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Progressive Brain Atrophy and Cortical Thinning in Schizophrenia after Commencing Clozapine Treatment

Mohamed Ahmed*[,1], Dara M Cannon[1], Cathy Scanlon[1], Laurena Holleran[1], Heike Schmidt[1], John McFarland[1], Camilla Langan[1], Peter McCarthy[2], Gareth J Barker[3], Brian Hallahan[1] and Colm McDonald[1]

[1]Clinical Neuroimaging Laboratory, Department of Psychiatry, College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Galway, Ireland; [2]Department of Radiology, College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Galway, Ireland; [3]Centre for Neuroimaging Sciences, Institute of Psychiatry, King's College London, London, UK

Despite evidence that clozapine may be neuroprotective, there are few longitudinal magnetic resonance imaging (MRI) studies that have specifically explored an association between commencement of clozapine treatment for schizophrenia and changes in regional brain volume or cortical thickness. A total of 33 patients with treatment-resistant schizophrenia and 31 healthy controls matched for age and gender underwent structural MRI brain scans at baseline and 6–9 months after commencing clozapine. MRI images were analyzed using SIENA (Structural Image Evaluation, using Normalization, of Atrophy) and FreeSurfer to investigate changes over time in brain volume and cortical thickness respectively. Significantly greater reductions in volume were detected in the right and left medial prefrontal cortex and in the periventricular area in the patient group regardless of treatment response. Widespread further cortical thinning was observed in patients compared with healthy controls. The majority of patients improved symptomatically and functionally over the study period, and patients who improved were more likely to have less cortical thinning of the left medial frontal cortex and the right middle temporal cortex. These findings demonstrate on-going reductions in brain volume and progressive cortical thinning in patients with schizophrenia who are switched to clozapine treatment. It is possible that this gray matter loss reflects a progressive disease process irrespective of medication use or that it is contributed to by switching to clozapine treatment. The clinical improvement of most patients indicates that antipsychotic-related gray matter volume loss may not necessarily be harmful or reflect neurotoxicity.
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INTRODUCTION

Structural brain abnormalities are well established in schizophrenia, with structural magnetic resonance imaging (MRI) consistently demonstrating reduced global cerebral volume, increased lateral ventricular volume, and reduced volume of frontotemporal cortical regions including the superior temporal gyrus and subcortical gray matter regions including the hippocampus, amygdala, and thalamus (Ellison-Wright *et al*, 2008; Haijma *et al*, 2013). Furthermore, several studies have found these structural brain abnormalities to be progressive over time (van Haren *et al*, 2007; Kempton *et al*, 2010; Fusar-Poli *et al*, 2013), with greater rates of progression potentially associated with a poorer clinical outcome (Cahn *et al*, 2006; van Haren *et al*, 2008). In addition, widespread cortical thinning of the cerebral cortex involving not just frontotemporal regions but also parietal and occipital regions has been demonstrated in individuals with schizophrenia compared with healthy controls (Nesvåg *et al*, 2008; Sprooten *et al*, 2013). It remains unclear whether these progressive changes are inherent to the pathophysiological disease processes underlying schizophrenia or whether they are related to or are accelerated by treatment with antipsychotic agents or other illness-related factors (Ho *et al*, 2011; Zipursky *et al*, 2013).

A number of longitudinal structural MRI studies have demonstrated an association between antipsychotic medication usage and regional brain volume changes, with a recent meta-analysis reporting an association between reduced gray matter volume and cumulative exposure to antipsychotic agents but not illness severity or duration of illness (Fusar-Poli *et al*, 2013). Typical and atypical antipsychotic agents and in particular clozapine have in some instances demonstrated differential effects on brain morphometry. For example, typical antipsychotic agents have consistently but not universally been associated with increased volume of the basal ganglia including the caudate (Scherk and Falkai, 2006; Ebdrup *et al*, 2013). However, clozapine monotherapy has consistently been associated with a reduction in caudate volume (Scheepers *et al*, 2001; van Haren *et al*, 2007), with this reduction in volume associated with clinical improvement (Scheepers *et al*, 2001). Similarly, in contrast to typical antipsychotic agents (Lieberman *et al*, 2005; van Haren *et al*, 2007), clozapine treatment has been associated in

*Correspondence: Dr M Ahmed, Clinical Neuroimaging Laboratory, Department of Psychiatry, College of Medicine, National University of Ireland, Galway, Galway, Ireland, Tel: +353 91 493555, Fax: +353 91 494563, E-mail: mohamed.ahmed@nuigalway.ie
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longitudinal studies with either an increase (Molina *et al*, 2005) or an attenuated loss of volume of the frontal lobe compared with typical antipsychotic agents (van Haren *et al*, 2007). Furthermore, a higher cumulative intake of typical antipsychotic agents has been associated with cortical thinning of the temporal and frontal cortical regions, with this cortical thinning attenuated when atypical antipsychotic agents including clozapine were administered (van Haren *et al*, 2011).

There is some evidence that clozapine has neuroprotective and neurogenesis stimulating properties (Halim *et al*, 2004), including through upregulation of B-cell lymphoma 2 (BCL2) (Bai *et al*, 2004). Given that most patients treated with clozapine have severe illness and treatment resistant to other antipsychotic agents, it is of considerable clinical importance to clarify whether this medication could ameliorate or arrest brain structural abnormalities associated with schizophrenia.

To date, there remain relatively few longitudinal MRI studies assessing whether morphological brain changes progress or are attenuated/reversed upon switching to clozapine treatment. Consequently, this study sought to assess the impact of clozapine therapy on regional morphometry and cortical thickness throughout the entire brain, utilizing complementary longitudinal MRI analysis techniques in patients over a 6–9-month period after commencing clozapine. In addition, we sought to assess whether any detected brain morphometric changes were associated with clinical characteristics including treatment response.

