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Title: Brain imaging studies of treatment-resistant schizophrenia: a systematic review

Running title: Treatment resistant neuroimaging review

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ABSTRACT

Background: Approximately 30% of patients with schizophrenia show an inadequate response to antipsychotics, termed treatment resistance. Neuroimaging studies may help elucidate the underlying neurobiological reasons that certain patients show inadequate treatment response, and help identify them earlier. In addition, studies examining the effect of clozapine on the brain may help identify which aspects of clozapine make it uniquely effective in treatment resistance.

Method: We performed a systematic search of PubMed between January 1980 and April 2015 in order to identify all neuroimaging studies that had examined treatment resistant patients, or longitudinally studied the effects of clozapine treatment.

Findings: The search identified 330 papers, of which 60 met inclusion criteria. Replicated differences in treatment resistant relative to responsive patients include reductions in gray matter and perfusion of frontotemporal regions and increases in white matter and basal ganglia perfusion. Clozapine treatment has been shown to lead to reductions in caudate nuclei volumes in three separate studies.

Interpretation: The available evidence supports the possibility that some of the neurobiological changes observed in resistant schizophrenia lie along a continuum with non resistant schizophrenia; while other differences may be more categorical in nature. There is, however, limited replication and in order for neuroimaging findings to be clinically translatable, future studies need to provide clear a priori hypotheses and test these rigorously.

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INTRODUCTION

Schizophrenia is a severe mental illness characterised by psychotic (positive), negative and cognitive symptoms, and has a prevalence of about 1%.\(^1\) Antipsychotic medication has revolutionised the treatment of schizophrenia.\(^2\) However, 20-30% of patients show limited response to anti-psychotic medication.\(^3\) Due to persistent symptoms such patients stay longer in hospital care, and have increased treatment costs in comparison to patients who have responded.\(^4\) Furthermore, the prognosis is worse the longer their symptoms do not show improvement.\(^5\)

Careful studies in the late 1980s and early 1990s demonstrated that less than 5% of patients who had not responded to two different first-line antipsychotics showed a response to a further antipsychotic, with the exception of clozapine.\(^6\) This has subsequently been confirmed in further clinical trials and naturalistic studies.\(^7\)

It has thus become clear that there is a group of patients whose illness does not respond to first-line treatment, and this has been termed treatment resistant schizophrenia (TRS).\(^8\) Studies of drug occupancy at D2/3 receptors have found comparable levels of D2 receptor occupancy in responders and non-responders, indicating that a failure to obtain adequate drug levels in the brain does not explain non-response.\(^9\)

These findings raise two questions. First, what is different about the underlying neurobiology in these patients that means antipsychotic drugs, other than clozapine, have little benefit? And second, what is it about clozapine that makes it uniquely
effective in these patients? Answering these questions is critical to developing new treatments for refractory schizophrenia. A further clinical need is the early identification of patients with TRS to allow them to start appropriate treatment without delay.\textsuperscript{10} Treatment guidelines recommend that patients should receive clozapine if they have not responded to two adequate antipsychotic trials.\textsuperscript{11} However in clinical practice there is generally a long delay before patients start clozapine.\textsuperscript{12} A biomarker that enabled the early identification of treatment resistance, potentially at first presentation, could obviate the current requirement for empirical trials of different antipsychotics.

The purpose of this paper is therefore to review the neuroimaging evidence regarding treatment resistant schizophrenia, and consider the implications for developing new treatments and biomarkers for treatment resistance.
METHODS

The search was conducted within PubMed looking for studies published between January 1980 to April 2015. In addition to the online database search results, reference lists of reviews and papers identified by the search were reviewed for additional studies.

The following key words were used as a search strategy:
(treatment resistant OR treatment refractory OR drug resistant)
AND
(schizophrenia OR psychosis)
AND
(magnetic resonance imaging OR MRI OR functional magnetic resonance imaging OR fMRI OR positron emission tomography OR PET OR magnetic resonance spectroscopy OR MRS OR EEG OR electroencephalography OR magnetoencephalography OR MEG OR event related potential OR ERP OR voxel based morphometry OR VBM OR diffusor tensor imaging OR DTI OR SPECT or SPECT or CT)

Studies were selected by two independent reviewers (EM & RM). To qualify for inclusion, studies must have been published in peer-reviewed journals as an original research paper in English language. We included all studies that recruited treatment-resistant patients and used in vivo brain imaging modalities. We also included longitudinal studies reporting neuroimaging findings pre and post clozapine treatment in patients with resistant schizophrenia (studies solely examining clozapine receptor occupancy, were not included).
The data extracted from each paper were: sample size, criteria for definition of
treatment-resistance, brain imaging modality, medication status, and diagnostic
criteria for the schizophrenia diagnosis. Where possible effect sizes for the contrasts
of interest were calculated, measured by Cohens’s $d$ for differences between means.

**RESULTS**

The search with the terms outlined above as well as reference list review identified
330 papers, of which 60 met the inclusion criteria (see Fig. 1). 14 of the studies
defined treatment resistance according to the criteria of Kane et al. The remainder
used a range of definitions, while eight studies did not specify any criteria (see Table
1).

*Studies comparing treatment-resistant patients with healthy control groups*

29 studies, comprising 680 patients and 714 controls, compared treatment-resistant
patients with healthy volunteers (see Table 2).

Ten structural studies were identified. Five reported overall gray matter volumes, and all but one reported significant reductions. Four studies reported specific regions
of gray matter reduction in TRS. Over 25 separate areas of reduction were
reported, with the left middle frontal, right precentral and right middle temporal gyri
being most consistently implicated. Regarding white matter volumes, one study of
haloperidol treated individuals reported overall increases in TRS, while a study of
clozapine treated individuals, and a study where medication status was not
specified,18 reported reductions. One study showed that resistant patients demonstrated enlargement in posterior sections of the corpus callosum, particularly the splenium.21 Complementing this finding, a diffusion tensor imaging study showed widespread disruptions to white matter tract integrity in TRS.22 This was especially apparent in the corpus callosum, and illness duration was negatively related to fractional anisotropy in the splenium.

Five studies used functional MRI (fMRI). Three resting state studies,23–25 and a study using a word generation task,26 have produced findings that while not necessarily incompatible are hard to draw coherent conclusions from as a whole. An arterial spin labeling (ASL) in individuals with resistant auditory hallucinations demonstrated increased cerebral blood flow in a variety of areas involved in speech processing.27

Seven studies used positron emission tomography (PET) or single photon emission computed tomography (SPECT). Six of these used radiotracers that allow the measurement cerebral metabolic rate (e.g. 18F-fluorodeoxyglucose (FDG)) or blood flow (Technetium-99m-exametazime (99mTc-HMPAO), Oxygen-15(15O), and technetium-99m-ethyl cysteinate diethylester (99mTc-ECD)), and all but one28 demonstrated a degree of hypofrontality in TRS. Three studies employing 99mTc-HMPAO SPECT demonstrated reduced perfusion of frontal areas in TRS.29–31 In one study,29 resistant patients also showed increased perfusion ratios in the basal ganglia (replicated in a second study30), while reduced perfusion of the right dorsolateral prefrontal cortex correlated with negative symptom severity. Another study used 99mTc-ECD SPECT, while participants performed the Wisconsin Card Sorting test. Individuals with TRS were had reduced rCBF in fronto-temporal regions at rest, and a

Reduced percentage increase during the task. A FDG PET study demonstrated reduced activity in cortical and subcortical regions in TRS. Another FDG PET study looking specifically at resistant hallucinations demonstrated increased metabolic activity in a range of language related areas.

Two studies employed magnetic resonance spectroscopy (MRS). One showed increased glutamate concentrations in the anterior cingulate cortex of individuals with TRS; while another showed increased glutamate+glutamine concentrations in the putamen.

