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Presynaptic dopamine capacity in patients with treatment resistant schizophrenia taking clozapine: an [¹⁸F]DOPA PET study

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Abstract

Some patients with schizophrenia show poor response to first-line antipsychotic treatments and this is termed treatment resistant schizophrenia. The differential response to first-line antipsychotic drugs may reflect a different underlying neurobiology. Indeed, a previous study found dopamine synthesis capacity was significantly lower in patients with treatment resistant schizophrenia. However, in this study, the treatment resistant patients were highly symptomatic whilst the responsive patients showed no or minimal symptoms. The study could not distinguish whether this was a trait effect or reflected the difference in symptom levels. Thus we aimed to test whether dopaminergic function is altered in patients with a history of treatment resistance to first-line drugs relative to treatment responders when both groups are matched for symptom severity levels by recruiting treatment resistant patients currently showed low symptom severity with the clozapine treatment.

Healthy controls (n=12), patients treated with clozapine (n=12) who had not responded to first-line antipsychotics and patients who had responded to first-line antipsychotics (n=12) were recruited. Participants were matched for age and sex, and symptomatic severity level in patient groups. Participants’ dopamine synthesis capacity was measured by using $[^{18}F]$DOPA PET.

We found that patients treated with clozapine show lower dopamine synthesis capacity than patients who have responded to first-line treatment (Cohen’s $d=0.9191$ (whole striatum), $0.7781$ (Associative striatum), $1.0344$ (Limbic striatum), $1.0189$ (Sensorimotor striatum)) in line with the hypothesis that the dopaminergic function is linked to treatment response.

This suggests that a different neurobiology may underlie treatment resistant schizophrenia.
and that dopamine synthesis capacity may be a useful biomarker to predict treatment responsiveness.
Introduction

A recent meta-analysis of presynaptic dopaminergic function as well as studies of dopamine receptor and transporter levels, identified elevated presynaptic dopamine synthesis and release capacity as the primary locus of dopaminergic abnormality in schizophrenia, with a large effect size (Howes et al., 2012a). Further support for this comes from studies of people at risk of psychosis that have also found elevated dopamine synthesis and release capacity (Howes et al., 2009b; Mizrahi et al., 2012; Stone et al., 2010), and studies linking this to the later onset of psychosis (Howes et al., 2011a; Howes et al., 2011b).

Antipsychotic drugs are central to the treatment of schizophrenia (Howes and Murray, 2014). All current antipsychotic drugs block dopamine receptors (Howes et al., 2009a) and their affinity for dopamine receptors is directly associated with their clinical effectiveness (Creese et al., 1976; Howes et al., 2009a). Furthermore, dopamine receptor blockade has been shown to be necessary for clinical response (Kapur et al., 2000; Nordstrom et al., 1993), and greater presynaptic dopamine dysfunction at baseline is associated with greater subsequent response to antipsychotic treatment (Abi-Dargham et al., 2000). Coupled with the evidence of elevated dopamine synthesis and release capacity in schizophrenia, this indicates that antipsychotics work by blocking the consequences of elevated dopaminergic neurotransmission (Howes et al., 2009a).

However, 15%-30% of patients with schizophrenia do not experience a significant reduction in symptoms with standard, first-line antipsychotic treatment (Kane et al., 1988). These patients are considered to be treatment resistant, defined as an inadequate response to adequate treatment trials with at least two different first-line antipsychotic drugs (Beck et al., 2014; Kane, 1989). Patients with treatment resistant schizophrenia are highly unlikely to
respond to further treatment with first-line antipsychotic drugs (Suzuki et al, 2007). Clozapine is the only antipsychotic drug with proven efficacy in patients with treatment resistant schizophrenia (Agid et al, 2013; Kumra et al, 2008). However, clozapine’s use is limited by poor tolerability in some patients and a complex monitoring regime (Howes et al, 2012b). There is, therefore, a need to understand the neurobiology of treatment resistant schizophrenia better to develop alternative treatments to clozapine.