MATERIALS AND METHODS

Participants

A total of 39 patients with treatment-resistant schizophrenia about to commence clozapine and 40 healthy volunteers were recruited from the in- and out-patient services of University Hospital Galway (UHG) and regional Health Services Executive (HSE) West of Ireland community mental health teams. Treatment resistance was defined as the failure to respond to at least two antipsychotic medications, including at least one atypical antipsychotic drug, with a prolonged period of moderate to severe positive and/or negative symptoms (mean duration of illness = 14 years, SD = 9 years, range 2–36 years) (see Table 1). All patients were diagnosed by trained psychiatrists, using the Structured Clinical Interview for DSM disorders (SCID) (First *et al*, 2002b), as meeting the criteria for schizophrenia as per the Diagnostic and Statistical Manual for Mental Disorders 4th Edition text revision (DSM-IV-TR) (American Psychiatric Association, 2000). All patients underwent symptomatic assessments around the time of scanning before commencing clozapine using the Positive and Negative Syndrome Scale (PANSS; Kay *et al*, 1987) and the Scales for Assessment of Positive Symptoms (SAPS; Andreasen 1984b) and Negative Symptoms (SANS; Andreasen, 1984a). Overall functioning was assessed using a Global Assessment of Functioning score (GAF; Endicott *et al*, 1976). All scales were completed by trained raters and findings are presented in Table 1. Patients were included if aged 18–60 years, and excluded if they had undergone a previous trial of clozapine treatment.

Healthy volunteers were recruited by local advertisement and were matched to patients for age and gender. They were screened using the SCID-Non-Patient Version (First *et al*, 2002a), and were excluded if there was a current or past axis I disorder (DSM-IV-TR) or any axis I psychotic disorder in a first-degree relative. Other exclusion criteria for both participant groups were the presence of neurological illness, intellectual disability, history of head injury resulting in loss of consciousness for >5 min, history of oral steroid use within the preceding 3 months, a DSM-IV-TR diagnosis of substance or alcohol abuse or dependence in the 12 months before assessment, and any contraindication for MRI scanning.

At follow-up after at least 6 months of treatment with clozapine, 33 patients were successfully re-recruited, scanned, and assessed using the PANSS, SANS, SAPS, and GAF. Of the six individuals not assessed at follow-up, three patients declined to participate in follow-up, one patient was unavailable, one patient had not commenced clozapine, and one patient discontinued clozapine because of severe neutropenia after 2 weeks of treatment. A 50% reduction from baseline total PANSS score was used as a cutoff to define treatment response at follow-up. Clinical details at follow-up included all pharmacological treatments, with daily and cumulative doses of clozapine and serum clozapine levels recorded. In all, 21 patients were on monotherapy antipsychotic medication before switching to clozapine (7 on olanzapine, 4 on quetiapine, 4 on aripiprazole, 1 on paliperidone, and 1 on depot risperidone). The remaining 12 patients were on combinations of antipsychotic drugs with 10 participants treated with a combination of two antipsychotic medications (in 7 cases olanzapine plus another antipsychotic), 1 treated with three antipsychotic medications (quetiapine, chlorpromazine, and flupenthixol depot), and 1 treated with four antipsychotic medications (sulpiride, thioridazine, aripiprazole, and quetiapine). Of the 33 patients successfully followed-up, 31 were taking clozapine monotherapy and 2 were taking additional olanzapine.

A total of 31 controls were successfully re-recruited for repeat scanning and rescreened using the SCID-Non-Patient Version (First *et al*, 2002b) to ensure no change in diagnosis status. All participants gave informed consent as approved by the UHG and the National University of Ireland Galway research ethics committees.

Structural MRI Acquisition

Structural MRI data were acquired at both baseline and follow-up on a 1.5 Tesla Siemens Magnetom Symphony (Erlangen, Germany) scanner using identical MRI protocols. For each individual, a two-dimensional midsagittal scan was used to position the subject so that the floor of the fourth ventricle was parallel to the y axis of the scanner coordinates system. The anterior–posterior (AP) axis was determined on the midsagittal slice using the anterior commisure–posterior commisure (AC-PC) line. Detailed high-resolution, whole-head contiguous axial slices (thickness 0.9 mm, field of view 230×230 mm) parallel to this axis were acquired using a set of three-dimensional (3D) T1-weighted magnetization prepared rapid gradient-echo (MPRAGE) sequences (relaxation time (TR): 1140 ms; echo time (TE): 4.38 ms; inversion time (TI): 600 ms; flip angle 15°; acquisition matrix 256×256, interpolated to 512×512, pixel resolution of 0.45 mm $\times$ 0.45 mm). Gross pathology was excluded after visual inspection by an experienced neuroradiologist blind to subject group.

