychotic treatment will be treatment-resistant using the current criteria and what the potential impact of clozapine is on these patients. In addition, the structural abnormalities observed in first-episode patients may be acute and related to the short-term treatment response.

It is therefore important to investigate whether there are differences in brain morphology associated with a lack of response to first-line antipsychotics and treatment resistance in schizophrenia also. A region-of-interest analysis has shown that treatment-resistant patients had significantly less GM in frontal and occipital lobes and significantly more WM in the frontal, parietal, and occipital regions compared with controls (C), whilst no significant differences between non-treatment-resistant patients and controls were observed (Molina et al., 2008). Conversely, this same study found that treatment-resistant patients who had commenced clozapine showed significant increases in frontal, parietal, and occipital GM over 6 months compared with controls, and a more marked decrease in frontal, parietal, and occipital WM, suggesting that clozapine may alter patterns of tissue loss. However, treatment-resistant and non-treatment-resistant patients were not directly comparable, as the former group had higher mean Positive and Negative Syndrome Scale (PANSS) scores at baseline and, in addition, only began clozapine at study entry, which may mean the findings were due to an acute response to medication. A second study using voxel-based morphometry (VBM) to directly compare treatment-resistant and non-treatment-resistant patients found smaller

volumes of the basal ganglia, precentral, and right medial occipital brain regions in treatment-resistant compared with non-treatment-resistant patients (Molina et al., 2010). However, none of the treatment-resistant patients were taking clozapine. More recently, a large study demonstrated reduced left dorsolateral prefrontal cortex in treatment-resistant patients compared with non-treatment-resistant patients (Zugman et al., 2013). However, the treatment-resistant patients were taking a variety of antipsychotics, including typical antipsychotics.

In summary, further studies investigating comparable groups of treatment-resistant and non-treatment-resistant patients and the role of clozapine are required to ascertain whether there are underlying differences in the pathophysiology of these patients. The specific aim of this study was to use 3 Tesla MRI to examine the brain volumes and patterns of GM loss in three distinct groups of patients with established schizophrenia who were matched for disease duration and psychopathology: (1) first-line atypical antipsychotic responders; (2) treatment-resistant but responding well to clozapine; and (3) ultra-treatment-resistant (clozapine-resistant). This is the first study to include a group of patients with ultra-treatment-resistant schizophrenia. We hypothesised that, despite clozapine therapy, treatment-resistant patients would show greater tissue loss compared with first-line antipsychotic responders, and that ultra-treatment-resistant patients would show greater tissue loss than both first-line responders and treatment-resistant (clozapine-responsive) patients. We also aimed to investigate the association between regional GM loss and psychopathology by regression with the PANSS and antipsychotic doses.

## Methods

### Participants

Fifty-two people diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders criteria (American Psychiatric Association, 2000) were recruited from inpatient and outpatient mental health services for a study investigating treatment-resistant schizophrenia. Twenty control subjects without histories of a psychiatric illness were directly recruited from the same geographic location through staff involved in the study. Selection of subjects for this study was based on a medication history that matched criteria for the treatment groups we were investigating (see below). All participants were aged between 18 and 45 years, and exclusion criteria included a history of any other Axis I disorder, history of a head injury (loss of consciousness greater than three minutes), other significant physical disorders that were uncontrolled and may have impacted brain structure or functioning (eg, hypertension), active substance dependence, and contraindications for MRI acquisition. All participants with schizophrenia were taking atypical antipsychotics and were clinically stable for at least six weeks at the time of the investigation to minimize the impact on data of acute relapse and/or large doses of medication. At the time of recruitment, average duration of antipsychotic treatment at the current dose was 378 days. The study was approved by the Northern X Regional Ethics Committee (Health and Disability Ethics Committee, New Zealand), and written informed consent was obtained from all participants after description of the study.

### Medication History and Clinical Assessment

Patients were assigned to one of three groups based on their treatment history and response to antipsychotic medication, which identified them as a first-line antipsychotic responder (FLR, atypical