Six studies used electroencephalography (EEG). The P300 is an event-related EEG component that occurs when a stimulus deviates from a preceding sequence of standard stimuli and is thought to index information processing efficiency. Two studies showed significant decreases in P300 amplitude in patients compared to controls. Two studies used the mismatch negativity (MMN) component which is believed to index the integrity of the pre-attentive sensory network. These showed decreased amplitudes in resistant patients. One study of thirteen patients was not in agreement with the above findings, both in terms of MMN and P300 components. Medication status of patients was not specified raising the possibility that this could explain the discrepancy.

*Studies comparing treatment-resistant with treatment-responsive patient groups*

Sixteen studies compared treatment-resistant (298 patients) and treatment-responsive groups (264 patients) (see Table 3).
Seven studies used structural MRI.\textsuperscript{14–16,19,20,40,41} All demonstrated reduced gray matter in frontal areas in resistant compared to responsive patients (although this was not significant in two studies\textsuperscript{14,40}). Two studies\textsuperscript{14,15} report increased white matter volumes in resistant patients, but only in one\textsuperscript{14} is this significant.

Two studies used fMRI. A rsMRI study demonstrated that resistant patients display greater functional connectivity between the dorsomedial prefrontal cortex and other frontotemporal areas, but reduced connectivity between the ventromedial prefrontal cortex and areas of the cingulate cortex.\textsuperscript{23} The ASL study described above demonstrated increased rCBF in the left superior temporal gyrus, right supramarginal gyrus and temporal polar cortex in patients with treatment resistant auditory hallucinations.\textsuperscript{27}

Three studies used PET or SPECT. In contrast to the ASL study discussed above,\textsuperscript{27} in a \textsuperscript{\textit{99m}}Tc-HMPAO SPECT study, no differences in perfusion between groups were reported.\textsuperscript{40} One FDG PET study used a haloperidol challenge and found that this caused widespread metabolic decreases in resistant but not treatment-responsive patients.\textsuperscript{42} Demjaha et al.\textsuperscript{43} used F-DOPA PET to show increased striatal dopamine synthesis capacity in responsive compared to resistant patients.

A sub-sample of the Demjaha et al. study\textsuperscript{43} was investigated using \textsuperscript{1}H-MRS.\textsuperscript{34} As described above they found that resistant patients had significantly higher anterior cingulate cortex glutamate levels compared to healthy controls,\textsuperscript{34} while responsive patients had similar levels to controls. Goldstein et al.\textsuperscript{35} showed that compared to individuals who have responded to first line antipsychotics, treatment resistant
patients who respond to clozapine show greater concentrations of glutamate+glutamine in the putamen, and reduced concentrations in the dorsolateral prefrontal cortex.

Four studies used EEG. One study found that treatment-resistant patients showed trend level P300 decreases compared to responsive patients. Another showed that treatment-resistant patients had a different connectivity pattern than treatment-responders, with a higher inter-hemispheric correlation between frontal electrodes. Gamma-beta correlations index a response to novel auditory stimuli. One study reported significant gamma and beta frequency increases in speech-related areas and a significant gamma-beta correlation in resistant but not responsive patients. A second study by the same group examined this effect in terms of dimensional complexity and found reduced neuronal synchronisation in the prefrontal cortex of resistant patients.

Longitudinal studies examining the effects of clozapine in treatment-resistant patients, and studies investigating predictors of clozapine response

We identified 33 papers, comprising a total of 844 patients and 322 controls (see Table 4).

Eleven studies used structural neuroimaging. Three early studies used computed tomography (CT) scans in an attempt to identify predictors of clozapine response. These consistently found that responders to clozapine have smaller prefrontal sulcal spaces compared to poor responders. Later findings that larger prefrontal and
temporal\textsuperscript{52} gray matter volumes are associated with a good response to clozapine are in keeping with the earlier CT studies. There have been some conflicting findings, one MRI study showed almost diametrically opposed results in that response was associated with larger sulcal spaces in the anterior superior temporal lobe. \textsuperscript{53} However, this study included treatment intolerant as well as treatment resistant patients, and this difference in patient population may explain this discrepancy. Another study, did not find any direct significant contrasts between individuals who had responded well to clozapine and clozapine non-responders.\textsuperscript{15}

Regarding the effects of clozapine treatment a longitudinal study demonstrated that over the course of a year patients started on clozapine showed a 10\% reduction in caudate nuclei volume, while those remaining on typical antipsychotics showed an 8\% increase.\textsuperscript{54} These findings were replicated by in a study showing that clozapine use led to caudate nuclei reductions over 24\textsuperscript{55} and 52 weeks.\textsuperscript{56} Furthermore, greater reductions in left caudate volume were seen in clozapine responders compared to non-responders. The findings of widespread reduced gray and increased white matter volumes in TRS reported by Molina et al. were attenuated during clozapine treatment.\textsuperscript{14} This is in contrast to a recent study showing gray matter losses in the prefrontal cortex were (non significantly) greater in patients treated with clozapine compared to healthy volunteers, although clozapine responders had less cortical thinning over the left medial frontal cortex and right middle temporal cortex compared to clozapine non-responders during this period.\textsuperscript{17}
Twelve PET/SPECT studies were identified. Two early FDG PET studies demonstrated increased metabolic rates in the basal ganglia\textsuperscript{57,58} and reduced rates in the frontal cortex\textsuperscript{58} following clozapine treatment. Two \textsuperscript{99m}Tc-HMPAO studies, however, suggested that clozapine response was predicted by pre-treatment increased basal ganglia and frontal cortex perfusion, and that treatment reduced perfusion.\textsuperscript{30,59} These differences may be accounted for by the fact that at the time of scanning individuals in the FDG studies had been antipsychotic free for at least 14 days while in \textsuperscript{99m}Tc-HMPAO studies individuals were taking antipsychotics, which have been shown to alter brain metabolism.\textsuperscript{60} A later \textsuperscript{99m}Tc-HMPAO study showed that clozapine treatment led to increased perfusion of the frontal cortex, and that this predicted response; again scanning occurred in this study following one week wash out as opposed to during antipsychotic treatment.\textsuperscript{61}

Some of the discrepancies’ between study findings may be accounted for by differences in participant medication status. However the likelihood that some of this heterogeneity is more intrinsic to the question under examination is well illustrated by two studies. One study reported individual patient findings, and described a number of patients showing reductions in perfusion, and others increases, in both the basal ganglia and frontal cortex following clozapine treatment.\textsuperscript{62} Second, a \textsuperscript{15}O-PET study showed that clozapine treatment led to increases in perfusion the dorsolateral part of the frontal cortex but decreases in the ventrolateral part.\textsuperscript{63}

A FDG PET study suggested that response to clozapine is modulated by different alleles of the DRD1 gene that codes for D1 receptors.\textsuperscript{64} It was found that cortical metabolic decreases were associated with clinical improvement for patients with the
DRD1 2,2 receptor genotype but not for patients with the heterozygous DRD1 1,2 genotype.

One study employed 1H-MRS to measure N-Acetylaspartic acid (NAA), a marker of neuronal integrity. It found lower NAA levels in the dorsolateral prefrontal cortex were associated with clinical improvement, while 8 weeks of clozapine increased NAA levels (though no correlation was found with clinical improvement). Another study suggested individuals on clozapine who show a good response have greater glutamate + glutamine levels in the putamen compared to those with a poor response.

Ten EEG studies were identified. One study found that clozapine normalised P300 and slow wave components, while another showed clozapine partially normalised P300 decreases, but did not have any effect on the MMN. These findings suggest that clozapine possibly affects attentive but not pre-attentive processing. Five studies used spectral analysis to assess effects of clozapine. Two early studies measured coherence and showed that resistant patients display interhemispheric and intrahemispheric dysconnectivity over anterior brain regions that clozapine partially normalised. These changes in coherence were also related to improvement in negative symptoms. Three studies demonstrated the widespread effects of clozapine on spectral power, indicating both increases in fast wave and slow wave power.