Table 1 Demographic, Clinical, and Global Characteristics of the Sample

Group Characteristics	Patients ($n=33$), mean ± SD, range	Controls ($n=31$), mean ± SD, range	*t*-test, F, or χ^2 (*p*-value)
Age (years)	36.4 ± 10.7 22–61	39.4 ± 10.6 23–59	1.10 (0.27)
Male/female (% female)	23/10 (30%)	20/11 (36%)	0.19 (0.66)
Follow-up duration (weeks)	31.8 ± 7.3 23–62	31.7 ± 8.9 24–69	−0.02 (0.98)
Duration of illness before commencing clozapine (years)	13.55 ± 8.76 2–36	NA	—
Number of psychotic episodes before commencing clozapine	4.8 ± 3.38 2–20	NA	—
Medications at baseline (n)			
Typical antipsychotics	12	NA	—
Atypical antipsychotics	33		
Clozapine	0		
Medications at follow-up (n)			
Typical antipsychotics	0	NA	—
Atypical antipsychotics	2		
Clozapine	33		
Mean daily dose of clozapine at follow-up (mg), range	349 ± 102 200–625	NA	—
Mean clozapine level at follow-up (ng/ml), range	433 ± 295 70–1360	NA	—
Clinical scales			
Total PANSS (baseline)[a]	53.9 (17.2)	NA	8.9 (<0.001)
Total PANSS (follow-up)[a]	27.6 (18.2)		
SAPS (baseline)	27.9 (16.3)		5.33 (<0.001)
SAPS (follow-up)	13.2 (10.9)		
SANS (baseline)	42.5 (20.6)		4.1 (<0.001)
SANS (follow-up)	27.8 (22.9)		
GAF (baseline)	46.8 (10.8)		−6.7 (<0.001)
GAF (follow-up)	64.9 (14.1)		
Global brain volumes (cm^3)			
GM (baseline)	666.80 (52.35)	686.71 (67.77)	0.91 (0.47)[b]
WM (baseline)	516.09 (612.80)	537.05 (67.32)	
CSF (baseline)	204.21 (98.29)	186.29 (92.78)	
GM (follow-up)	650.35 (63.13)	681.04 (68.02)	2.47 (0.054)[b]
WM (follow-up)	520.79 (64.13)	535.64 (69.37)	
CSF (follow-up)	228.98 (102.84)	188.64 (94.84)	

Abbreviations: CSF, cerebrospinal fluid; GAF, Global Assessment of Functioning; GM, gray matter; PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scales for the Assessment of Positive Symptoms; WM, white matter.
[a]PANSS was scored on the 0–6 scale.
[b]Multivariate analysis for global metrics inclusive of GM, WM, and CSF between patients and controls.

Image processing: measuring regional brain volume change. SIENA (structural image evaluation, using normalization, of atrophy) (Smith *et al*, 2001) was utilized to estimate percentage brain volume change in each subject over time. SIENA analysis first segmented brain from nonbrain tissue in both baseline and follow-up images. This was followed by registration of both images while using the skull images to constrain scaling and skew. Images were then segmented

into different tissue types (gray matter, white matter, and cerebrospinal fluid) using the FMRIB's automated segmentation tool (FAST) component of SIENA analysis. Finally, the percentage brain volume change was estimated based on calculating surface motion and tissue edge point movement. Once SIENA output was obtained, an 'edge flow image in standard edge space' was created for each subject, with these images merged into a single 4D image before the creation of the design matrix and the subsequent voxel-wise cross-subject statistical analysis.

Image processing: measuring cortical thickness change and global volumes. FreeSurfer (Dale *et al*, 1999; Reuter *et al*, 2012) was utilized to estimate percentage cortical thickness change. In brief, the cerebral white matter (WM) was segmented, divided into two hemispheres with the brain stem, and cerebellum removed. Tessellation was performed producing a triangle-based mesh of the WM surface that was refined to minimize the voxel-based nature of the initial curvature. The WM surfaces are deformed outward to generate the pial (GM/CSF intersection) surface with topologic defects in the surface corrected using an automated topology fixer. Visual quality checks were performed and inaccuracies manually edited and corrected by reprocessing. The cortical surface was then spherically inflated to expose the entire cortical surface, including deep tissue inside the sulci. Using combined information from the pial and WM surfaces, cortical thickness was calculated at each vertex.

To measure changes in cortical thickness between the baseline and follow-up scans, images were then processed using the longitudinal stream in FreeSurfer (http://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalProcessing) (Reuter *et al*, 2012) to create a within-subject template space and unbiased image with respect to any time point utilizing a robust inverse consistent registration. Processing steps include skull stripping, Talairach transformations, and atlas registration with spherical surface maps initialized with common information from the within-subject template to significantly increase reliability and statistical power (Reuter *et al*, 2012). At each vertex the symmetrized percentage change (SPC) was calculated as the rate of thickness change with respect to the average thickness; SPC = rate/mean, where rate = (thick2 − thick1)/(time2 − time1) and mean = 0.5 × (thickness 1+thickness 2). Subject surface data are then smoothed by using a 20-mm FWHM 2-D Gaussian kernel and mapped to an average surface.

Statistical Analysis

A general linear model was applied at each voxel (for SIENA) and each surface vertex (for FreeSurfer) to determine effect of 'group' (patient or healthy volunteer) on change in measurement parameter controlling for age and gender. For SIENA analysis, threshold-free cluster enhancement permutation analysis with 10 000 random permutations was used to correct for multiple comparisons (Smith and Nichols, 2009). FreeSurfer's statistical analysis tool utilized a Monte-Carlo permutation cluster analysis, a significance value of $p < 0.05$, a cluster threshold of 0.05, and 10 000 random permutations (Reuter *et al*, 2012).

Based on identified brain regions with volume change, a comparison was conducted between patients who had responded (RS) or not responded (NR) at follow-up. A general linear model was used to determine the effect of 'group' on average brain cluster change (regional brain volume or cortical thickness changes), adding age and gender as covariates. Correlation analyses were carried out to determine whether identified brain changes were associated with age, clinical symptoms, duration of illness, number of psychotic episodes, daily and cumulative doses of clozapine, and serum clozapine levels.

RESULTS

Longitudinal Structural Changes in Patients Compared with Healthy Controls

The SIENA analysis detected significant regional brain tissue edge movements representing clusters of brain volume reduction in the right prefrontal cortex (RPFC) (mean = − 0.21-mm, SD = 0.26 mm), left prefrontal cortex (LPFC) (mean = − 0.20 mm, SD = 0.24 mm) and periventricular area (PVA) (mean = − 0.20 mm, SD = 0.14 mm) in the patient group compared with healthy controls (mean = − 0.004 mm, SD = 0.08 mm; mean = − 0.02 mm, SD = 0.07 mm; and mean = − 0.01 mm, SD = 0.04 mm, respectively). RPFC and LPFC volume reductions included the dorsolateral, prefrontal, medial prefrontal, and frontal pole subdivisions. The detected PVA volume loss was confined to the border voxels surrounding the lateral and third ventricles (Figure 1).