non-clozapine antipsychotic), treatment-resistant (TR, responding to clozapine), or ultra-treatment-resistant (UTR, clozapine-resistant; Table 1). UTR participants were put on alternative or additional antipsychotics because their symptoms did not respond to clozapine alone, and was not due to clozapine-induced side effects that led to a decrease in clozapine dose and the subsequent need for additional antipsychotics. Treatment resistance was defined as a lack of significant symptom improvement following at least two trials of different antipsychotic agents at therapeutic doses for a minimum of 6 weeks each (American Psychiatric Association, 2004). Each patient's antipsychotic dose at the time of assessment was converted to chlorpromazine equivalents (CPZEs) using formulae with power transformation (Andreasen et al., 2010), except for amisulpride, which in the absence of a power formula was calculated using expert consensus regarding antipsychotic dosing (Gardner et al., 2010). Duration of illness was calculated as the interval between first contact with psychiatric services and study assessment. Psychotic symptoms were evaluated at the time of MRI using the PANSS (Kay et al., 1987). Only four patients had been ill for less than three years (three FLR and one TR), indicating that most patients were chronically ill. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; WHO ASSIST Working Group, 2002) was used to assess past and present substance use; all participants provided a urine sample to test for current recreational drug use.

### Magnetic Resonance Imaging

All participants underwent MRI in a Siemens 3T Skyra scanner to obtain a T1-weighted MPRAGE. A 32-channel head coil was used for all acquisitions except five, in which a 20-channel head coil was used (2 FLR, 1 TR, 2 UTR). This was due to some participants having a larger head size, which led to discomfort when using the slightly smaller 32-channel coil. Acquisition parameters were: repetition time = 1900 ms; echo time = 2.39 ms; inversion

time = 900 ms; flip angle 9°; one repetition; parallel imaging (GRAPPA) factor of 2; field-of-view 230 x 230 mm; matrix = 256 x 256; resulting in 0.9 x 0.9 x 0.8 mm voxels. Three-dimensional gradient distortion correction was applied to images to correct for non-linear changes in the magnetic field that could lead to image warping. All subjects had good GM/WM contrast and no or minimal artefact.

MRI data was analyzed with FSL version 5.0.2 (<http://fsl.fmrib.ox.ac.uk/fsl>). Whole brain, GM, and WM tissue volumes, normalized for subject head sizes, were estimated with SIENAX (Smith et al., 2002). Voxel-wise differences in GM volume between groups were investigated using FSL-VBM (Douaud et al., 2007). Structural images were brain-extracted and the GM was segmented before being registered to MNI-152 standard space using non-linear registration. The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific GM template. All native GM images were subsequently non-linearly registered to this study-specific template and modulated to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated GM images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (~7 mm FWHM).

### Statistical Analyses

Analyses of demographic and clinical variables and brain volumes were performed using SPSS version 20.0 (SPSS Inc.). Age, sex, and disease duration were matched between groups at the design stage. Group differences in demographic and clinical data were analyzed using a one-way between-subjects ANOVA with Tukey post hoc tests for continuous variables, and Fisher's Exact Test for categorical variables. Linear regression models were employed to compare differences in brain volumes between groups, using a group indicator and including age and sex as covariates. VBM

**Table 1.** Demographic Data of Different Schizophrenia Treatment Groups and Controls.

	Controls (n = 20)	First-line antipsychotic responders (n = 18)	Treatment- resistant (n = 19)	Ultra-treatment-resistant (n = 15)	
Age (years)	33.3 (8.4)	32.2 (7.9)	33.3 (8.0)	34.3 (7.1)	F(3, 68) = 0.187, p = 0.905
Sex (M:F)	17:3	14:4	14:5	13:2	p = 0.766 (FET)
Education (years)	13.9 (2.0)	12.3 (2.8)	11.1 (2.6)	12.1 (2.0)	F(3, 68) = 4.498, p = 0.006 (C > TR)
Illness duration (years)	-	10.0 (7.9)	13.0 (6.9)	11.4 (4.7)	F(2, 49) = 0.899, p = 0.414
Duration of prodromal phase (months)*	-	12.5 (14.7)	10.4 (17.8)	23.4 (26.0)	F(2, 49) = 2.060, p = 0.138
Pre-morbid IQ (z-score)	-0.52 (0.97)	-1.04 (1.13)	-0.79 (0.99)	-1.46 (1.14)	F(3, 68) = 2.428, p = 0.073
PANSS	-	60 (11)	57 (12)	62 (11)	F(2, 49) = 0.788, p = 0.460
PANSS positive	-	13 (5)	11 (4)	13 (5)	F(2, 49) = 1.106, p = 0.339
PANSS negative	-	17 (6)	18 (7)	20 (7)	F(2, 49) = 1.093, p = 0.343
PANSS general	-	29 (6)	28 (6)	29 (4)	F(2,49) = 0.210, p = 0.811
ASSIST score	26.4 (17.3)	50.2 (21.4)	36.4 (21.1)	45.7 (23.4)	F(3,68) = 4.853, p = 0.004
Current antipsychotics used	-	Olanzapine (n = 8) Risperidone (n = 6) Aripiprazole (n = 3) Amisulpride (n = 1)	Clozapine (n = 19)	Clozapine + Aripiprazole (n = 4) Clozapine + Amisulpride (n = 4) Clozapine + Risperidone (n = 3) Clozapine + Quetiapine (n = 2) Aripiprazole + Quetiapine (n = 2)	
Current antipsychotic medication dosage†	-	421.6 (191.6)	459.9 (221.9)	847.4 (342.7)	F(2,49)=13.785, p < 0.001 (FLR & TR < UTR)