It has been proposed that the differential response to first-line antipsychotic drugs seen in patients reflects a different underlying neurobiology, and, specifically, that patients with treatment responsive schizophrenia show elevated dopamine synthesis and release capacity, which is not seen in patients with treatment resistant schizophrenia (Howes and Kapur, 2014). Supporting this, studies of plasma dopamine metabolites show that patients with lower baseline levels are less likely to respond to first-line antipsychotic drugs (Ottong and Garver, 1997; Yoshimura et al, 2003). Furthermore, a post-mortem study comparing dopaminergic markers between patients who had histories of good and poor response found that patients with a history of poor response showed fewer dopaminergic synapses identified by the immunochemical localization of tyrosine hydroxylase (Roberts et al, 2009). There is also evidence from a PET study that found dopamine synthesis capacity was significantly lower in patients with treatment resistant schizophrenia, who remained highly symptomatic and functionally impaired despite adequate treatment trials with at least two different first-line antipsychotic drugs, when compared to patients who had responded to first-line antipsychotic treatment (Demjaha et al, 2012). Taken together with the previous literature that there is a positive association between symptomatic severity and dopamine synthesis capacity in schizophrenia (Howes et al, 2007), this finding suggests that dopaminergic function is
different in treatment resistant schizophrenia. However, in this study, the treatment resistant patients were highly symptomatic whilst the responsive patients showed no or minimal symptoms. The study could not, therefore, distinguish whether this was a trait effect or a reflection of the difference in symptom levels.

Thus, we aimed to test whether dopaminergic function is altered in patients with a history of treatment resistance to first-line drugs relative to treatment responders when both groups are matched for symptom severity levels. In line with the proposal that treatment resistant schizophrenia has a non-dopaminergic basis (Howes and Kapur, 2014), we hypothesized that patients with treatment resistant schizophrenia would have lower dopamine synthesis capacity compared to patients who have responded to first-line antipsychotic drugs when the symptom state is matched. To match the symptom state, we recruited patients with treatment resistant schizophrenia who had responded to clozapine and compared them to patients who had responded to first-line antipsychotic drugs using $^{18}$F]DOPA PET to measure presynaptic dopamine synthesis capacity. Additionally, we included a matched healthy volunteer group to provide a comparison with normal dopamine synthesis capacity.

**Materials and Methods**

This study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008.

**Participants**

Participants (aged 18 to 65 years) received a full explanation of the study and provided written informed consent to participate. Screening procedures included physical examination,
checking vital signs, laboratory tests (hematology, blood chemistry, and urinalysis), and a 12-lead electrocardiogram. Subjects with any medically significant abnormalities and/or psychiatric diseases (except schizophrenia in patients group) were excluded. Symptoms were rated using the Positive and Negative Syndrome Scale (PANSS), and chlorpromazine equivalent doses for antipsychotic treatment were calculated using the formula described by Andreasen et al (2010).

Patients with schizophrenia

Patients were recruited from the outpatient clinic in the Seoul National University Hospital. Patients who met the following inclusion criteria were invited to participate in the study: (1) Patients who met DSM-IV criteria for schizophrenia (2) Patients who had a total score of less than or equal to 80 in the PANSS and no items with a score above 4 on the positive subscale of the PANSS (3) patients who have received first-line antipsychotic drugs including risperidone, olanzapine and paliperidone (first-line AP group) or clozapine (clozapine group) for at least 12 weeks (4) The first-line AP group had to have no history of being given clozapine or being refractory to first-line antipsychotic drug treatments (5) Based on chart review, the clozapine group had to have history of no response to at least two different first-line antipsychotic drugs. Twelve patients were enrolled for each group.

Healthy controls

Twelve healthy controls (control group) were recruited via advertisement and were matched to the patients with schizophrenia on the basis of age (within 3 years) and sex. A psychiatric interview for the presence of DSM-IV axis I disorders was conducted using the Structured Clinical Interview for DSM-IV Axis I Disorders, Non-Patient edition (SCID-I/NP) (First et al, 2002).
Positron Emission Tomography

All the patients except one who took a long-acting injectable risperidone were taking their antipsychotic drugs at night once a day. The patients were instructed to take their antipsychotic medication at 9 p.m. a day before the scan. The date of the PET scan was randomly assigned to participants to avoid a possible bias by consecutive scans from the same group. The PET scan was performed at 2 p.m. and participants were required to fast and abstain from smoking and drinking from midnight on the day of the scan and received 150 mg carbidopa and 400 mg entacapone orally 1 hour prior to scanning to reduce the formation of radiolabeled metabolites (Turkheimer et al., 1999).