Widespread cortical thinning was identified longitudinally across the brain in the patient group compared with controls, with a higher rate of change affecting both hemispheres across the prefrontal, posterior temporal, cingulate cortex, and parietal and occipital lobes (Figure 2).

Global Brain Volumes and Cortical Thickness at Baseline and Follow-Up

As illustrated in Table 1, GM, WM, or CSF volumes were not significantly different between patients and controls at baseline ($F = 0.91$, $p = 0.47$) or at follow-up ($F = 2.47$, $p = 0.054$); however, nonsignificant reductions in GM ($F = 3.5$, $p = 0.07$) and increases in CSF ($F = 2.88$, $p = 0.09$) were noted in the patient group compared with controls at follow-up. There was no difference in cortical thickness between patients and controls at baseline controlling for age and correcting for multiple comparisons (FDR = 0.05).

Clinical Response to Clozapine and Structural Brain Changes

A total of 20 patients (61%) were deemed to have responded and 13 not responded to clozapine treatment. The NR subgroup was prescribed a significantly higher daily dose of clozapine at follow-up (mean = 383.3 mg, SD = 105.5 mg) than the RS subgroup (mean = 321.3 mg, SD = 87.5 mg; $t = 89.0$, $p = 0.04$). Cumulative clozapine dose was significantly higher in the NR group (mean = 92 575 mg, SD = 37 114 mg) compared with the RS group (mean = 71 246 mg, SD = 22 890 mg; $t = 2.05$, $p = 0.049$). No significant difference in serum clozapine levels or other clinical variables were detected between these two groups.

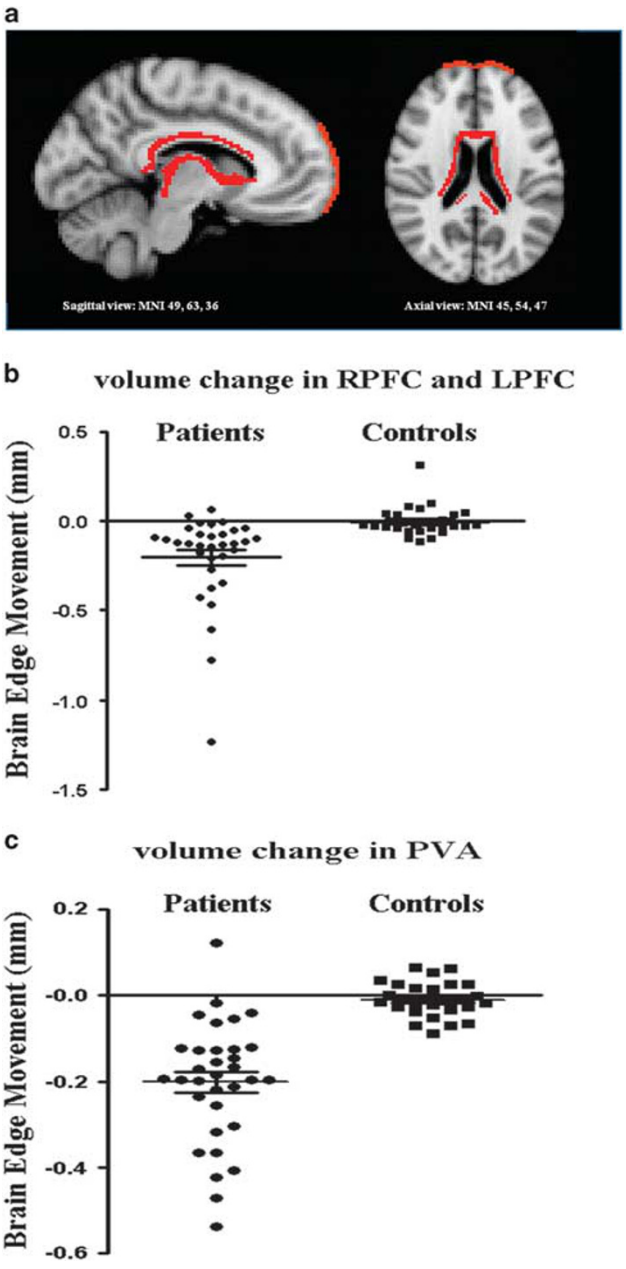

Figure 1 (a) Masks applied to highly significnt clusters of RPFC, LPFC, and PVA identified with volume loss at follow-up in the patient group ($n=33$) compared with the control group ($n=31$). (b, c) Significant differences at follow-up in SIENA analysis between patients and controls in regional brain tissue edge movements (as a measure of regional brain volume change) in RPFC (MWW = 141, $p<0.001$), LPFC (MWW = 149, $p<0.001$), and PVA (MWW = 61, $p<0.001$). No difference noted between responders ($n=20$) and nonresponders ($n=13$) to clozapine. LPFC, left prefrontal cortex; MWW, Mann–Whitney *U*-test; PVA, periventricular area; RPFC, right prefrontal cortex.

NR patients demonstrated marginally greater cortical thinning of the left medial frontal cortex (LMFC) and the right middle temporal cortex (RMTC) compared with RS patients ($F=4.1$, $p=0.05$) as shown in Table 2 and Figure 3. No volumetric differences were demonstrated between these two groups using SIENA analysis (Table 2). There was no significant correlation between the detected changes in the regional or global brain volumes and daily or cumulative clozapine doses or serum clozapine levels.