All data given as mean (SD) except sex and antipsychotics used. Bolded p values are statistically significant at p < 0.05.

ASSIST, Alcohol, Smoking and Substance Involvement Screening Test; C, control; FET, Fisher's Exact Test; FLR, first-line antipsychotic responder; PANSS, Positive and Negative Syndrome Scale; TR, treatment-resistant; UTR, ultra-treatment-resistant.

\*Time prior to first psychiatric contact based on treating physician's notes and self-report. †Chlorpromazine mg equivalents.

analyses to investigate voxel-wise differences in the GM density between groups were performed using permutation-based non-parametric testing (5000 permutations) implemented using Randomise (FSL version 5.0.2). Threshold-free cluster enhancement was used, and family-wise error (FWE)-corrected values (to correct for multiple comparisons across space) of  $p < 0.05$  were considered significant. Age and gender were used as covariates in VBM analyses. To obtain the anatomical localization of significant cluster peaks, the Harvard-Oxford cortical structural atlas was used (Desikan et al., 2006), and only clusters consisting of more than 20 voxels are reported. In addition, we performed separate regression analyses using Randomise with: (1) the antipsychotic dosage (CPZE) within the patient group, to explore whether current medication dose is related to structural abnormalities; and (2) PANSS scores, to explore whether psychopathology is related to structural abnormalities.

## Results

Mean age, sex distribution, illness duration, PANSS scores, and pre-morbid IQs did not differ significantly between our subject groups (Table 1). A significant difference in years of education was found, with the treatment-resistant group having fewer years of education than control subjects. In addition, a significant difference in total ASSIST scores was shown, with the FLR and UTR groups having higher scores than control subjects. When looking at the different classes of recreational drugs, use of tobacco, cannabis, inhalants, and hallucinogens were found to be significantly different between groups (tobacco  $C < FLR$  and  $C < UTR$ ; cannabis  $C < FLR$ ; inhalants  $C < TR$ ; hallucinogens  $C < UTR$ ). There were no significant differences between groups in scores for alcohol, cocaine, amphetamines, sedatives, or opioids.

### Whole Brain Differences

The mean brain volumes in controls and the three treatment groups are presented in Table 2, adjusted for age and sex. Significantly smaller whole brain and WM volumes were seen in all patient groups compared with controls, and GM volumes

were significantly smaller in TR and UTR treatment groups compared with both controls and FLR. Only UTR patients showed significantly larger ventricular CSF volumes compared with controls and the FLR group.

### VBM GM Differences Between Schizophrenia Treatment Groups and Controls

VBM analyses revealed an extensive bilateral pattern of decreased GM volume in UTR patients compared with controls, with the two largest clusters bilaterally including the superior and middle temporal gyri, Heschl's gyrus, central and parietal operculum, post-central gyrus, and insula, but with clusters also seen in the left cerebellum, bilateral ventromedial prefrontal cortex, right lateral occipital cortex, and bilateral anterior cingulate gyrus (Table 3 and Figure 1). A less widespread pattern of reduced GM was seen in TR patients compared with controls, with significant clusters observed in the right central operculum (MNI peak coordinates  $x = 64, y = -8, z = 10$ ; 136 voxels;  $p = 0.019$ ) and right inferior temporal gyrus (MNI peak coordinates  $x = 56, y = -36, z = -20$ ; 106 voxels;  $p = 0.033$ ). In FLR, increased GM volume was observed compared with control subjects in the right lateral occipital cortex (MNI peak coordinates  $x = 42, y = -72, z = -14$ ; 30 voxels;  $p = 0.037$ ). Reverse contrasts showed no regions where TR or UTR had significantly more GM volume than controls, or FLR had significantly less GM volume than controls.