Participants underwent a short computed tomography (CT) for attenuation correction and PET imaging on a Biograph 40 Truepoint PET/CT scanner (Siemens, Knoxville, Tennessee, USA) for 95 minutes after an intravenous bolus injection of approximately 370 MBq (10mCi) of $^{18}$F-DOPA with minimum specific activity of $1.30 \times 10^7$ Ci/mol. Head movement was monitored with a mark and minimized using a light head strap. After routine corrections for uniformity, decay corrections and attenuation (using the CT), the PET imaging data acquired in a list mode were reconstructed with a filtered back-projection using a Gaussian filter. Images were collected in a three-dimensional mode with 148 axial slices, an image size of 256 x 256, a pixel size of 1.3364 x 1.3364 mm$^2$ and a slice thickness of 3 mm. The dynamic volumetric images were sequenced using the following framing: 2 x 30, 4 x 60, 3 x 120, 3 x 180, and 15 x 300 sec.

For the analysis of volume effects in the striatum, high-resolution T1-weighted magnetic resonance images (MRIs) were also acquired after the PET scan (TE = 1.89 ms, TR = 1670 ms, flip angle = 9°, 208 slices, matrix = 256 x 256, FOV = 250 mm).
**Image analysis**

PET image analysis was conducted as previously described (Bloomfield *et al.*, 2014). Inter-frame correction for head movement during the scan was performed by denoising the non-attenuation-corrected dynamic images using a level 2, order 64 Battle-Lemarie wavelet filter. Frames were hence realigned to a single ‘reference’ frame, acquired 8 min post-injection, employing a mutual information algorithm (Turkheimer *et al.*, 1999). The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images, creating a movement-corrected dynamic image. Subsequently, the realigned images were spatially normalized by registering their summed image to the $[^{18}\text{F}]\text{DOPA}$ template created in a previous study (McGowan *et al.*, 2004). Region-of-interest (ROI) time-activity curves (TACs) were hence extracted using atlas maps for the whole striatum, and its associative, limbic, and sensorimotor sub-regions (Martinez *et al.*, 2003). The cerebellum was used as the reference region as it is a region with minimal dopaminergic projections (Hammers *et al.*, 2003). Finally, using the cerebellar TAC as a reference region input, the Gjedde-Patlak plot (Patlak and Blasberg, 1985) was applied at ROI and voxel level to derive the influx rate constants ($k_{\text{ic}}$) relative to the cerebellum, for the regions of interest and individual parametric maps respectively. The analysis was performed using a combination of SPM5 package (http://www.fil.ion.ucl.ac.uk/spm) and in-house code based on Matlab2012b$^\text{®}$ (The Mathworks Inc., MA, USA). A previous test retest study has found this approach to have high reliability for striatum (Egerton *et al.*, 2010). Average parametric images in each group were derived from the individual parametric images normalized into Montreal Neurological Institute standard space (matrix dimension: 91x109x91; voxel size: 2mm isotropic) using the participant’s PET summation image and the $[^{18}\text{F}]\text{DOPA}$ template.
Volumetric segmentation in each high-resolution T1 weighted MRI was performed using the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/) (Fischl, 2012) to extract striatal volumes.

**Statistical analysis**

After confirming the data were normally distributed by using the Kolmogorov-Smirnov test, we used independent t-test and analysis of variance test to compare demographic variables and striatal volumes between groups. Pearson’s Chi-square test was used to test difference in sex ratio between groups. A mixed effects model was employed in a repeated measures analysis to test whether there was a group effect on \( k_i^{\text{cer}} \) with the ROI (Region: modeled as a dummy variable: 1=associative, 2=limbic, 3=sensorimotor subregions) and the group (Group: modeled as a dummy variable: 1=control group, 2=first-line AP group, 3=clozapine group) as fixed effects and subjects as random effects. Pearson correlation analysis was employed to investigate the relationship between \( k_i^{\text{cer}} \) and symptoms measured by using PANSS and between the striatal volume and the duration of illness or duration of exposure to current antipsychotics.