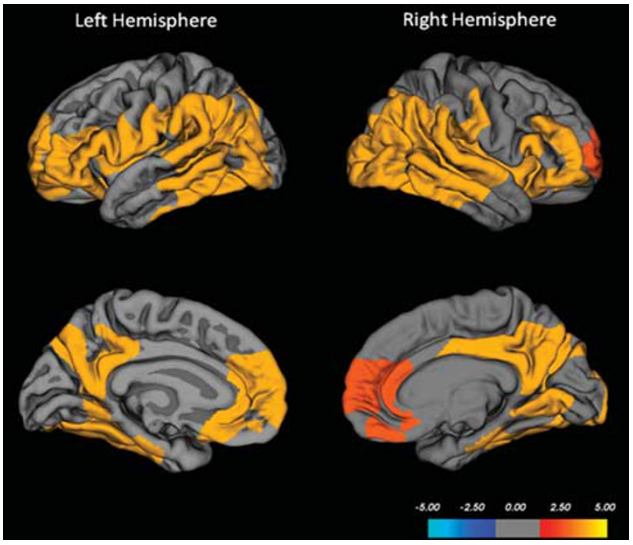

Figure 2 Differential changes in cortical thickness in patients ($n=33$) and controls ($n=31$) demonstrating regions of comparatively greater cerebral cortex thinning in patients. The significance in this display is a $-\log(10)$ *p*-value. Orange/yellow indicates greater thinning in patients than controls, whereas blue represents greater thinning in controls than patients.

Correlation Between Structural Brain Changes and Clinical Variables in Patients

As volume changes in the RPFC and LPFC were found to be highly correlated in patients ($r=0.89$, $p<0.001$), both regions were combined for the purpose of investigating other clinical correlates. Volume changes for the total PFC ($r=0.45$, $p=0.009$), but not the PVA ($r=-0.07$, $p=0.71$), demonstrated a significant positive correlation with age. There were no significant correlations between detected regional brain volume loss of the PFC or PVA and PANSS or SANS scores, duration of illness, and number of psychotic episodes.

Given the extent of the detected changes in cortical thickness and to mitigate against multiple comparisons, the most significant region in each hemisphere (the LMFC and RMTC) were extracted for further examination. There were no significant correlations between cortical thinning and any of the clinical variables investigated in patients in this study.

DISCUSSION

Progressive regional loss of brain volume in the PFC and PVA and global cortical thinning were demonstrated in patients compared with healthy controls in this 6–9-month longitudinal MRI study of patients switched to clozapine treatment. Our findings of progressive regional brain volume loss are consistent with some previous studies of individuals with schizophrenia treated with antipsychotic medications, many of which included patients treated with clozapine as well as other antipsychotic agents (van Haren *et al*, 2007; Ho *et al*, 2011; Fusar-Poli *et al*, 2013), and do not support a differential attenuation/reversal of gray matter loss with

Table 2 Regional Brain Tissue Edge Movements (as a Measure of Regional Brain Volume Change) and Cortical Thickness Change Detected in patients Who Responded (RS) Compared with Those Who Did Not (NR)

Brain area	Measure	Mean edge movement (mm) ± SD		Comparing means, Mann–Whitney test (*p*-value)
		RS ($n=20$)	NR ($n=13$)	
RPFC	Brain tissue edge movement (SIENA analysis)	−0.23 ± 0.28	−0.17 ± 0.24	100 (0.27)
LPFC		−0.22 ± 0.23	−0.18 ± 0.24	104 (0.34)
PVA		−0.18 ± 0.14	−0.23 ± 0.13	170 (0.14)
LMFC	Cortical thickness change (FreeSurfer analysis)	−0.01 ± 0.1	−0.14 ± 0.15	**F (*p*-value)**
RMTC				4.1 (0.05)

Abbreviations: LMFC, left medial frontal cortex; LPFC, left prefrontal cortex; PVA, periventricular area; RMTC, right middle temporal cortex; RPFC, right prefrontal cortex. Treatment response was defined as a 50% reduction in PANSS score at follow-up.

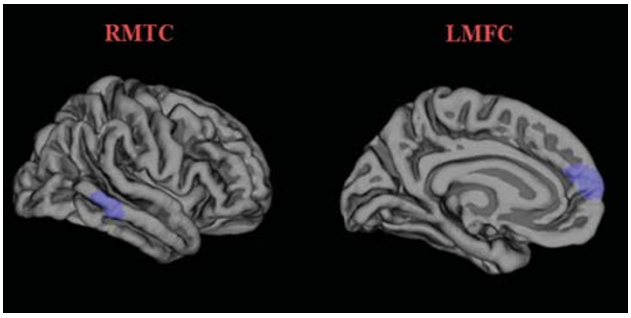

Figure 3 Greater cortical thinning of LMFC and RMTC in NR patients ($n=13$) compared with RS ($n=20$). LMFC, left medial frontal cortex; NR, nonresponders; RMTC, right middle temporal cortex; RS, responders to clozapine.

clozapine use suggested by some studies (Molina *et al*, 2005; van Haren *et al*, 2007; van Haren *et al*, 2011). Indeed, both typical and atypical antipsychotic agents have been linked to brain tissue loss in animal models (rats and macaque monkeys) treated with antipsychotic agents (Dorph-Petersen *et al*, 2005; Konopaske *et al*, 2007; Vernon *et al*, 2011).

A recent longitudinal cortical thickness study in patients treated with clozapine noted cortical thinning in the superior temporal cortex in the interscan interval, after controlling for the association of thinning in this region with poorer clinical and functional outcome (van Haren *et al*, 2011), a finding consistent with the present study. Our finding of cortical thinning is also consistent with a small study of patients with childhood-onset schizophrenia who demonstrated a similar cortical thinning trajectory when treated with either olanzapine or clozapine, with the authors noting that patients treated with clozapine displayed a region of significant thinning compared with patients treated with olanzapine in the RPFC (Mattai *et al*, 2010). These studies tentatively suggest that patients treated with clozapine may suffer greater cortical gray matter loss than other patients with schizophrenia; however, as these studies like ours are observational in design, it is not possible to identify whether clozapine treatment is the pathophysiological factor involved in the gray matter loss, or whether other factors such as more malignant illness with associated accentuated tissue loss are causative.