### VBM GM Differences Between Schizophrenia Treatment Groups

Both TR and UTR patient groups showed areas of significantly less GM compared with the FLR group. Areas showing significantly less GM in TR patients compared with FLRs included the superior, middle, and inferior temporal gyri, pre- and post-central gyri, middle and superior frontal gyri, supramarginal gyrus, and lateral occipital cortex (Table 4, Figure 2). Two significant clusters were observed when comparing UTR patients with FLRs, in the right parietal operculum (MNI peak coordinates  $x = 50, y = -26, z = 18$ ; 594 voxels;  $p = 0.008$ ) and the left cerebellum (MNI

Table 2. Normalized Volumes and Significant Group Differences.

	Controls (n = 20)	Antipsychotic responders (n = 18)	Treatment-resistant (n = 19)	Ultra-treatment-resistant (n = 15)	Mean difference (cm <sup>3</sup> ) (95% CI, p-value)
Whole brain volume (cm <sup>3</sup> )	1603.0 (58.5)	1560.8 (66.0)	1530.1 (68.9)	1501.7 (55.7)	C > FLR 42.2 (8.6–75.8, $p = 0.015$ ) C > TR 72.9 (39.8–106.1, $p < 0.001$ ) C > UTR 101.4 (66.2–136.5, $p < 0.001$ ) FLR > UTR 59.1 (22.9–95.5, $p = 0.002$ )
Gray matter volume (cm <sup>3</sup> )	830.3 (40.8)	817.3 (46.6)	781.5 (38.5)	764.1 (27.5)	C > TR 48.8 (30.7–67.0, $p < 0.001$ ) C > UTR 66.1 (46.9–85.4, $p < 0.001$ ) FLR > TR 35.9 (17.3–54.4, $p < 0.001$ ) FLR > UTR 53.2 (33.3–73.1, $p < 0.001$ )
White matter volume (cm <sup>3</sup> )	772.8 (32.7)	743.5 (37.7)	748.7 (42.7)	737.5 (38.1)	C > FLR 29.3 (5.0–53.5, $p = 0.019$ ) C > TR 24.1 (0.1–48.1, $p = 0.049$ ) C > UTR 35.2 (9.8–60.7, $p = 0.007$ )
Peripheral cortex gray matter volume (cm <sup>3</sup> )	676.7 (35.5)	664.9 (40.8)	628.5 (35.1)	620.2 (23.8)	C > TR 48.2 (32.7–63.8, $p < 0.001$ ) C > UTR 56.6 (40.1–73.0, $p < 0.001$ ) FLR > TR 36.4 (20.5–52.3, $p < 0.001$ ) FLR > UTR 44.7 (27.7–61.7, $p < 0.001$ )
Ventricular CSF volume (cm <sup>3</sup> )	29.3 (10.2)	30.4 (9.7)	32.0 (12.7)	38.6 (13.0)	C < UTR -9.4 (-16.0 to -2.7, $p = 0.007$ ) FLR < UTR -8.3 (-15.2 to -1.4, $p = 0.019$ )

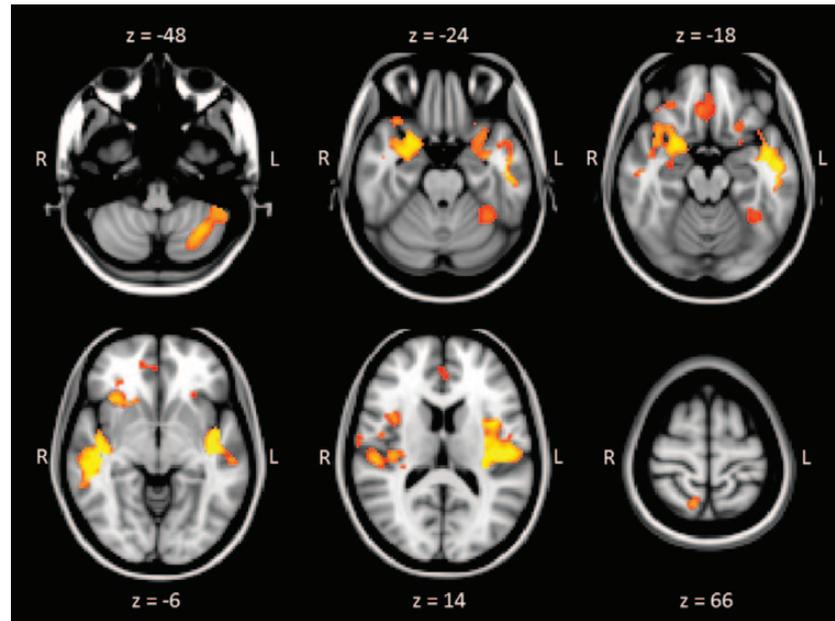
Data are given as mean (SD). Volumes adjusted for age and sex.