**Results**

Twelve participants were recruited in each group. One smoking patient was enrolled in the clozapine group and there was no participant with a history of alcohol abuse and/or dependence. Table 1 shows demographic characteristics of participants. The mean age (±s.d.) of participants was 30.9±8.6 years. There was no significant difference in age between the groups (F=0.05, df=2,33, p=0.951). The gender distribution was not significantly different
between groups ($\chi^2=0.262$, df=2, $p=0.877$). The mean PANSS total score ($\pm$s.d.) was 31.7$\pm$1.1 in control group, 50.3$\pm$11.1 in the first-line AP group and 49.7$\pm$7.9 in the clozapine group. As expected, there was a significant effect of group on PANSS scores ($F=21.75$, df=2, 33, $p<0.001$). Pairwise comparison using Bonferroni’s correction revealed significantly lower scores in the control group than in the first-line AP group (mean difference=18.67, s.e.=3.21, df=33, $p<0.001$) and in the clozapine group (mean difference=18.00, s.e.=3.21, df=33, $p<0.001$), but no significant difference in the total PANSS score between the first-line AP group and the clozapine group (mean difference=0.67, s.e.=3.21, df=33, $p=1.000$).

Antipsychotic drugs in the first-line AP group were risperidone for 5 patients, paliperidone for 3 patients and olanzapine for 4 patients. The mean chlorpromazine-equivalent doses ($\pm$s.d.) were 285.4$\pm$153.2 mg in the first-line AP group and 261.4$\pm$117.5 mg in the clozapine group. The chlorpromazine equivalent doses were not significantly different between the first-line AP group and the clozapine group ($t=-0.43$, df=22, $p=0.671$).

The mean striatal volumes ($\pm$s.d.) were 21953.6$\pm$1896.3 mm$^3$ in the control group, 23685.6$\pm$2787.8 mm$^3$ in the first-line AP group and 22576.8$\pm$2529.8 mm$^3$ in the clozapine group, and there was no significant difference in the striatal volume between the groups ($F=1.56$, df=2, 33, $p=0.225$). There was no correlation between the striatal volume and the duration of illness ($r=0.066$, $p=0.756$) or duration of exposure to current antipsychotics ($r=-0.104$, $p=0.627$). The mean injected dose ($\pm$s.d.) of [18F]DOPA was 10.0$\pm$0.7 mCi in the control group, 9.8$\pm$0.9 mCi in the first-line AP group and 9.9$\pm$0.7 mCi in the clozapine group and there was no significant difference in the injected dose between the groups ($F=0.33$, df=2, 33, $p=0.719$). Figure 1A shows average parametric images of [18F]DOPA ($k_i^{cer}$) in each group and table 2 shows $k_i^{cer}$ values. The $k_i^{cer}$ values were significantly different according to the
The $k_i^{\text{cer}}$ difference according to the group was still significant after incorporating the striatal volume as a covariate (Group: $F=12.88$, df=2, 94.4, $p<0.001$; Region: $F=34.69$, df=2, 59.95, $p<0.001$). In pairwise comparison, the clozapine group showed significantly lower $k_i^{\text{cer}}$ value than the control group (mean difference=$-0.00154$, s.e.=0.00028, df=95.0, $p<0.001$) and the first-line AP group (mean difference=$-0.00110$, s.e.=0.00028, df=95.0, $p<0.001$) (Figure 1B). However, the $k_i^{\text{cer}}$ value in the first-line AP group was not significantly different from that in the control group (mean difference=$-0.00044$, s.e.=0.00028, df=95.0, $p=0.367$) (Figure 2). There was no correlation between $k_i^{\text{cer}}$ from the whole striatum and the total scores of PANSS in either patient group ($r=0.244$, $p=0.445$ for first line AP group; and $r=-0.231$, $p=0.470$ for clozapine group).