Reduced PFC volume is a consistent finding in individuals with chronic schizophrenia (Molina *et al*, 2004; Schuster *et al*, 2012). Studies examining the association between PFC gray matter loss and antipsychotic medication have yielded heterogeneous results. No difference was reported in PFC volume between patients with schizophrenia treated with typical antipsychotic agents compared with those who were neuroleptic naive (Keshaven *et al*, 1994), and between patients prescribed typical compared with atypical antipsychotic agents (Schuster *et al*, 2012).

Individuals in the present study had been diagnosed with schizophrenia for a considerable period of time, and there is some evidence that regional brain volume changes are more likely to occur early in the disease process of schizophrenia (Pantelis *et al*, 2003), although this finding is not universal (van Haren *et al*, 2011). In the present study, the regional brain volume loss of the left and right PFC was associated with younger participant age, consistent with younger individuals being more susceptible to gray matter loss. This association with age was not demonstrated for the PVA. Atrophy at the PVA was confined to the border voxels surrounding the lateral and third ventricles, suggesting this atrophy is a proxy measure for ventricular enlargement. Ventricular expansion has, unlike other brain regions, previously been demonstrated to be independent of age or illness duration and also to be progressive in nature (Kempton *et al*, 2010). Such expansion may be secondary to disproportionate reductions or tissue shrinkage in unidentified, localized periventricular structures (Harrison, 1999). Possible mechanisms include neuronal apoptosis, necrosis, and synaptic pruning (Ho *et al*, 2003), or a decrease in the number or size of one or several types of neurons including glia cells (Dorph-Petersen *et al*, 2005). Atypical antipsychotic medications have also been associated with ventricular expansion (Crespo-Facorro *et al*, 2008); however, a recent meta-analysis demonstrated no association between cumulative antipsychotic medications and ventricular expansion longitudinally (Fusar-Poli *et al*, 2013).

Most gray matter tissue is present in the cortex and thus excessive cortical thinning may explain or significantly contribute to decreases demonstrated in gray matter volume in chronic schizophrenia. In this study, widespread cortical thinning was demonstrated longitudinally in the patient group compared with controls, a finding consistent with previous literature (Nesvåg *et al*, 2008; Sprooten *et al*, 2013). Cortical thinning has been reported to be largely unrelated to the type of antipsychotic medication prescribed including

clozapine (Nesvåg *et al*, 2008; Mattai *et al*, 2010; Hirjak *et al*, 2014); however, atypical antipsychotic agents and in particular clozapine have also been associated with an attenuation of cortical thinning in the frontal lobe (van Haren *et al*, 2011). In this study, treatment (NRs had greater cortical thinning in the right middle temporal and left medial frontal cortex. This subgroup of patients was prescribed higher dosages of clozapine, on average, compared with clozapine responders. This finding possibly supports previous evidence that clozapine use is associated with increased cortical thinning (Ho *et al*, 2011). However, it is also possible that in treatment responders, clozapine has greater neuroprotective and neurogenesis stimulating properties (Halim *et al*, 2004), resulting in an attenuation of cortical thinning.

Alternatively, this finding of greater cortical thinning in the clozapine NR group may reflect that more severe psychotic illness is associated with greater cortical thinning, a hypothesis consistent with findings of excessive brain volume loss and a poorer clinical outcome in both chronic schizophrenia (Davis *et al*, 1998) and first-episode psychosis (Cahn *et al*, 2002). Nevertheless, a greater clinical improvement in schizophrenia has also been associated with higher rates of gray matter reduction (Gur *et al*, 1998; Sporn *et al*, 2003).

Findings of putative antipsychotic-related regional brain volume loss may not necessarily be harmful to patients or reflect neurotoxicity. Dendritic and synaptic pruning of malfunctioning neurons in schizophrenia has been suggested as the mechanism underlying gray matter loss associated with response to atypical antipsychotics including clozapine (Sporn *et al*, 2003; Bardgett *et al*, 2006). Chronic frontal hypoperfusion leading to reductions in metabolic activity secondary to antipsychotic medications (typical and atypical) (Miller *et al*, 2001) could also be a mechanism underlying the association between antipsychotic medications and the regional brain volume loss (Ho *et al*, 2011).

Caution is required in interpreting the results of this study as dissecting the effects of progressive brain changes relating to illness pathophysiology and those related to antipsychotic treatment is complex (Navari and Dazzan, 2009). Brain morphometric changes due to antipsychotic medications may be difficult to disentangle from those due to illness progression as antipsychotic medications are associated with reducing regional brain volumes in those regions also noted to be reduced in individuals with schizophrenia who are antipsychotic naive, such as the temporal and frontal cortices and striatum (Haijma *et al*, 2013). Furthermore, different antipsychotic medications may have differential effects on the brain, and all patients in this study had previously been treated with both typical and atypical antipsychotic agents. The precise mechanisms whereby treatment with or withdrawal of antipsychotic medications may potentially influence regional brain volumes and cortical thinning have yet to be elucidated. Potential causes include changes in gene expression, receptor density, or blood flow in response to receptor blockade (Scherk and Falkai, 2006; Navari and Dazzan, 2009).