DISCUSSION
The Neurobiology of treatment resistance

Two main schools of thought exist regarding the neurobiology of TRS. One, which can be characterised as the continuum hypothesis, posits that the same pathophysiological processes underlie symptoms in both responsive and resistant patients, but that these processes occur to a greater degree in resistant patients and so treatment is less effective. The other, that can be considered the categorical hypothesis, is that resistant schizophrenia has a fundamentally different pathophysiology to responsive schizophrenia, and thus current treatments are ineffective as they target the wrong processes.70

Figure 2 summarises the findings that have support from more than a single study. When resistant patients are compared to healthy controls, structural studies uniformly show gray matter reductions relative to controls, which is consistent with findings seen in schizophrenia in general.71 It is important to note, however, that volume reductions may not be universally detrimental, with one study showing an association between symptom severity and larger orbitofrontal cortex volumes.72 Functional changes were also similar to those reported in schizophrenia in general.26,27

In comparing resistant and responsive patients the most replicated finding was a greater reduction in gray matter in resistant patients, predominantly in frontal areas. One fMRI27 and one EEG study14 also suggested a continuum of pathology – with differences observable in the treatment responders compared to controls, but more marked in the resistant group.
In terms of neurochemistry, two PET studies are consistent in suggesting that resistant patients might have different dopaminergic functioning relative to responsive patients.\textsuperscript{42,43} One F-DOPA PET study showed raised dopamine synthesis capacity in schizophrenia patients in general but no evidence of increased capacity in resistant patients.\textsuperscript{43} A FDG PET study of the effect of a haloperidol challenge showed marked metabolic decreases in resistant but not responsive patients.\textsuperscript{42} This can be interpreted as being secondary to responsive patients having elevated presynaptic dopamine reserves, and thus being able to accommodate the antidopaminergic effects of haloperidol; while treatment-resistant patients do not, resulting in decreased metabolism. If resistant patients do indeed have a normally functionally dopaminergic system, this raises the question of what neurochemical abnormalities underlie treatment resistance. Glutamatergic dysfunction has been implicated in the development of schizophrenia, in relation to both positive and negative symptoms.\textsuperscript{73–81} The two 1H-MRS studies were consistent in showing glutamatergic elevations in resistant compared to responsive patients.\textsuperscript{34,35} As elevations in glutamate have been associated with excitotoxicity and structural brain changes\textsuperscript{82}, glutamate elevations in resistant patients could account for the relative gray matter reductions found in some studies of resistant patients. Whilst this supports the idea that glutamatergic dysfunction underlies resistance, it needs to be tested further.

Support for both continuum and categorical hypotheses can be found outside of neuroimaging. Recent research has suggested a potential genetic framework on which categorically different schizophrenia subtypes could sit,\textsuperscript{83} and neurochemically it is possible that categorical differences in dopaminergic and glutamatergic function could account for differences in treatment response.\textsuperscript{70} Conversely other studies
provide support for a continuum - demonstrating that patients with greater exposure to both environmental and genetic risk factors are more likely to be treatment resistant.

Recent studies have employed multimodal imaging techniques to more precisely delineate the neurobiological processes underlying psychotic disorders. An expansion of this approach to include both thorough phenotypic characterization, and measurement of environmental and genetic factors may be needed to gain a fuller understanding of the causative factors leading to treatment resistance.

Treatment resistant patients will, by definition, have greater symptom severity but may also have longer illness duration, and greater cumulative antipsychotic exposure than responsive patients. Long term exposure to antipsychotics has been shown to cause both increases in basal ganglia volume and atrophy of cortical gray matter. As such the brain differences observed could reflect these confounds, as opposed to identifying pathophysiologically different illness types.

*The effects of clozapine on brain structure and function and predictors of clozapine response*

Two studies showed an association between clozapine treatment and reductions in caudate volume, while other antipsychotics were associated with enlargement. In addition, reductions in caudate volume were associated with a good clinical response. Furthermore, two SPECT studies demonstrated response to clozapine is predicted by
increased pre-treatment perfusion of the basal ganglia, that decreases with successful treatment.\textsuperscript{30,59}

This suggests that clozapine’s superior efficacy may be related to its normalising effect on striatal structure and function, consistent with its reduced affinity for the D2 receptor.\textsuperscript{92} Some patients seem to show an initial good response to antipsychotic treatment and then develop treatment resistance after a number of years of treatment.\textsuperscript{8,93} It has been suggested that this secondary treatment resistance is due to D2/3 receptor supersensitivity due to receptor up-regulation or other changes. Antipsychotic exposure is associated with dopamine D2/3 receptor up-regulation in rodents\textsuperscript{94} and, whilst the degree to which this happens in humans is unclear, antipsychotic treatment is associated with changes in striatal volume and functional indices in patients.\textsuperscript{89} Clozapine has a relatively low affinity for and fast dissociation from the D2/3 receptor.\textsuperscript{92} Thus, putatively, these actions at the D2/3 receptor could allow D2/3 supersensitivity to resolve, and underlie clozapine’s efficacy for individuals who have developed secondary treatment resistance following sustained antipsychotic treatment. Whilst this is consistent with the normalisation of the striatal functional and structural changes seen with clozapine, this needs testing in patients. Moreover this is unlikely to explain all of clozapine’s clinical efficacy, not least because enhanced efficacy in treatment resistance is not seen with quetiapine, which also shows relatively low affinity for D2/3 receptors.\textsuperscript{95}

Clozapine has effects on a large number of other neurotransmitter systems, including glutamate.\textsuperscript{96} Given the glutamatergic abnormalities that have been associated with
resistant schizophrenia this is a element of its pharmacology which could may contribute to its superior efficacy.

The apparent inconsistency between the two studies that showed increased perfusion following clozapine treatment and the others can potentially be explained by differences in participants’ medication status at the time of scan. The findings of Lahti et al. neatly illustrate the fact that striatal perfusion increases with greater D2 antagonism. Therefore if a baseline scan is performed while participants are receiving non-clozapine antipsychotics and are then scanned again when receiving clozapine a reduction in perfusion might be expected (due to a relative reduction in D2 antagonism). If, however, the baseline scan occurs when participants are receiving no antipsychotic treatment, the scan following clozapine treatment might be expected to show increased perfusion, due to the relative increase in D2 antagonism.

In terms of predicting response, early studies suggested that individuals with the most marked frontal atrophy were less likely to benefit from clozapine treatment; but later studies have produced conflicting results. The findings regarding clozapine’s longitudinal effects on global gray matter studies are too inconsistent to draw conclusions from. Electrophysiological studies showed that clozapine has widespread effects on spectral power and connectivity. It appears that a good clinical response to clozapine is accompanied by normalization of various EEG measures towards the values seen in healthy controls. No consistent findings, however, show markers predicting treatment response at baseline.

Current research limitations and future directions
Our review highlights the heterogeneity that permeates neuroimaging research into treatment resistance. Some of this may be inherent to the problem under examination. There may be many ways for an illness to be treatment resistant, but only one route to treatment response. In particular individuals with similar clinical presentations may show treatment resistance due to different pharmacokinetic and pharmacodynamic factors, and/or vary markedly in the underlying pathoetiiology. Some of the heterogeneity is, however, as a result of the methods used. The cohorts studied vary widely in illness duration, in previous drug treatment and treatment at time of scan, in sample sizes, in imaging techniques, and in analysis methods used. A further issue contributing to heterogeneity is that many studies were underpowered to detect even moderate effect sizes (e.g. Cohen’s d=0.5). Another limitation is the variable definition of treatment-resistance among studies. It is important that, for research purposes, treatment-resistance is defined using standardised, quantifiable criteria. This would allow for more direct comparisons across studies, which is particularly important in the identification of biomarkers.