**Discussion**

Our study is the first to report dopamine synthesis capacity in a group of patients solely treated with clozapine. Elkashef et al. (2000) also measured dopamine synthesis capacity in patients treated with clozapine. However, they reported the mean value of dopamine synthesis capacity in a group including patients treated with either first-line antipsychotic drugs or clozapine (Table 3). Our main finding is that patients who have responded to clozapine with the history of treatment resistance show lower dopamine synthesis capacity than patients who have responded to first-line treatment in line with the hypothesis that the dopaminergic function is linked to treatment response (Howes and Kapur, 2014). Both patient groups showed low symptom severity levels and were well matched for total symptom severity ratings. In view of this, our finding extends a previous study where symptom severity differed between treatment resistant and responsive groups (Demjaha et al., 2012), to indicate
that lower dopamine synthesis capacity is likely to reflect a trait, rather than state, aspect of treatment resistant schizophrenia. This is consistent with the hypothesis that treatment resistant schizophrenia has a different neurobiological basis to schizophrenia that has responded to first-line antipsychotic treatment (Howes and Kapur, 2014).

We observed no difference in dopamine synthesis capacity between the first-line AP group and the control group, in contrast to the majority of previous radiolabeled DOPA PET studies in schizophrenia, which report an elevation in schizophrenia (Table 3). It could be due to small sample size to detect difference in dopamine synthesis capacity between schizophrenia and controls. Our study was powered (power>0.8) to detect the difference between treatment resistant and responsive patients based on the effect size>1.2 reported in the only prior study (Demjaha et al, 2012). The Cohen’s d effect size difference in dopamine synthesis capacity between schizophrenia and controls was 0.8 (Howes et al, 2012a). Thus the sample size might be small to detect differences between controls and schizophrenia. Another possibility is that it reflects a state effect in this group, either due to phase of illness or to antipsychotic treatment (Grace, 1992; Grace and Bunney, 1986; Grunder et al, 2003; Vernaleken et al, 2006). Most studies using radiolabeled DOPA were conducted in drug-naïve or drug-free patients and reported consistently higher levels of presynaptic dopamine function in schizophrenia compared with healthy controls (Table 3). In contrast, studies in patients treated with antipsychotic drugs showed mixed results. The primary outcome in the current study was the influx rate constant normalized to the distribution volume of the cerebellum. The blood flow changes induced by antipsychotic treatment have been reported in the frontal cortex (Miller et al, 2001), basal ganglia (Miller et al, 1997) and hippocampus (Medoff et al, 2001). However, the results are inconsistent in this aspect and the cerebellum, which served
as the reference region in our study, showed no blow flow change associated with antipsychotic treatment (Miller et al., 2001). Thus antipsychotic treatment is unlikely to affect the primary outcome by changing the distribution volume of the cerebellum. To our knowledge, there have been five studies which examined dopamine synthesis capacity in patients treated with antipsychotic drugs and compared it with healthy controls (Table 3) (Demjaha et al., 2012; Elkashef et al., 2000; Howes et al., 2013; McGowan et al., 2004; Shotbolt et al., 2011). Two reported higher level of presynaptic dopamine function in patients (Howes et al., 2013; McGowan et al., 2004), and two reported no difference between patients treated with antipsychotic drugs and healthy controls (Elkashef et al., 2000; Shotbolt et al., 2011) and Demjaha et al. (2012) reported higher level of presynaptic dopamine function in antipsychotic responders and no difference in antipsychotic-refractory patients. The patients included in these studies were similar to our patient groups in being more chronic and less symptomatic than the studies of drug-naive/free patients. There is some evidence that presynaptic dopamine dysfunction varies with phase of illness, increasing with acute psychosis (Howes et al., 2011a; Laruelle et al., 1999). Thus taken together, this inconsistency in the findings in chronic treated patients may be due to an effect of treatment and/or state of illness, which requires a prospective study to address this issue.

We found that the clozapine group showed a significantly lower $k_{i_{\text{cer}}}$ value than the control group, with an effect size (Cohen’s $d$) of 1.3. This is in contrast to the previous report by Demjaha et al. (2012), which showed no difference in dopamine synthesis capacity between the healthy controls and the treatment-resistant patients with schizophrenia. A difference between the studies is that the treatment resistant patients in our study were treated with clozapine. This is the first study reporting lower dopamine capacity in patients treated with
clozapine. Clozapine is reported to reduce extracellular dopamine level after chronic administration (Shilliam and Dawson, 2005). This may account for the difference between the study by Demjaha et al (2012) and our study, though the mechanism of action reducing dopamine synthesis still remains obscure. It is also important to note that clozapine is reported to increase the activity of aromatic L-amino acid decarboxylase, the enzyme that convert L-DOPA (and radiolabeled DOPA in the PET scan) to dopamine (Neff et al, 2006). This could influence $k_{i}^{cer}$ values in the clozapine group, although increased enzyme activity would be expected to increase $k_{i}^{cer}$ values, whereas we saw lower $k_{i}^{cer}$ in the clozapine group.