The strengths of this study include the assessment of a relatively homogenous sample of patients with treatment-resistant schizophrenia, all of whom were switched to clozapine for a similar time period, thus removing variability associated with use of different antipsychotic medications during the follow-up period. Participants were assessed with identical MR sequences and scanner over the assessment period and complementary imaging techniques optimized for longitudinal data including SIENA that takes into account baseline volumetric measures and is sensitive to detecting volumetric change (de Bresser *et al*, 2011). A limitation of the study is the lack of an additional patient control group treated with a different antipsychotic agent in an effort to delineate disease effects from treatment effects. However, treatment-resistant schizophrenia may be associated with a more malignant disease process with more progressive gray matter loss, and perhaps the only way to confirm that medical treatment rather than the underlying disease process produced the gray matter loss identified in the present study is through a randomized controlled trial incorporating MRI measurements. The functional significance of the brain tissue loss is also unclear and additional functional measurements such as cognitive testing could have enabled assessment of what, if any, negative consequences emerged alongside the progressive gray matter loss detected.

In conclusion, this longitudinal MRI study of patients with treatment-resistant schizophrenia demonstrated that patients experience a differential reduction in the PFC and PVA and generalized cortical thinning in the months after switching to clozapine treatment. These findings indicate that switching to clozapine treatment is not associated with arrest or reversal of brain volume loss or cortical thinning in schizophrenia and may even contribute to these morphological abnormalities. The clinical improvement of most patients indicates that gray matter loss associated with clozapine treatment does not necessarily reflect a harmful or neurotoxic process.

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REFERENCES

American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn, Text Revised. Washington, DC.

Andreasen NC. *Scale for the Assessment of Negative Symptoms (SANS)*. University of Iowa; 1984a.

Andreasen NC. *Scale for the Assessment of Positive Symptoms (SAPS)*. University of Iowa; 1984b.

Bai O, Zhang H, Li XM (2004). Antipsychotic drugs clozapine and olanzapine upregulate bcl-2 mRNA and protein in rat frontal cortex and hippocampus. *Brain Res* **1010**: 81–86.

Bardgett ME, Griffith MS, Foltz RF, Hopkins JA, Massie CM, O'Connell SM (2006). The effects of clozapine on delayed spatial

alternation deficits in rats with hippocampal damage. *Neurobiol Learn Mem* **85**: 86–94.

Cahn W, Hulshoff Pol HE, Lems EB, van Haren NE, Schnack HG, van der Linden JA *et al* (2002). Brain volume changes in first episode schizophrenia: a 1-year follow-up study. *Arch Gen Psychiatry* **59**: 1002–1010.

Cahn W, van Haren NE, Hulshoff Pol HE, Schnack HG, Caspers E, Laponder DA *et al* (2006). Brain volume changes in the first year of illness and 5-year outcome of schizophrenia. *Br J Psychiatry* **189**: 381–382.

Crespo-Facorro B, Roiz-Santiáñez R, Pérez-Iglesias R, Pelayo-Terán JM, Rodríguez-Sánchez JM, Tordesillas-Gutiérrez D *et al* (2008). Effect of antipsychotic drugs on brain morphometry: a randomized controlled one-year follow-up study of haloperidol, risperidone and olanzapine. *Prog Neuropsychopharmacol Biol Psychiatry* **32**: 1936–1943.

Dale AM, Fischl B, Sereno MI (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* **9**: 179–194.

Davis KL, Buchsbaum MS, Shihabuddin L, Spiegel-Cohen J, Metzger M, Frecska E *et al* (1998). Ventricular enlargement in poor-outcome schizophrenia. *Biol Psychiatry* **43**: 783–793.

de Bresser J, Portegies MP, Leemans A, Biessels GJ, Kappelle LJ, Viergever MA (2011). A comparison of MR based segmentation methods for measuring brain atrophy progression. *Neuroimage* **54**: 760–768.

Dorph-Petersen KA, Pierri JN, Perel JM, Sun Z, Sampson AR, Lewis DA (2005). The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: a comparison of haloperidol and olanzapine in macaque monkeys. *Neuropsychopharmacology* **30**: 1649–1661.

Ebdrup BH, Nørbak H, Borgwardt S, Glenthøj B (2013). Volumetric changes in the basal ganglia after antipsychotic monotherapy: a systematic review. *Curr Med Chem* **20**: 438–447.

Ellison-Wright I, Glahn DC, Laird AR, Thelen SM, Bullmore E (2008). The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. *Am J Psychiatry* **165**: 1015–1023.

Endicott J, Spitzer RL, Fleiss JL, Cohen J (1976). The global assessment scale: a procedure for measuring overall severity of psychiatric disturbance. *Arch Gen Psychiatry* **33**: 766–771.

First MB, Spitzer RL, Gibbon M, Williams JB (2002a). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP)*. Biometrics Research, New York State Psychiatric Institute: New York.

First MB, Spitzer RL, Gibbon M, Williams JB (2002b). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P)*. Biometrics Research, New York State Psychiatric Institute: New York.

Fusar-Poli P, Smieskova R, Kempton MJ, Ho BC, Andreasen NC, Borgwardt S (2013). Progressive brain changes in schizophrenia related to antipsychotic treatment? A meta-analysis of longitudinal MRI studies. *Neurosci Biobehav Rev* **37**: 1680–1691.

Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W *et al* (1998). A follow-up magnetic resonance imaging study of schizophrenia: relationship of neuroanatomical changes to clinical and neurobehavioral measures. *Arch Gen Psychiatry* **55**: 145–152.

Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, Kahn RS (2013). Brain volumes in schizophrenia: a meta-analysis in over 18000 subjects. *Schizophr Bull* **39**: 1129–1138.

Halim ND, Weickert CS, McClintock BW, Weinberger DR, Lipska BK (2004). Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. *Neuropsychopharmacology* **29**: 1063–1069.

Harrison PJ (1999). The neuropathology of schizophrenia: a critical review of the data and their interpretation. *Brain* **122**: 593–624.

Hirjak D, Wolf RC, Stieltjes B, Hauser T, Seidl U, Schröder J *et al* (2014). Cortical signature of neurological soft signs in recent onset schizophrenia. *Brain Topogr* **27**: 296–306.