C, control; CSF, ; FLR, first-line antipsychotic responder; TR, treatment-resistant; UTR, ultra-treatment-resistant.

**Table 3.** Local Peaks of Significant Clusters Showing Reduced Gray Matter in the Ultra-Treatment-Resistant Group Compared with the Controls.

Cortical region	Side	Cluster size	MNI peak coordinates (mm)	Local maximum p value*
Superior temporal gyrus	Right	3896	48 -22 -6	0.002
Superior temporal gyrus (planum polare)	Left	3121	-42 -6 -18	<0.001
Cerebellum	Left	815	-32 -60 -48	0.010
Ventromedial prefrontal cortex	Bilateral	363	6 32 -16	0.021
Cerebellum	Left	217	-32 -50 -24	0.027
Lateral occipital cortex	Right	178	12 -58 66	0.018
Anterior cingulate gyrus	Bilateral	55	0 42 14	0.039

\*With family-wise error correction for multiple comparisons across space.  $p < 0.05$  and  $>20$  voxels.



**Figure 1.** Reduced gray matter in ultra-treatment-resistant patients compared with controls, overlaid on the MNI-152 template brain.

peak coordinates  $x = -42$ ,  $y = -40$ ,  $z = -44$ ; 145 voxels;  $p = 0.016$ ). Direct comparison of the two treatment-resistant patient groups showed no significant differences. Reverse contrasts showed no regions where TR or UTR schizophrenia groups had significantly greater GM volume than the FLR group.

### Correlation of GM with Antipsychotic Dosage and PANSS Scores

There was no association between GM volumes and either antipsychotic dose or PANSS scores (total, positive, negative, or general) in patients.

### Discussion

This study investigated structural brain differences between people with schizophrenia whose symptoms responded to first-line conventional antipsychotic medication and those who are treatment-resistant (but clozapine-responsive) and ultra-treatment-resistant (clozapine-resistant). Our main finding is that TR and UTR schizophrenia patients show a widespread GM volume reduction, including areas of the temporal, parietal, frontal, and occipital lobes, compared with those people with schizophrenia who respond to first-line atypical antipsychotic medication. Our study is unique in several key areas compared with previous MRI

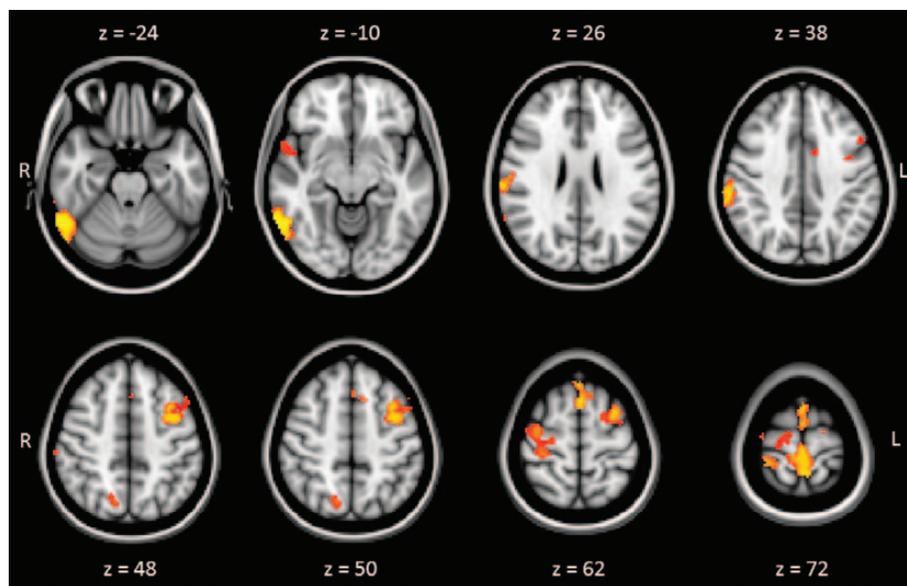
studies investigating treatment response and treatment-resistance: (1) we defined our schizophrenia treatment groups according to current guidelines on treatment resistance, and included a separate group of patients who were treatment-resistant to clozapine; (2) all patients who were treatment-resistant were receiving clozapine (or had received clozapine in the past if ultra-treatment-resistant); (3) schizophrenia treatment groups were matched for disease duration and psychopathology; and (4) we used advanced imaging techniques, including 3T MRI and unbiased VBM analytical methods.