Clinical implications

Lower presynaptic dopamine capacity observed in the clozapine group may reflect a different underlying pathophysiology of schizophrenia in patients resistant to first-line antipsychotic drugs. Evidence that glutamate levels are higher in treatment resistant patients (Demjaha et al, 2014; Mouchlianitis et al, doi:10.1093/schbul/sbv151), suggests that glutamatergic differences could underlie the pathophysiology of treatment resistance, and is consistent with findings that clozapine acts on the glutamate system (Duncan et al, 1998; Javitt et al, 2005; Lopez-Gil et al, 2007). Other neurochemical systems may also be involved in treatment resistance (Selvaraj et al, 2014).

Furthermore, the finding supports the early initiation of clozapine treatment as it suggests there is less neurochemical rational for dopaminergic blockade. Reducing unnecessary exposure to first-line antipsychotic drugs could lower the risk of adverse effects as well. There is already some clinical evidence that clozapine initiation soon after the first episode of psychosis improves outcome (Agid et al, 2007; Remington et al, 2013). Our findings suggest that dopamine synthesis capacity may be a useful biomarker for predicting treatment
resistance, although sensitivity and specificity will need to be tested (Bose et al, 2008).

Limitations

This is a cross-sectional study and we did not measure presynaptic dopamine capacity before the administration of antipsychotic drugs or from illness onset. Though our result is consistent with lower dopamine synthesis capacity being a trait of treatment resistant schizophrenia, a prospective study is required for confirmation. Though it was not statistically significant, the average duration of exposure to current antipsychotic drugs was longer in the clozapine group than in the first-line AP group (Table 1). Moreover, the clozapine group had a period of treatment with first-line antipsychotic medication prior to clozapine treatment. As such, it is not unexpected that the patients in the clozapine group might have longer exposure to antipsychotics, though they did not respond to first-line antipsychotic drugs. Considering that the duration of exposure to antipsychotic drugs may affect dopamine synthesis capacity (Grace, 1992; Shilliam and Dawson, 2005), this needs to be taken into consideration when interpreting the result. According to the inclusion/exclusion criteria, we excluded participants with history of drug abuse or dependence. However, one smoking patient was enrolled in the clozapine group. There are some reports that cigarette smoking can influence dopamine synthesis capacity, though the results are inconsistent (Bloomfield et al, 2014; Rademacher et al, 2015; Salokangas et al, 2000). Nonetheless, re-analysis without the data from the patient with the history of cigarette smoking in the clozapine group found the same results and therefore smoking is unlikely to affect the result. Concomitant medication could have affected the outcome. We did not take the concomitant medication into consideration when interpreting results. This could be a limitation for the study. However, as seen in Table 1, the concomitant medication does not seem different.
between two patient groups, hence is unlikely to affect the group difference in the outcome. The specific activity of $[^{18}\text{F}]$DOPA was not routinely measured in the current study and we were not able to compare the specific activity between groups. The difference in specific activity between groups could have affected the result. However, we randomly assigned the date of scan to participants to avoid the possible bias and visual inspection into individual data acquired on the same day found similar trends of lower $k_i^{\text{cer}}$ values in the clozapine group than in the first-line AP group, which may suggest the bias is unlikely to affect the result.

Conclusions

Dopamine synthesis capacity is lower on average in patients resistant to first-line antipsychotic drugs relative to patients who have responded to first-line antipsychotic drugs, suggesting that a different neurobiology may underlie treatment resistant schizophrenia and that dopamine synthesis capacity may be a useful biomarker to predict treatment responsiveness.

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Figure Legends

Figure 1. Average parametric images (A) of $[^{18}\text{F}]\text{DOPA (}k_i^{\text{cer}}\text{)}$ from the healthy controls (Control group), and patients treated with the first-line antipsychotic drugs (First-line AP group) or clozapine (Clozapine group) and statistical parametric mapping outputs (B) comparing control group and clozapine group (Left) and first-line AP group and clozapine group (Right). The statistical parametric mapping outputs are thresholded at $p<0.001$ uncorrected and the blue hairline indicates the voxels with highest $t$ value.