Ho BC, Andreasen NC, Nopoulos P, Arndt S, Magnotta V, Flaum M (2003). Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. *Arch Gen Psychiatry* **60**: 585–594.

Ho BC, Andreason NC, Ziebell S, Pierson R, Magnotta V (2011). Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Arch Gen Psychiatry* **68**: 128–137.

Kay SR, Fiszbein A, Opler LA (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* **13**: 261–276.

Kempton MJ, Stahl D, Williams SCR, DeLisi LE (2010). Progressive lateral ventricular enlargement in schizophrenia: a meta-analysis of longitudinal MRI studies. *Schizophr Res* **120**: 54–62.

Keshavan MS, Bagwell WW, Haas GL, Sweeney JA, Schooler NR, Pettegrew JW (1994). Changes in caudate volume with neuroleptic treatment. *Lancet* **344**: 1434.

Konopaske GT, Dorph-Petersen KA, Pierri JN, Wu Q, Sampson AR, Lewis DA (2007). Effect of chronic exposure to antipsychotic medication on cell numbers in the parietal cortex of macaque monkeys. *Neuropsychopharmacology* **32**: 1216–1223.

Lieberman JA, Tollefson GD, Charles C, Zipursky R, Sharma T, Kahn RS *et al* (2005). Antipsychotic drug effects on brain morphology in first-episode psychosis. *Arch Gen Psychiatry* **62**: 361–370.

Mattai A, Chavez A, Greenstein D, Clasen L, Bakalar J, Stidd R *et al* (2010). Effects of clozapine and olanzapine on cortical thickness in childhood-onset schizophrenia. *Schizophr Res* **116**: 44–48.

Miller DD, Andreasen NC, O'Leary DS, Watkins GL, Boles Ponto LL, Hichwa RD (2001). Comparison of the effects of risperidone and haloperidol on regional cerebral blood flow in schizophrenia. *Biol Psychiatry* **49**: 704–715.

Molina V, Sanz J, Sarramea F, Benito C, Palomo T (2004). Lower prefrontal gray matter volume in schizophrenia in chronic but not in first episode schizophrenia patients. *Psychiatry Res* **131**: 45–56.

Molina V, Reig S, Sanz J, Palomo T, Benito C, Sanchez J *et al* (2005). Increase in gray matter and decrease in white matter volumes in the cortex during treatment with atypical neuroleptics in schizophrenia. *Schizophr Res* **80**: 61–71.

Navari S, Dazzan P (2009). Do antipsychotic drugs affect brain structure? A systematic and critical review of MRI findings. *Psychol Med* **39**: 1763–1777.

Nesvåg R, Lawyer G, Varnäs K, Fjell AM, Walhovd KB, Frigessi A *et al* (2008). Regional thinning of the cerebral cortex in schizophrenia: effects of diagnosis, age and antipsychotic medication. *Schizophr Res* **98**: 16–28.

Pantelis C, Velakoulis D, McGorry P, Wood SJ, Suckling J, Phillips LJ *et al* (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet* **361**: 281–288.

Reuter M, Schmansky NJ, Rosas HD, Fischl B (2012). Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* **61**: 1402–1418.

Scheepers FE, de Wied CC, Hulshoff Pol HE, van de Flier W, van der Linden JA, Kahn RS (2001). The effect of clozapine on caudate nucleus volume in schizophrenic patients previously treated with typical antipsychotics. *Neuropsychopharmacology* **24**: 47–54.

Scherk H, Falkai P (2006). Effects of antipsychotics on brain structure. *Curr Opin Psychiatry* **19**: 145–150.

Schuster C, Schuller AM, Paulos C, Namer I, Pull C, Danion JM *et al* (2012). Gray matter volume decreases in elderly patients with

schizophrenia: a voxel-based morphometry study. *Schizophr Bull* **38**: 796–802.

Smith SM, De Stefano N, Jenkinson M, Matthews PM (2001). Normalized accurate measurement of longitudinal brain change. *J Comput Assist Tomogr* **25**: 466–475.

Smith SM, Nichols TE (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* **44**: 83–98.

Sporn AL, Greenstein DK, Gogtay N, Jeffries NO, Lenane M, Gochman P *et al* (2003). Progressive brain volume loss during adolescence in childhood-onset schizophrenia. *Am J Psychiatry* **160**: 2181–2189.

Sprooten E, Papmeyer M, Smyth AM, Vincenz D, Honold S, Conlon GA *et al* (2013). Cortical thickness in first-episode schizophrenia patients and individuals at high familial risk: a cross-sectional comparison. *Schizophr Res* **151**: 259–264.

van Haren NE, Hulshoff Pol HE, Schnack HG, Cahn W, Brans R, Carati I *et al* (2008). Progressive brain volume loss in schizophrenia over the course of the illness: evidence of maturational abnormalities in early adulthood. *Biol Psychiatry* **63**: 106–113.

van Haren NE, Hulshoff Pol HE, Schnack HG, Cahn W, Mandl RC, Collins DL *et al* (2007). Focal gray matter changes in schizophrenia across the course of the illness: a 5-year follow-up study. *Neuropsychopharmacology* **32**: 2057–2066.

van Haren NE, Schnack HG, Cahn W, van den Heuvel MP, Lepage C, Collins L *et al* (2011). Changes in cortical thickness during the course of illness in schizophrenia. *Arch Gen Psychiatry* **68**: 871–880.

Vernon AC, Natesan S, Modo M, Kapur S (2011). Effect of chronic antipsychotic treatment on brain structure: a serial magnetic resonance imaging study with ex vivo and postmortem confirmation. *Biol Psychiatry* **69**: 936–944.

Zipursky RB, Reilly TJ, Murray RM (2013). The myth of schizophrenia as a progressive brain disease. *Schizophr Bull* **39**: 1363–1372.