Reduced whole brain, WM, and GM volumes in patients with schizophrenia relative to control subjects were observed, although the reduction in GM volume in first-line antipsychotic responders did not reach statistical significance. VBM corroborated our findings of GM volume reduction in TR and UTR schizophrenia treatment groups compared with controls, whereby a similar pattern of regional GM tissue loss as in other studies of schizophrenia was seen, focused on the superior and middle temporal gyri but also including medial frontal areas and the anterior cingulate cortex (Honea et al., 2005; Meisenzahl et al., 2008; Palaniyappan et al., 2010; Tanskanen et al., 2010; Colibazzi et al., 2013; Zierhut et al., 2013; Liu et al., 2014; Ohtani et al., 2014). Our results are comparable with those of Molina et al. (2008), who found that TR patients had significantly less GM in the frontal and occipital regions relative to controls, whilst

**Table 4.** Local Peaks of Significant Clusters Showing Reduced Gray Matter in the Treatment-Resistant Patients Compared with the First-Line Antipsychotic Responders.

Cortical region	Side	Cluster size	MNI peak coordinates (mm)	Local maximum <i>p</i> value*
Inferior temporal gyrus	Right	1685	58 -56 -24	0.001
Post-central gyrus	Bilateral	1193	-2 -46 72	0.006
Middle frontal gyrus	Left	797	-36 2 48	0.007
Anterior supramarginal gyrus	Right	656	66 -24 26	0.010
Superior frontal gyrus	Bilateral	625	0 14 62	0.006
Superior temporal gyrus (temporal pole)	Right	175	60 10 -10	0.036
Lateral occipital cortex	Right	74	14 -70 50	0.028
Supplementary motor cortex	Left	35	-12 2 38	0.039

\*With family-wise error correction for multiple comparisons across space.  $p < 0.05$  and  $>20$  voxels.



**Figure 2.** Reduced gray matter in treatment-resistant patients compared with first-line antipsychotic responders, overlaid on the MNI-152 template brain.

there were no significant differences between non-treatment-resistant patients and controls. The more extensive pattern of GM loss seen in TR patients in the current study may be due to the fact that VBM rather than region-of-interest analysis was used, or that our patient groups were matched for PANSS scores. The most extensive region of GM loss in UTR patients was seen in the superior temporal gyrus (STG), a region that has been implicated in studies of first-episode schizophrenia (Kasai et al., 2003; Asami et al., 2012), suggesting that this is a relatively stable deficit but that extensive tissue loss in this area may be associated with more severe symptomatology and treatment resistance. A meta-analysis of longitudinal studies of schizophrenia found a significantly higher volumetric decrease over time in the left and right STG, left Heschl's gyrus, and left planum temporal (Vita et al., 2012). Similarly, a review investigating the trajectory of brain structural impairments in schizophrenia found the STG was already impaired at the onset of symptoms, which worsened during the acute disease phase followed by stabilization and subsequent age-related progression (Chiapponi et al., 2013). The STG is critical for auditory processing, and has therefore been proposed as the region responsible for symptoms such as auditory hallucinations and thought disorder in schizophrenia (Modinos et al., 2013; Zierhut et al., 2013). We also found two regions of reduced GM in the cerebellum in the UTR group

compared with controls, a structure that has been implicated in first-episode and schizophrenia patients (Rasser et al., 2010; Tanskanen et al., 2010). Recent evidence suggests that the cerebellum plays a role in cognition (Andreasen and Pierson, 2008), and deficits in a variety of cognitive domains are known symptoms of schizophrenia which do not necessarily improve with the administration of antipsychotics. Cognitive dysfunction plays a critical role in the pathogenesis and prognosis of schizophrenia, and reduced volume in the cerebellum in TR schizophrenia may reflect a poorer prognosis in these patients.

The group of FLRs showed no significant regions of volume reduction compared with controls, and actually showed a region of increased volume in the right lateral occipital cortex. The fact that we did not find extensive regions of GM tissue loss in this group of patients, despite a mean disease duration of 10 years, may reflect responsiveness to medication. Studies showing tissue loss in schizophrenia are likely to have included patients that exhibited a wide range of responses to their antipsychotic medication that could have masked minimal volume changes in some subjects. Antipsychotic drugs might also act to reverse pathology in the dopaminergic pathway during the early treatment phase of schizophrenia (Wang et al., 2004; Kippin et al., 2005), and this may be more pronounced in early treatment responders compared with those who are treatment resistant.