Figure 2. $[^{18}\text{F}]\text{DOPA }k_i^{\text{cer}}$ values in the whole striatum according to the group. Each dot represents an individual $k_i^{\text{cer}}$ value and each vertical bar indicates the mean and the standard deviation for the corresponding group. Asterisks indicate significant differences between two groups ($p<0.001$).
Table 1. Demographic characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Control group, n=12</th>
<th>First-line AP group, n=12</th>
<th>Clozapine group, n=12</th>
<th>p value</th>
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<tr>
<td>Age (yr±s.d.)</td>
<td>30.3 ± 8.4</td>
<td>31.1 ± 9.8</td>
<td>31.3 ± 8.1</td>
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<td>Sex (male/female)</td>
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<td>8 / 4</td>
<td>9 / 3</td>
<td>0.877</td>
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<td>PANSS total score (±s.d.)</td>
<td>31.7 ± 1.1</td>
<td>50.3 ± 11.1</td>
<td>49.7 ± 7.9</td>
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<td>Positive scale score</td>
<td>7.0 ± 0.3</td>
<td>10.8 ± 2.7</td>
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<td>12.8 ± 2.8</td>
<td>&lt;0.001</td>
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<td>General psychopathology score</td>
<td>17.3 ± 1.0</td>
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<td>Duration of illness (mont±s.d.)</td>
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<td>Paliperidone (3)</td>
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<td></td>
<td>Olanzapine (4)</td>
<td>Clozapine (12)</td>
<td></td>
</tr>
<tr>
<td>Antipsychotic dose (mg±s.d.)</td>
<td>-</td>
<td>Risperidone: 4.0±1.5</td>
<td>Paliperidone: 8.0±1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine: 11.9±12.1</td>
<td>282.3 ± 126.9</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalent dose (mg±s.d.)</td>
<td>-</td>
<td>285.4 ± 153.2</td>
<td>261.4 ± 117.5</td>
<td>0.671</td>
</tr>
<tr>
<td>Duration of exposure to current antipsychotics (mont±s.d.)</td>
<td>-</td>
<td>64.1 ± 18.2</td>
<td>76.3 ± 12.4</td>
<td>0.584</td>
</tr>
<tr>
<td>Concomitant mediation (n)</td>
<td>-</td>
<td>None (6)</td>
<td>SSRI (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzodiazepine (3)</td>
<td>SSRI (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antiparkinsonian agent (4)</td>
<td>Benzodiazepine (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antiparkinsonian agent (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The chlorpromazine equivalent dose was calculated based on the formula from Andreasen et al (2010)

One patient treated with risperidone was given long-acting injectable risperidone. The dose of long-acting injectable risperidone was converted to oral equivalent.(Bai et al, 2007)

SSRI includes escitalopram and fluoxetine in the first-line AP group and escitalopram, sertraline and fluoxetine in the clozapine group.

Benzodiazepine includes lorazepam and clonazepam in the first-line AP group and lorazepam,
alprazolam and clonazepam in the clozapine group.

Antiparkinsonian agent indicates medication for treating extrapyramidal symptoms including propranolol, benztropine and trihexyphenidyl in both groups.
Table 2. $[^{18}\text{F}]\text{DOPA} \ k_{i}^{\text{cer}}$ values (min$^{-1}$) (±s.d.) of regions-of-interest in the healthy controls (Control group) and patients treated with first-line antipsychotic drugs (First-line AP group) or clozapine (Clozapine group).