Cross-sectional comparisons of treatment resistant and responsive patients can potentially indicate differences that may underlie treatment resistance. They cannot, however, determine causality. Furthermore, it is difficult to exclude certain confounders in cross-sectional studies, for example, the finding that higher clozapine doses correlate with greater gray matter loss is confounded by the association of higher doses with disease severity. In view of this, where there are differences, we cannot exclude that they may be secondary to other factors. We did not identify any studies that prospectively investigated brain structure or function from illness onset to the development of treatment resistance. A logical strategy is to start with cross-
sectional studies to identify brain differences between responders and resistant patients but, ultimately, prospective studies from illness onset are needed to identify what biologically underlies treatment resistance. Furthermore, prospective studies will be required to evaluate whether any neurobiological markers have the potential for clinically relevant prediction of treatment resistance.

The available imaging evidence provides some limited support for both continuum and categorical hypotheses. This suggests a hybrid of both hypotheses may best describe the neurobiology of resistant schizophrenia, with some aspects such as structural changes on a continuum, whilst other aspects, such as presynaptic dopamine function, may be categorically different. It is also apparent, that whilst there have been fifty-nine imaging studies of resistant schizophrenia, few have attempted to replicate prior findings. Well controlled, ideally prospective, studies from illness onset are required to definitively determine the key aspects of the neurobiology underlying treatment resistance and identify reliable biomarkers for treatment resistance.
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**Authors** | **Year** | **Sample** | **Resistance criteria** | **Modality** | **Medication at time of scan** | **Diagnostic Criteria**
---|---|---|---|---|---|---
Ahmed et al. | 2015 | 33 TR, 31 HC | Failed ≥2 Aps (≥1 atypical), Prolonged positive or negative symptoms of moderate severity | MRI – Structural | Pre and post clozapine | DSM-IV-TR
Afonso-Solis et al. | 2015 | 19 TR-AVH, 14 R, 20 HC | Daily AVH AND failed ≥2 Aps (at dose equiv ≥600mg clozapine/day) | fMRI – resting state | Typical/Atypical Aps | DSM-IV-TR
Anderson et al. | 2015 | 15 CNR, 19 TR, 18 R, 20 HC | Lack of significant response despite trials (adequate dose and≥6 wk duration) of ≥2 Aps | MRI- structural | Atypical Aps (including clozapine) | DSM-IV-TR
Arango et al. | 2003 | 45 TR | Residual positive (≥8 BPRS psychotic) or negative symptoms (≥20 SANS) despite ≥2 6wk AP trials. Prospective trial fluphenazine 20mg/day – subjects with >30% improvement excluded. | MRI – Structural | Clozapine or Haloperidol | DSM-III-R
Bartlett et al. | 1998 | 7 TR, 7 R | Unmedicated BPRS ≥ 50 or medicated BPRS ≥ 42 AND no worsening when unmedicated. Prospective 4-wk AP trial for patients with no records | FDG-PET PET (haloperidol challenge) | Not specified | DSM-III-R
Buchsbaum et al. | 1992 | 12 Sz | Not specified | FDG-PET | Pre/post clozapine/thioxene | Not specified
Chakos et al. | 1995 | 8 clozapine, 7 typical Aps | Not specified | MRI- Structural | Clozapine and typical Aps | Not specified
Ergun et al. | 2010 | 20 TR | Treatment refractory or AP intolerant | 99mTc-HMPAO SPECT | Pre and post clozapine | DSM-IV
Ertugrul et al. | 2009 | 22 TR | On clozapine due to treatment resistance or intolerance to previous Aps | 99mTc-HMPAO SPECT/ 1H-MRS | Typical and atypical Aps | DSM-IV
Fitzgerald et al. | 2007 | 3 TR, 4HC | Persistent severe refractory hallucinations that had not responded to ≥2 adequate courses of Aps | IMRI (word generation) | Clozapine, amisulpride, sertraline, valproate, diazepam | Not specified
Friedman et al. | 1991 | 34 TR | Failure to respond to ≥2 different class Aps (each for ≥6 weeks, ≥ 800mg CPZ equiv), ≥4 on BPRS positive items | CT Scan | Clozapine | RDC
Gallyt et al. | 2005 | 15 TR, 14 HC | Not specified | EEG | Pre and post clozapine | DSM-IV
Gross et al. | 2004 | 16 TR | Kane et al (1988) | EEG | Risperidone or olanzapine | SCID + chart review
Holleran et al. | 2014 | 19 TR, 19 HC | Failure to respond to ≥2 Aps (≥1 atypical), prolonged moderate/severe positive or negative symptoms. | MRI- DTI | Atypical Aps, antidepressants | DSM-IV
Honer et al. | 2014 | 42 TR (inc 3 Schizo/affective) | Poor response to adequate AP dose for ≥6 months. May et al. (1988) scale. | CT scan | Antipsychotic class not specified | DSM-III-R
Hoptman et al. | 2005 | 49 TR | Kane et al (1988) | MRI-Structural | Typical and atypical Aps (including clozapine) | SCID + chart review
Horton et al. | 2011 | 21 TR, 19 HC | Not specified | EEG | Clozapine | DSM-IV and SCID
Kikuchi et al. | 2014 | 26 TR | Poor tolerance or poor response despite ≥2 Aps (≥1 atypical), ≥ 4 weeks and ≥600mg CPZ equiv. | EEG | Pre and post clozapine treatment | Not specified
Klirova et al. | 2013 | 15 TR-AVH, 19HC | Non response to both typical and atypical Aps + ≥5 episodes AVH per day in the last month | FDG PET | Aps, Antidepressants, anticonvulsants | DSM-IV
Kubera et al. | 2014 | 10 TR-AVH, 10 NAVH, 14 HC | Persistent AVH despite ≥2 AP trials (adequate dose, ≥6 wks) | MRI Structural | Clozapine and other Aps | DSM-IV
Lacroix et al. | 1995 | 10 TR, 10 NR | 35% or more and a 30% or less reduction, respectively, on the Brief Psychiatric Rating Scale (BPRS) | EEG | Not specified | DSM-IV