Direct comparison showed that TR and UTR groups had significantly less GM both globally and regionally compared with the group of FLRs. Regional GM differences were similar in location to those shown between TR/UTR and control subjects, including the inferior and superior temporal gyri, middle frontal gyrus, lateral occipital cortex, and cerebellum. This finding agrees with that of [Zugman et al. \(2013\)](#) who found a significant reduction in the left dorsolateral prefrontal cortex in TR schizophrenia in comparison to non-treatment-resistant schizophrenia. There is some prior evidence to support the view that GM reduction is associated with poor treatment response and poor outcomes in schizophrenia. Some studies have found significantly greater loss of frontal, parietal, and occipital GM in treatment-resistant patients compared with non-treatment-resistant patients, and that the best predictor of response to clozapine treatment was temporal and dorsolateral prefrontal GM volume ([Molina et al., 2003, 2008, 2010](#)). Moreover, patients with poor outcomes (based on Global Assessment of Functioning) had significantly greater decreases in cortical thickness within the left middle temporal cortex, superior temporal cortex, Heschl's gyrus, and anterior cingulate over a 5-year period ([van Haren et al., 2011](#)). Regions where we found reductions in the TR and UTR groups compared with the FLRs have also been implicated in studies investigating the response to antipsychotics in those with first-episode schizophrenia. Over a 16-week period, non-responders to olanzapine or risperidone were found to have significant cortical thinning in occipital and prefrontal regions compared with responders ([Szeszko et al., 2012](#)). Likewise, a 12-week follow-up of first-episode patients showed that non-responders to antipsychotics showed hypoglycemia in the insula, superior frontal, middle frontal, inferior and superior temporal cortices, and temporal pole ([Palaniyappan et al., 2013](#)). Whilst we do not propose that GM volume loss is directly related to treatment resistance, and our study cannot determine whether the differences we observed indirectly contribute to or are a consequence of treatment resistance, these studies in first-episode patients suggest there may be a neurobiological underpinning in people with treatment-resistant schizophrenia. One could also propose that people with treatment-resistant schizophrenia may have experienced a greater number of relapses and/or relapse duration and that these may have had a toxic effect on the brain ([Andreasen et al., 2013](#); [Hyza et al., 2014](#)). However, it has been shown that brain volume change is not associated with duration of untreated illness ([Boonstra et al., 2011](#)). It is still undetermined whether there is an independent pathophysiological process taking place in treatment-resistant schizophrenia indicating a distinct group of patients, or whether treatment resistance represents an accelerated form of the same underlying disease process.

We found no evidence that the two treatment-resistant groups (clozapine-responsive vs clozapine-resistant) differed from each other. These results are somewhat unexpected, as ultra-treatment-resistant (clozapine-resistant) schizophrenia may be considered a more severe form of schizophrenia than treatment-resistant (clozapine-responsive) schizophrenia. Previous studies investigating the response to clozapine in treatment-resistant patients showed that there was significantly greater prefrontal sulcal prominence in those that did not respond to clozapine compared with those who did ([Friedman et al., 1991](#); [Konicki et al., 2001](#)). In addition, [Honer et al. \(1995\)](#) found that an increased size of the posterior frontal and lateral temporal sulci was related to a poor response to clozapine. However, these studies used computed tomography to assess specific brain measurements, rather

than the unbiased whole-brain MRI method of analysis we employed, which may reveal brain changes more sensitively. More recently, two MRI studies found that whilst there were no differences in caudate volume cross-sectionally ([Scheepers et al., 2001a](#)), there was a significant decrease in volume of the left caudate over 1 year in responders to clozapine relative to non-responders ([Scheepers et al., 2001b](#)). Patients were taking typical antipsychotics prior to initiation of clozapine, and therefore it was postulated that clozapine had a "correcting" effect on the increase in caudate volume that has been observed in patients after treatment with typical antipsychotics. The patients in our study had been taking atypical antipsychotic treatment for some time, and it is therefore unlikely that we would have observed this restoring effect on the caudate in either treatment group. The absence of volume differences between TR and UTR groups in our investigation indicates that non-response to clozapine is not related to GM volume. It must also be considered that if the trajectory of volume changes in TR and UTR groups are different, this would not be apparent due to the cross-sectional nature of the study. However, investigation of a cohort of patients from the same treatment-resistant study using diffusion tensor imaging found that the UTR group did not show any significant deficits in fractional anisotropy compared with the TR group; instead, significantly higher fractional anisotropy (better WM integrity) was shown in the right superior longitudinal fasciculus in the UTR group compared with the TR group (unpublished data).