<table>
<thead>
<tr>
<th>Region</th>
<th>Control group</th>
<th>First-line AP group</th>
<th>Clozapine group</th>
<th>Cohen’s $d$ between either patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole striatum</td>
<td>0.01521±0.00121</td>
<td>0.01465±0.00112</td>
<td>0.01351±0.00135</td>
<td>0.9191</td>
</tr>
<tr>
<td>Associative striatum</td>
<td>0.01483±0.00131</td>
<td>0.01420±0.00126</td>
<td>0.01318±0.00136</td>
<td>0.7781</td>
</tr>
<tr>
<td>Limbic striatum</td>
<td>0.01439±0.00115</td>
<td>0.01411±0.00076</td>
<td>0.01315±0.00107</td>
<td>1.0344</td>
</tr>
<tr>
<td>Sensorimotor striatum</td>
<td>0.01647±0.00135</td>
<td>0.01596±0.00132</td>
<td>0.01446±0.00161</td>
<td>1.0189</td>
</tr>
<tr>
<td>Authors</td>
<td>Controls</td>
<td>Patients</td>
<td>CPZ equivalents (mg/day) (±s.d.)</td>
<td>Total symptom score (±s.d.)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------</td>
<td>----------------------</td>
<td>---------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
<td>N (Male/Female)</td>
<td>Age (±s.d.)</td>
<td>N (Male/Female)</td>
<td>Age (±s.d.)</td>
</tr>
<tr>
<td>Reith et al (1994)</td>
<td>13 (9/4)</td>
<td>36 (±13)</td>
<td>5 (5/0)</td>
<td>38 (±4)</td>
</tr>
<tr>
<td>Hietala et al (1995)</td>
<td>8 (6/2)</td>
<td>27 (±7)</td>
<td>7 (4/3)</td>
<td>26 (±7)</td>
</tr>
<tr>
<td>Dao-Castellana et al (1997)</td>
<td>7 (na)</td>
<td>25 (±5)</td>
<td>6 (na)</td>
<td>26 (±9)</td>
</tr>
<tr>
<td>Hietala et al (1999)</td>
<td>13 (8/5)</td>
<td>30.4 (±9.4)</td>
<td>10 (4/6)</td>
<td>29.6 (±8.8)</td>
</tr>
<tr>
<td>Lindstrom et al (1999)</td>
<td>10 (8/2)</td>
<td>na</td>
<td>12 (10/2)</td>
<td>31 (na)</td>
</tr>
<tr>
<td>Elkashef et al (2000)</td>
<td>13 (8/5)</td>
<td>34.6 (±10.8)</td>
<td>19 (15/4)</td>
<td>36.3 (na)</td>
</tr>
<tr>
<td>Meyer-Lindenberg et al (2002)</td>
<td>6 (5/1)</td>
<td>34 (na)</td>
<td>6 (5/1)</td>
<td>35 (na)</td>
</tr>
<tr>
<td>McGowan et al (2004)</td>
<td>12 (12/0)</td>
<td>38.3 (±7.1)</td>
<td>16 (16/0)</td>
<td>39.9 (±11.3)</td>
</tr>
<tr>
<td>Kumakura et al (2007)</td>
<td>15 (15/0)</td>
<td>37.3 (±6.4)</td>
<td>8 (8/0)</td>
<td>37.3 (±6.3)</td>
</tr>
<tr>
<td>Nozaki et al (2009)</td>
<td>20 (10/10)</td>
<td>35.1 (±9.5)</td>
<td>18 (10/8)</td>
<td>35.6 (±7.4)</td>
</tr>
<tr>
<td>Howes et al (2009b)</td>
<td>12 (8/4)</td>
<td>24.3 (±4.6)</td>
<td>7 (5/2)</td>
<td>36.0 (±14.7)</td>
</tr>
<tr>
<td>Shotbolt et al (2011)</td>
<td>20(10/10)</td>
<td>39 (±14)</td>
<td>6 (na)</td>
<td>43 (±12)</td>
</tr>
<tr>
<td>Study</td>
<td>Treatment-Resistant</td>
<td>Treatment-Responder</td>
<td>PANSS: Treatment-Resistant</td>
<td>CASH:</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>---------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Denjaha et al (2012)</td>
<td>12 (5/7)</td>
<td>12 (5/7)</td>
<td>396.1 (±57.5)</td>
<td>77.6  (±47.6)</td>
</tr>
<tr>
<td>Howes et al (2013)**</td>
<td>29 (22/7)</td>
<td>29 (26/3)</td>
<td>104.3 (±10.6)</td>
<td></td>
</tr>
<tr>
<td>**: Fourteen antipsychotic treated patients and twelve controls in Howes et al 2013 have been included in McGowan et al 2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.
Figure 2.