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<th>Study</th>
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<th>Sample Characteristics</th>
<th>MRI Characteristics</th>
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<td>Lahti et al.</td>
<td>2003, 2004</td>
<td>6 partially responsive, 10 HV</td>
<td>Not specified</td>
<td>15O-PET</td>
<td>Pre/post clozapine</td>
<td>DSM-III-R</td>
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<tr>
<td>Lauriello et al.</td>
<td>1998</td>
<td>21 TR</td>
<td>Treatment intolerant or inadequate response.</td>
<td>MRI-Structural</td>
<td>Typical Aps</td>
<td>DSM-III-R</td>
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<tr>
<td>Lee et al.</td>
<td>2006, 2008</td>
<td>25 TR-AVH, 23 nAVH</td>
<td>Persistent AVH for ≥2yrs</td>
<td>EEG</td>
<td>Conventional neuroleptics</td>
<td>DSM-IV</td>
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<tr>
<td>Mallier et al.</td>
<td>2012</td>
<td>52 TR, 182 MDD, 76 HC</td>
<td>Not specified</td>
<td>MRI-Structural</td>
<td>Not specified</td>
<td>DSM-IV</td>
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<tr>
<td>Molina et al.</td>
<td>1996</td>
<td>24 TR</td>
<td>Lack of adequate response to ≥2 chemically different Aps, ≥800mg CPZ equiv</td>
<td>99mTc-HMPAO SPECT</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
<td></td>
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<tr>
<td>Molina et al.</td>
<td>1997b</td>
<td>39 TR (includes Molina et al. 1996 sample), 28 HC</td>
<td>Lack of response to ≥2 dissimilar Aps (≥800mg CPZ equiv), each one for ≥2 months over last year.</td>
<td>99mTc-HMPAO SPECT</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
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<tr>
<td>Molina et al.</td>
<td>2003</td>
<td>25 TR</td>
<td>Lack of response to ≥2 different Aps for ≥6 weeks in past 12 mths, dose ≥800mg CPZ equiv. Significant positive or disorganisation residual symptoms</td>
<td>MRI-structural</td>
<td>Pre/post clozapine</td>
<td>DSM-III-R</td>
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<tr>
<td>Molina et al.</td>
<td>2005, 2007</td>
<td>23 TR, 17NN, 18HC</td>
<td>Lack of adequate response to ≥2 Aps for ≥4 weeks in preceding 12 months, dose ≥800mg CPZ equiv. All had haloperidol for ≥4wks before scan</td>
<td>99mTc-HMPAO SPECT</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
<td></td>
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<tr>
<td>Molina et al.</td>
<td>2008a</td>
<td>30 TR, 19 R and 44 HC</td>
<td>Kane et al (1988)</td>
<td>MRI-structural, EEG</td>
<td>Haloperidol prior to first MRI, then olanzapine or clozapine</td>
<td>DSM-IV</td>
<td></td>
<td></td>
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<tr>
<td>Molina et al.</td>
<td>2008b</td>
<td>10 TR, 10 HC</td>
<td>A poor response during the previous year to haloperidol or risperidone followed by lack of response to 4 week trial of risperidone</td>
<td>99mTc-HMPAO SPECT</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potkin et al.</td>
<td>1994</td>
<td>18 Scz</td>
<td>Not specified</td>
<td>FDG PET</td>
<td>Pre/post clozapine</td>
<td>Not specified</td>
<td></td>
<td></td>
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<tr>
<td>Potkin et al.</td>
<td>2003</td>
<td>15 TR</td>
<td>Not specified</td>
<td>FDG PET</td>
<td>Pre/post clozapine</td>
<td>Not specified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarantelli et al.</td>
<td>2014</td>
<td>20 (TR + CNR), 15 R, 16 HC</td>
<td>&lt;20 % improvement AND total &gt; 45 on BPRS AND ≥4 in ≥2 BPRS psychotic items AND ≥2 yrs poor functioning despite 6-8 weeks with ≥2 Aps and good adherence.</td>
<td>MRI – structural</td>
<td>Typical and atypical Aps (including clozapine)</td>
<td>DSM-IV-TR</td>
<td></td>
<td></td>
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<tr>
<td>Ravani et al.</td>
<td>2015</td>
<td>47 TR, 66 HC</td>
<td>Kane et al (1988) criteria</td>
<td>EEG (auditory evoked)</td>
<td>Pre/Post clozapine</td>
<td>DSM-IV</td>
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<tr>
<td>Scheepers et al.</td>
<td>2001a</td>
<td>26 TR</td>
<td>No response (CGI≥4) to ≥1 typical AP for ≥4 weeks OR severe EPSeS or TD</td>
<td>MRI-Structural</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
<td></td>
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</tr>
<tr>
<td>Scheepers et al.</td>
<td>2001b</td>
<td>28 TR</td>
<td>No response (CGI≥4) to ≥1 typical AP for ≥4 weeks OR severe EPSeS or TD</td>
<td>MRI-Structural</td>
<td>Pre/post clozapine</td>
<td>DSM-IV</td>
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<tr>
<td>Tsekou et al.</td>
<td>2015</td>
<td>7 TR</td>
<td>Brenner et al (1990) criteria AND ≥70 on PANSS, and schizophrenia diagnosis for≥2 years</td>
<td>Sleep EEG</td>
<td>Pre/post clozapine</td>
<td>DSM-III-R</td>
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</tr>
<tr>
<td>Umbricht et al.</td>
<td>1998</td>
<td>11 TR, 6 R, 13 HC</td>
<td>Partially refractory –≥4 on any of the 4 BPRS positive symptom items</td>
<td>EEG</td>
<td>Clozapine and haloperidol</td>
<td>DSM-III-R</td>
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<tr>
<td>Vercammen et al.</td>
<td>2010</td>
<td>27 TR-AVH, 27 HC</td>
<td>Daily AVH, ≥2 adequate AP trials</td>
<td>MRI-Resting state</td>
<td>Aps and benzodiazepines</td>
<td>DSM-IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhao et al.</td>
<td>2006</td>
<td>21 TR, 40 HC</td>
<td>Andreasen’s negative symptom profile</td>
<td>SPECT</td>
<td>Medication free, follow up on clozapine</td>
<td>DSM-IV</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 1: Study characteristics**