Although current antipsychotic doses were significantly higher in the UTR group compared with both the TR and FLR groups, we found no significant association between regional GM volume and antipsychotic dose in patients. However it is possible that both past and current exposure to antipsychotic medication may have had an effect on brain tissue volume. A longitudinal study of 211 people with first-episode schizophrenia found that antipsychotic treatment was significantly related to a decrease in GM and WM volumes after correcting for the effects of follow-up duration, illness severity, and substance misuse ([Ho et al., 2011](#)), whilst a meta-analysis found that longitudinal GM volume decreases in patients with schizophrenia were associated with higher cumulative exposure to antipsychotics over time ([Fusar-Poli et al., 2013](#)). Conversely, in 105 patients with schizophrenia no association was observed between cumulative antipsychotic exposure and brain volume change over 5 years ([Collin et al., 2012](#)). Moreover, in people with chronic schizophrenia who have had little or no exposure to antipsychotic medication similar regional brain volume reductions as those seen in our study have been reported ([Liu et al., 2014](#)), and first-episode patients who are medication-naive show structural brain changes that suggest the differences we observed are unlikely to be solely due to medication effects ([Lui et al., 2009](#); [Radua et al., 2012](#)). There was also no significant difference in GM between our TR and UTR groups, despite it being likely that UTR schizophrenia patients had higher cumulative antipsychotic exposure, suggesting that our findings are related to treatment resistance rather than the effects of antipsychotic medication exclusively. Studies have also suggested that clozapine may in fact preserve or reverse GM volume and cortical thickness, which would strengthen our finding that greater GM loss is in fact related to treatment resistance rather than medication effects ([Molina et al., 2005, 2008](#); [van Haren et al., 2011](#)). Additionally, no association was observed between regional GM volume and PANSS scores in our patients, contrary to some previous studies ([Palaniyappan et al., 2010](#); [Zierhut et al., 2013](#)). This also suggests that the differences in brain morphology observed

in the current study are related to treatment resistance rather than psychopathology per se.

Whilst we have already mentioned the unique and advantageous features of this study, which potentially make it a valuable addition to the current literature on treatment-resistant schizophrenia, there are some limitations. This was a cross-sectional study, so we could not determine the trajectory of tissue loss between our treatment groups or draw any conclusions regarding causal relationships between brain volume loss, treatment resistance, and antipsychotic medication. In addition, as with most studies investigating patients with established schizophrenia, it is likely that patients differed in the amount of medication they had used before inclusion in the study, and we were unable to reliably calculate lifetime chlorpromazine equivalents based on patients' medical charts. Similarly, it was difficult to assess medication adherence in our population, which had a mean disease duration of 10 years. Whilst we attempted to minimize these effects by only including clinically stable patients taking atypical antipsychotics at the time of investigation (and therefore not receiving large doses of medication) it was not possible to assess whether GM volumes were related to the long-term effects of antipsychotic medication. Due to the unique way that we differentiated our patients with schizophrenia, we had relatively small sample sizes within each group. However, this was a preliminary study of treatment resistance and our results indicate that larger studies should be performed. Although we had to use a 20-channel head coil rather than the 32-channel head coil for five of our participants, these subjects were distributed across the three patient groups and analyses run whilst excluding these participants showed no changes in our main findings. A recent study has also shown VBM results were not significantly affected when MPRAGE images were acquired using a 12-channel versus a 32-channel head-coil (Streitburger et al., 2014). Finally, our patients with schizophrenia showed some differences in recreational drug history compared with control subjects and six of our participants tested positive for tetrahydrocannabinol (three FLR, one TR, and two UTR) at the time of assessment, which could have affected our results. However, these six subjects were distributed across the three patient groups and analyses were run again excluding these participants with no major changes in our findings. Moreover, there were no significant differences in ASSIST scores between patient groups, suggesting that our main finding of differences in GM volume between treatment-resistant and non-treatment-resistant patients remains valid.

In conclusion, this study has shown that there is decreased GM volume in people with treatment-resistant and ultra-treatment-resistant schizophrenia relative to those who respond to first-line atypical antipsychotic medication. The discovery of factors associated with treatment resistance will aid in understanding the underlying causes of treatment resistance and may assist in identifying and treating people with treatment-resistant schizophrenia more effectively; regional GM volume loss may be one such factor. Large-scale prospective longitudinal studies following people from the first episode to treatment response or resistance are required to confirm our findings and the true relationship between brain tissue loss, medication, and treatment resistance.

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## Statement of Interest

None

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