1H-MRS – proton magnetic resonance spectroscopy; AP – antipsychotic; AVH – Auditory verbal hallucinations; BPRS – Brief Psychiatric Rating Scale; CNR – clozapine non responder; CPZ equiv – Chlorpromazine equivalents; DSM – Diagnostic and Statistical Manual of Mental Disorders; EEG – electroencephalogram; HC- healthy controls; MDD – major depressive disorder; MRI – Magnetic Resonance Imaging; MRS – Magnetic Resonance Spectroscopy; TR – Treatment resistant; TD – Treatment refractory; Tc – Technetium-99m; SPECT – Single Photon Emission Computed Tomography; FDG – Fluorodeoxyglucose; MRI – Magnetic Resonance Imaging.
Resonance Imaging; PET – Positron Emission Tomography; Individuals with schizophrenia without auditory hallucinations; R – antipsychotic responders; Scz- unspecified whether responder/resistant; SPECT- single-photon emission computed tomography; TR – treatment resistant; TR-AVH, treatment resistant auditory verbal hallucinations.
The Lancet Psychiatry 

**Effect of interest**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Modality</th>
<th>Effect of interest</th>
<th>Effect size (d)</th>
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</thead>
<tbody>
<tr>
<td>Ahmed et al.</td>
<td>2015</td>
<td>Structural MRI</td>
<td>Raw brain volumes</td>
<td>0.33 (ns)</td>
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<tr>
<td>Anderson et al.</td>
<td>2015</td>
<td>Structural MRI</td>
<td>Normalised brain volumes</td>
<td>GM: TR = 1.23</td>
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<tr>
<td>Cachia et al.</td>
<td>2008</td>
<td>Structural MRI</td>
<td>Cortical folding in TR</td>
<td>Left Frontal (middle) 0.75</td>
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<tr>
<td>Hoptman et al.</td>
<td>2005</td>
<td>Structural MRI</td>
<td>Larger left OFC GM volumes and bilateral OFC WM volumes were associated with greater aggression</td>
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<tr>
<td>Kubera et al.</td>
<td>2014</td>
<td>Structural MRI</td>
<td>GM in TR in predominantly of lateral prefrontal, temporal and parietal regions.</td>
<td></td>
</tr>
<tr>
<td>Maller et al.</td>
<td>2012</td>
<td>Structural MRI</td>
<td>Raw brain volumes</td>
<td>0.56</td>
</tr>
<tr>
<td>Molina et al.</td>
<td>2008a</td>
<td>Structural MRI</td>
<td>Normalised brain volumes</td>
<td>GM volume in TR Frontal: 1.59</td>
</tr>
<tr>
<td>Quaranatelli et al.</td>
<td>2014</td>
<td>Structural MRI</td>
<td>Global GM (normalised volumes) in TR, GM at left post central gyrus and dorsolateral superior frontal gyrus; right Rolandic operculum, inferior frontal gyrus, insula and amygdala; and bilateral precentral and middle frontal gyrus.</td>
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<tr>
<td>Sun et al.</td>
<td>2009</td>
<td>Structural MRI</td>
<td>Total normalised CC volume in TR</td>
<td>1.9</td>
</tr>
<tr>
<td>Zugman et al.</td>
<td>2013</td>
<td>Structural MRI</td>
<td>GM in TR in in left: orbitofrontal, middle temporal, fusiform, caudal middle frontal, STG, lingual areas; and right: precentral, pars triangularis, middle temporal and lateral occipital areas.</td>
<td></td>
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<tr>
<td>Holleran et al.</td>
<td>2014</td>
<td>Structural MRI – DTI</td>
<td>Fractional anisotropy in TR: genu, body, and splenium of CC; Temporal ILF, SLF, external capsule, temporal UF, posterior PC, left anterior LIC, fornix, cerebellar peduncles and corticospinal tract.</td>
<td>1.23</td>
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<tr>
<td>Alonso-Solis et al.</td>
<td>2015</td>
<td>rsMRI</td>
<td>FSC in TR between pIPL and occipital fusiform gyrus, ligual gyrus and L occipital pole.</td>
<td></td>
</tr>
<tr>
<td>Vercammen et al.</td>
<td>2010</td>
<td>rsMRI</td>
<td>FC in TR-AVH between the Left TPJ and Right IFG. Severity of hallucinations correlated with coupling between the left TPJ and: bilateral anterior cingulate and amygdala.</td>
<td></td>
</tr>
<tr>
<td>Wolf et al.</td>
<td>2011</td>
<td>rsMRI</td>
<td>Speech-related network: tConnectivity in bilateral temporal and tConnectivity in L. anterior cingulate displayed by TR Attention network: tConnectivity in R MFG in TR Executive function network: tConnectivity in L. precuneus, R.MFG, SFG in TR</td>
<td></td>
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<tr>
<td>Wolf et al.</td>
<td>2012</td>
<td>MRI – Arterial spin labelling</td>
<td>tCBF in TR in the left IFG, the left ACC, the SMA) in a cluster including the left MTG and STG, the left insula, the right MTG and the right SMG, extending to the right TPC</td>
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<tr>
<td>Fitzgerald et al.</td>
<td>2007</td>
<td>fMRI (word generation)</td>
<td>tActivation in TR in medial frontal regions and greater activation in left caudal precentral gyrus.</td>
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<tr>
<td>Demjaha et al.</td>
<td>2012</td>
<td>[18F]-DOPA PET</td>
<td>Healthy volunteers and TRS show no differences in striatal dopamine synthesis capacity</td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Method</td>
<td>Findings</td>
<td></td>
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<td>---------</td>
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<tr>
<td>Klirova et al.</td>
<td>2013</td>
<td>rsMRI</td>
<td>Perfusion in TR in lentiform nucleus, thalamus, postcentral gyrus, left parahippocampal gyrus and right superior frontal gyrus. In left acoustic-linguistic cortex ↑ found in MTG and TPJ.</td>
<td></td>
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<tr>
<td>Molina et al.</td>
<td>1997a</td>
<td>99mTc-HMPAO SPECT</td>
<td>Right posterior temporal 1.52 Left ventral prefrontal 0.63 Left dorsolateral 1.56 ↑ Perfusion in TR and right basal ganglia</td>
<td></td>
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<tr>
<td>Molina et al.</td>
<td>1997b</td>
<td>99mTc-HMPAO SPECT</td>
<td>Right basal ganglia perfusion ↓ in HC, ↑ in CNR and ↑ in TR. Thalamus and left basal ganglia perfusion similar between TR and HC, CNR however shows ↓ perfusion in these regions. TR and CNR show ↑ perfusion compared to HC in left lower prefrontal dorsolateral cortex. TR shows ↑ perfusion in upper dorsolateral cortex compared to HC or UTC.</td>
<td></td>
</tr>
<tr>
<td>Molina et al.</td>
<td>2007</td>
<td>FDG-PET</td>
<td>Clozapine treated TRS show ↓ activity in, Dorsolateral cortex, OFC, ACC, insular cortex and head of caudate nuclei.</td>
<td></td>
</tr>
<tr>
<td>Molina et al.</td>
<td>2008b</td>
<td>99mTc-HMPAO SPECT</td>
<td>Risperidone treated TR showed ↓ activity in the medial prefrontal, middle cingulate and insula. TR showed ↑ perfusion in brainstem and hippocampus, and a small part of left posterior occipital and temporal region.</td>
<td></td>
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<tr>
<td>Zhao et al.</td>
<td>2004</td>
<td>99mTc-ECD SPECT</td>
<td>↓ rCBF at rest and ↓ percentage increase during Wisconsin card sorting test in TR. Left Frontal Lobe 1.48 Right Frontal Lobe 1.40 Left temporal Lobe 1.31 Right Temporal Lobe 1.48</td>
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<tr>
<td>Demjaha et al.</td>
<td>2014</td>
<td>1H-MRS</td>
<td>TR show increased ACC glutamate concentrations compared to HC 1.45</td>
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<tr>
<td>Goldstein et al.</td>
<td>2015</td>
<td>1H-MRS</td>
<td>TR clozapine responders have higher Glx/Cr than HC in the putamen (although this does not survive multiple comparisons correction) 3.68</td>
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<tr>
<td>Gallety et al.</td>
<td>2005</td>
<td>EEG</td>
<td>TR compared to HC (prior to clozapine) show ↓ Midline N1, P300, parietal slow wave activity</td>
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<tr>
<td>Horton et al.</td>
<td>2011</td>
<td>EEG</td>
<td>Frequency deviant conditions: ↓ MMN latencies TR 0.93 ↑ MMN amplitude for TR 1.19 Duration deviant conditions No difference in MMN latency 0.59 ↑ MMN amplitude for TR 3.14</td>
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<tr>
<td>Milovan et al.</td>
<td>2004</td>
<td>EEG</td>
<td>↓ MMN amplitude in TR 0.98 lateral electrode 0.89 midline electrode 0.89</td>
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<tr>
<td>Molina et al.</td>
<td>2008a</td>
<td>EEG</td>
<td>↓ P300 amplitude in TR 2.94</td>
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<tr>
<td>Ravan et al.</td>
<td>2015</td>
<td>EEG</td>
<td>Machine learning investigation of EEG responses to auditory odd ball task able to classify HC and TRS with 81.4% accuracy</td>
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<tr>
<td>Umbricht et al.</td>
<td>1998</td>
<td>EEG</td>
<td>↓ MMN amplitudes in TR 0.99</td>
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</table>

Table 2. Treatment resistant versus healthy control studies
ACC – anterior cingulate cortex; CC – corpus callosum; CNR – clozapine non responder; Cr – creatinine; CSF – cerebrospinal fluid; FC– functional connectivity; Glx – (glutamate + glutamine); GM – Gray matter; ICV – Intracranial volume; IFG – inferior frontal gyrus; ILF – inferior longitudinal fasciculus; ITG – inferior temporal gyrus; LIC - limb of the internal capsule; MMN - mismatch negativity; MTG - middle temporal gyrus; ns – not statistically significant; OFC - orbitofrontal cortex; pIPL- posterior inferior parietal lobule; SLEF – superior longitudinal fasciculus; SMA– supplementary motor area; SMG – supramarginal gyrus; STG – superior temporal gyrus; TPC – temporoparietal cortex; TPJ – temporoparietal junction; UF- uncinate fasciculus; rsMRI – resting state MRI; VMPFC – ventromedial prefrontal cortex; WM- white matter
Table 3. Treatment resistant versus treatment responder studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Modality</th>
<th>Effect of interest</th>
<th>Effect size</th>
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<tbody>
<tr>
<td>Anderson et al.</td>
<td>2015</td>
<td>Structural MRI</td>
<td>↓ global GM in TR/CNR</td>
<td>0.84 (TR vs R)</td>
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<td></td>
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<td></td>
<td>↓ GM in TR vs R in TG, PCG, MFG, SFG, SMG gyrus and lateral occipital cortex</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↓ GM CNR vs R in right parietal operculum and left cerebellum</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TR vs CNR: no significant differences</td>
<td></td>
</tr>
<tr>
<td>Kubera et al.</td>
<td>2014</td>
<td>Structural MRI</td>
<td>↓GM in TR-AVH compared to nAVH across a structural network involving predominantly</td>
<td>0.41 (ns)</td>
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<td></td>
<td></td>
<td></td>
<td>medial frontal, orbitofrontal and superior temporal regions.</td>
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</tr>
<tr>
<td>Lawrie et al.</td>
<td>1995</td>
<td>Structural MRI</td>
<td>↓Whole brain volume in TR</td>
<td>0.46 (ns)</td>
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<td>↓Right temporal lobe volume in TR</td>
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<tr>
<td>Mitelman et al.</td>
<td>2005</td>
<td>Structural MRI</td>
<td>↓GM in posterior cingulate and retrosplenial cortices in TR</td>
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<tr>
<td>Molina et al.</td>
<td>2008a</td>
<td>Structural MRI and EEG</td>
<td>↓GM at baseline in TR</td>
<td>Frontal: 0.87</td>
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<td>↑WM at baseline in TR</td>
<td>Occipital: 0.81</td>
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<td>↑in GM longitudinally in TR compared to R</td>
<td>Frontal: 1.13</td>
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<td>↑in WM longitudinally in TR compared to R</td>
<td>Parietal: 1.35</td>
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<td>↓in WM longitudinally in TR compared to R</td>
<td>Occipital: 1.81</td>
</tr>
<tr>
<td>Quaranatelli et al.</td>
<td>2014</td>
<td>Structural MRI</td>
<td>↓GM in TR at left PCG and SFG (dorsolateral); and bilateral middle frontal gyrus</td>
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<tr>
<td>Zugman et al.</td>
<td>2013</td>
<td>Structural MRI</td>
<td>↓GM in DLPFC in TR</td>
<td></td>
</tr>
<tr>
<td>Alonso-Solis et al.</td>
<td>2015</td>
<td>MRI - Resting state</td>
<td>↑FC in TR between dMPFC; and central opercular cortex, insular cortex, precentral</td>
<td>0.31 (TR vs R)</td>
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<td></td>
<td></td>
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<td>cortex and STG; and between the temporal pole: and cerebellum.</td>
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<tr>
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<td></td>
<td>↑FC in TR between vMPFC; and paracingulate cortex, ACC, and subcallosal cortex;</td>
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<tr>
<td>Wolf et al.</td>
<td>2012</td>
<td>MRI – Arterial spin labelling</td>
<td>↑CBF in TR-AVH compared to nAVH in the left STG and right SMG, TPC.</td>
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<tr>
<td>Bartlett et al.</td>
<td>1998</td>
<td>FDG PET (haloperidol challenge)</td>
<td>↓Whole brain metabolic rate in TR</td>
<td>1.2</td>
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<td>↓Left DLPFC metabolic rate in TR</td>
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<td>↓Right DLPFC metabolic rate in TR</td>
<td>0.87</td>
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<td>↓Left Temporal Cortex metabolic rate in TR</td>
<td>1.19</td>
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<td>Demjaha et al.</td>
<td>2012</td>
<td>F-DOPA PET</td>
<td>↓whole striatum dopamine synthesis capacity in TR</td>
<td>1.11</td>
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<td>↓ associative subdivision dopamine synthesis capacity in TR</td>
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<td>↓limbic subdivision dopamine synthesis capacity in TR</td>
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<td>sensorimotor subdivision (ns)</td>
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<tr>
<td>Lawrie et al.</td>
<td>1995</td>
<td>99mTc-HMPAO SPECT</td>
<td>No significant differences in perfusion between TR and R.</td>
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<tr>
<td>Goldstein et al.</td>
<td>2015</td>
<td>1H-MRS</td>
<td>TR shows ↑and CNR shows ↑↑ Glx/CR in DLPFC</td>
<td>3.99 (CNR vs R)</td>
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<td>↑Glx/CR in TR (clozapine responders) compared to R and CNR in putamen.</td>
<td>0.31 (TR vs R)</td>
</tr>
<tr>
<td>Demjaha et al.</td>
<td>2014</td>
<td>1H-MRS</td>
<td>↑ anterior cingulate glutamate in TR compared to R</td>
<td>4.00 (TR vs CNR)</td>
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<tr>
<td>Lee et al.</td>
<td>2006</td>
<td>EEG</td>
<td>↑Beta1 in TR</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑Beta2 in TR</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>gamma-beta2 and beta3 correlation in TR but not RS in posterior and anterior</td>
<td>range of r=0.42-0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>electrodes</td>
<td></td>
</tr>
<tr>
<td>Lee et al.</td>
<td>2008</td>
<td>EEG</td>
<td>↑gamma frequency in TR at D2 (i.e. more chaotic) in right frontal electrode</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P3</td>
<td></td>
</tr>
<tr>
<td>Molina et al.</td>
<td>2008a</td>
<td>EEG</td>
<td>TR have ↑P300 amplitude</td>
<td>0.53 (ns)</td>
</tr>
<tr>
<td>Ramos et al.</td>
<td>2001</td>
<td>EEG</td>
<td>TR have ↑temporal alpha2, ↑ temporal beta1, ↓temporal beta2, ↑ occipital beta2</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>TRS have ↑intrahemispheric correlation in Fp2-P4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TRS have ↑intrahemispheric correlation between F8-T4</td>
<td></td>
</tr>
</tbody>
</table>

ACC – anterior cingulate cortex; CNR – clozapine non responder; dMPFC – dorsomedial prefrontal cortex; MFG - Middle frontal gyrus; nAVH – individuals not experiencing auditory hallucinations PCC – posterior cingulate cortex; PCG – post central gyrus, RS –
Goldstein et al. (2015). Structural MRI

Author et al. (Year) Modality Effect of interest Effect size
Ahmed et al. (2015) Structural MRI ↓GM over 6-12 months greater in TR treated with clozapine than HC

Right prefrontal cortex 1.06
Left prefrontal cortex 1.02
Periventricular area 1.85

- Cortical thickness of LMFC and RMTG in CNR compared to CR. 1.07

Anderson et al. (2015) Structural MRI No significant structural differences between TR and CNR

Arango et al. (2003) Structural MRI In clozapine treated patients, ↑pretreatment right prefrontal GM vol associated with ↑response whereas converse true in haloperidol treated patients.

Chakos et al. (2003) Structural MRI Patients scanned at baseline and then again 55 wks post clozapine, showed 10% ↓in caudate nuclei vol, while those remaining on typical antipsychotics showed 8% ↑0.94 (change within clozapine group)

Foner et al. (2019) CT scan Cortical sulcal spaces in clozapine responders compared to nonresponders

Friedman et al. (1991) CT scan ++ responders have ↓prefrontal sulcal spaces than + responders who in turn have ↑than non-responders

Konicki et al. (2001) CT scan ↑prefrontal sulcal spaces in clozapine responders compared to poor responders 3.80

Lauriello et al. (1998) Structural MRI No correlation between change in BPRS and sulcal CSF or GM volumes in PFC and frontal cortex. ↓Sulcal CSF volumes in anterior superior temporal lobe were associated with clinical improvement.

Molina et al. (2003) Structural MRI Improvement in positive symptoms related to temporal GM vol. Improvement in negative symptoms predicted by DLPCF vol. Improvement in disorganised dimension predicted by intracranial and hippocampal vol.

Molina et al. (2008a) Structural MRI TR showed longitudinal changes compared to HC over (41)approx…28 months – ↑GM in frontal, parietal and occipital regions; and ↓in WM in frontal, parietal and occipital regions

Scheepers et al. (2001a, b) Structural MRI Clozapine use led to significant ↓in caudate nucleus volume over 24 wks. This was not related to clinical response at 24 weeks but when patients followed up for 52 weeks the change in left caudate volume was significantly greater in responders compared to non-responders. 0.23 (change in caudate over 24wks)

0.56 (responders vs non-responders)

Buchsbaum et al. (1992) FDG-PET Clozapine ↑and thioxene & metabolic rates in the basal ganglia; these effects most marked on right side. Baseline metabolic rates predicted clinical medication response, with right inferior caudate metabolic rates differentiating clozapine and thioxene responders.

Ergun et al. (2010) 99mTc-HMPAO SPECT After 6 wks of clozapine treatment, changes in blood flow seen in 12/20 pts, mostly in basal ganglia or frontal cortex.

Ertugrul et al. (2009) 99mTc-HMPAO SPECT ↑in CR perfusion ratio of Right and Left (sup and medial) frontal/caudate ↑with treatment. This change not seen in CNR. Change in perfusion ratio correlates with improvements in cognitive testing. Response to clozapine predicted by baseline right frontal/thalamus perfusion. 0.56 (CR vs CNR)

Lahti et al. (2003, 2004) 11C-PET Clozapine ↑and haloperidol ↑↑CBF in the striatum. Clozapine ↑↑CBF to ACC, dorsolateral frontal cortex and occipital cortex more than haloperidol. Both drugs led to ↑CBF in the hippocampus, ventrolateral frontal cortex and right middle temporal cortex.

Molina et al. (2003) Structural MRI, FDG-PET Improvement of positive symptoms predicted by ↑Temporal gray matter at baseline Improvement of disorganised symptoms predicted by smaller intracranial and hippocampal volume Improvement of negative symptoms predicted by DLPCF volume and activity

Molina et al. (2005) FDG-PET 6 mths of Clozapine treatment leads to metabolic ↓in DLPCF, mPFC, basagla and left inferior temporal cortex. Leads to metabolic ↑↑in occipital cortex. ↓activity in basal ganglia correlates with ↑↑improvement in negative symptoms. ↑↑activity in motor area relates to ↓in disorganisation symptoms. ↑↑activity in primary visual area correlates with ↑↑in positive symptoms.

Molina et al. (1996, 1997b) 99mTc-HMPAO SPECT Prior to clozapine CR showed ↑↑in perfusion in thalamus, basal ganglia, left lower and right upper DLPFC. CR subsequently showed ↓↓in perfusion post clozapine in L basal ganglia and bilateral thalamus. Post clozapine treatment responders showed perfusion decrease in thalamus, basal ganglia, and dorsolateral cortex. Non responders did not show significant changes in any perfusion values.

Molina et al. (2008b) 99mTc-HMPAO SPECT Following 1 mth of clozapine pts no longer showed ↓activity in cingulate or insular regions although pts still showed ↓in perfusion in MFC. Hyperactivity in brainstem, temporolateral and occipital areas still present.

Potkin et al. (1994) FDG PET Clozapine responders showed greater increase in perfusion following clozapine treatment in: medial occipital cortex and caudate head. A decrease was found in the posterior cortex and hippocampus.

Potkin et al. (2003) FDG PET D1 heterozygotes show widespread metabolic decreases following clozapine treatment and good clinical response – while this is not observed for D1 -1.2 heterozygotes. Interestingly, heterozygotes showed worsening of symptoms which was associated with metabolic decreases in the left prefrontal cortex, bilateral temporal and an increase in right inferior temporal cortices

Zhao et al. (2006) 99mTc-ECD SPECT Clozapine had no effect on rCBF either during resting state or during Wisconsin card sorting test, although behavioural performance in the task occurred

Ertugul et al. (2009) 1H-MRS Nearly significant increase in NAA/Cr ratio in Left DLPCF after clozapine treatment.

Goldstein et al. (2015) 1H-MRS CR show greater Glx/Cr than CNR in putamen. 4.00
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>EEG</th>
<th>Change in EEG Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallety et al.</td>
<td>2005</td>
<td>EEG</td>
<td>Clozapine treatment was associated with normalisation of P3 and late slow waves and partial normalisation of N1 amplitude.</td>
</tr>
<tr>
<td>Gross et al.</td>
<td>2004</td>
<td>EEG</td>
<td>Clozapine treatment associated with increase in theta power in midline which correlates with clinical improvement.</td>
</tr>
<tr>
<td>Kikuchi et al.</td>
<td>2014</td>
<td>EEG</td>
<td>39% of patients treated with clozapine developed EEG abnormalities. Individuals who developed abnormalities were more likely to be younger and have a shorter duration of illness.</td>
</tr>
<tr>
<td>Knott et al.</td>
<td>2001</td>
<td>EEG</td>
<td>Clozapine treatment decreases relative alpha power and mean beta/total spectrum frequency, and increases absolute total and delta/theta power.</td>
</tr>
<tr>
<td>Knott et al.</td>
<td>2002</td>
<td>EEG</td>
<td>Clozapine treatment normalises some of the inter- and intrahemispheric coherence abnormalities present at baseline.</td>
</tr>
<tr>
<td>Lacroix et al.</td>
<td>1995</td>
<td>EEG</td>
<td>Clozapine treatment led to increases in theta and alpha bands. Low responders show a greater beta1 increase than high responders. High responders show increased coherence between a wide variety of regions (centred on the right anterior-medial temporal region and in the theta band) that is not observed in low responders.</td>
</tr>
<tr>
<td>MacCrimmon et al.</td>
<td>2012</td>
<td>EEG</td>
<td>Baseline EEG compared with second EEG taken on average 1.4 years after starting clozapine. Clozapine augments power in delta and theta bands globally (particularly in frontal areas). Beta3 power reduced. Alpha shows a frontal increase and posterior decrease.</td>
</tr>
<tr>
<td>Ravan et al.</td>
<td>2014</td>
<td>EEG</td>
<td>CR EEG become indistinguishable from HV EEG following clozapine treatment, whereas CNR remain markedly different.</td>
</tr>
<tr>
<td>Tsekou et al.</td>
<td>2015</td>
<td>EEG</td>
<td>Stage 2 sleep increased with clozapine treatment, whereas CNR remain markedly different.</td>
</tr>
<tr>
<td>Umbricht et al.</td>
<td>1998</td>
<td>EEG</td>
<td>Clozapine partially normalises P300 decreases but does not affect MMN.</td>
</tr>
</tbody>
</table>

**Table 4: Longitudinal studies of treatment resistant patients pre- and post-clozapine, and studies predicting clozapine response**

CR – clozapine responder; CNR – clozapine non responder; Cr - creatinine ; DLPFC- dorsolateral prefrontal cortex; LMFC – left medial frontal cortex; mPFC – medial prefrontal cortex; NAA- N-acety aspartate; pts –patients; rCBF – regional cerebral blood flow; RMTC – right medial temporal cortex
Records identified through database searching (n=304)

Additional records identified through other sources (n=26)

Records screened (n=330)

Full text articles assessed for eligibility (n=330)

Full text articles excluded
Did not use brain imaging (n=46)
Not specific to treatment resistance (n=225)

Studies included in qualitative synthesis (n=60)

Studies comparing TRS with HC (n=29)

Structural MRI (n=10)
Ahmed, Anderson, Cachia, Holleran, Kubera, Maller, Molina 2008a, Quarantelli, Sun, Zugman

fMRI (n=5)

SPECT/PET (n=7)
Demjaha 2012, Kirova, Molina 1997a,b, 2007, 2008b, Zhao

EEG (n=6)
Gallety, Horton, Molina 2008a, Milovan, Ravan, Umbricht

MRS (n=2)
Goldstein, Demjaha 2014

Studies comparing TRS with medication responders (n=16)

Structural MRI (n=7)
Anderson, Kubera, Lawrie, Mitelman, Molina 2008a, Quarantelli, Zugman

fMRI (n=2)
Alonso-Solis, Wolf 2012

PET/SPECT (n=3)
Bartlett, Demjaha 2012, Lawrie

MRS (n=2)
Goldstein, Demjaha 2014

Studies examining effect of clozapine in TRS (n=33)

Structural MRI/CT (n=11)
Ahmed, Anderson, Arango, Chakos, Honer, Friedman, Konicki, Lauriello, Molina 2003, 2008a, Scheepers 2001a, b

PET/SPECT (n=12)

MRS (n=2)
Ertugal, Goldstein

EEG (n=10)

Figure 1. Flow diagram of study selection and study characteristics.
Some studies use multiple imaging techniques and examine multiple populations – hence are represented more than once.