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Treatment-resistant schizophrenia: current insights on the pharmacogenomics of antipsychotics

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REVIEW

Treatment-resistant schizophrenia: current insights on the pharmacogenomics of antipsychotics

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Abstract: Up to 30% of people with schizophrenia do not respond to two (or more) trials of dopaminergic antipsychotics. They are said to have treatment-resistant schizophrenia (TRS). Clozapine is still the only effective treatment for TRS, although it is underused in clinical practice. Initial use is delayed, it can be hard for patients to tolerate, and clinicians can be uncertain as to when to use it. What if, at the start of treatment, we could identify those patients likely to respond to clozapine – and those likely to suffer adverse effects? It is likely that clinicians would feel less inhibited about using it, allowing clozapine to be used earlier and more appropriately. Genetic testing holds out the tantalizing possibility of being able to do just this, and hence the vital importance of pharmacogenomic studies. These can potentially identify genetic markers for both tolerance of and vulnerability to clozapine. We aim to summarize progress so far, possible clinical applications, limitations to the evidence, and problems in applying these findings to the management of TRS. Pharmacogenomic studies of clozapine response and tolerability have produced conflicting results. These are due, at least in part, to significant differences in the patient groups studied. The use of clinical pharmacogenomic testing – to personalize clozapine treatment and identify patients at high risk of treatment failure or of adverse events – has moved closer over the last 20 years. However, to develop such testing that could be used clinically will require larger, multicenter, prospective studies.

Keywords: personalized medicine, pharmacogenetics, treatment resistant psychosis, clozapine, pharmacokinetic, pharmacodynamic

Introduction

Treatment-resistant schizophrenia (TRS) affects ~30% of people with a diagnosis of schizophrenia.[1] TRS is defined as nonresponse to at least two trials of antipsychotic medication of adequate dose and duration,[2] at which point, the antipsychotic clozapine is indicated. Interestingly, clozapine does not work better than other antipsychotics in first-episode cases.[3] Recent work suggests that different underlying mechanisms are responsible for the symptoms in TRS.[4] The changes in presynaptic dopamine transmission usually seen in schizophrenia are absent in TRS,[5] but we do see changes in anterior cingulate glutamate activity.[6] It is therefore not surprising that other antipsychotics, which all have their main effects on dopamine receptors, fail to work in people with TRS. This may come to be seen less as treatment resistance and more as a failure to direct treatment toward the relevant underlying problem.

Clozapine is unique as it is the only evidence-based treatment for TRS,[2,7,8] with 60%–70% of those treated showing a response.[9] However, some patients with TRS do not respond to clozapine. At present, we can identify neither those who will

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improve on clozapine nor those who will not respond to other antipsychotics.

Despite its unique efficacy in TRS, clozapine is underprescribed in most countries. Levels of use are far less than the ~50%–60% of TRS patients who could benefit from it.[10–12] The evidence suggests that it is only used after a delay of several years.[13] The reasons for this include a fear of side effects, and the inconvenience of therapeutic blood monitoring. This means that many who could benefit from clozapine do not.[14,15] Moreover, when clozapine is not used in TRS, patients are often treated with nonevidence-based, high-toxicity, high-dose antipsychotic treatments, and polypharmacy.[13] If clozapine does work, it can be transformative, improving psychotic symptoms, function, and longevity. However, the process of establishing its efficacy (or otherwise) can be lengthy (up to a year) and grueling for the patient. This evaluation of efficacy and side effects is more difficult because there is really no alternative medication for TRS.

There are currently no evidence-based pharmacotherapies for the 30% of TRS patients who fail to respond to clozapine[9,16] or those who discontinue clozapine due to adverse events.[17,18] As well as facilitating the use of clozapine, pharmacogenomics and personalized medicine could support the development of new medications.

Personalized medicine and pharmacogenomics in TRS

Personalized, or precision medicine uses "genetic or other biomarker information to improve the safety, effectiveness, and health outcomes of patients via more efficiently targeted risk stratification, prevention, and tailored medication and treatment management approaches".[19]

In TRS, by considering a person's individual genomic, epigenetic, molecular, cellular, clinical, behavioral, and environmental characteristics, it should be possible to tailor appropriate preventative and therapeutic interventions to that individual. This would allow for the safer and timelier introduction of clozapine in patients where it is likely to be effective.

The potential benefits and function of pharmacogenomics in schizophrenia are listed in Table 1.

Terminology

Pharmacogenomics

Pharmacogenomics looks at how genes control drug pharmacokinetics and pharmacodynamics.[20] The term is often used interchangeably with the term pharmacogenetics. Pharmacogenetics usually refers to how a specific gene or a set of genes can influence a patient's response to medicine(s).

Table 1 Uses of tools provided by pharmacogenomic biomarkers in TRS

Avoid toxicity and subsequent ADRs
Avoid underdosing and subsequent lack of efficacy
Avoid drug use by hypersensitive individuals
Improve clinical diagnosis
Rescue drugs previously withdrawn because of ADRs

Note: Reproduced with permission of Royal College of Psychiatrists via PLSClear, Lally J, MacCabe JH. Personalised approaches to pharmacotherapy for schizophrenia.[117]

Abbreviations: TRS, treatment-resistant schizophrenia; ADRs, adverse drug reactions.

Pharmacogenomics looks at how a person's whole genetic makeup can influence his/her responses to medicine(s). Early studies focused on pharmacogenetic approaches, looking at groups of genes that seemed to be likely to be involved with a particular disorder – "candidate" genes. Now, with newer technologies, pharmacogenomic approaches are more common.

In this article, we use the term pharmacogenomics to refer to both approaches.

Epigenetics

Epigenetics refers to the regulation of translation of DNA. This is mainly through changes in DNA methylation and chromatin structure, histone modification, RNA editing, and nontranscriptional gene silencing via micro-RNAs.[21,22]

Pharmacogenomics in TRS

Patients with TRS have an excess of rare disruptive variants both in gene targets of antipsychotics and in genes with evidence for a role in antipsychotic efficacy.[23,24] A number of studies in TRS patients have looked at the effects of variations in the genes responsible for the effectiveness, adverse effects, and metabolism of clozapine.[25–28] Newer genomic approaches using genome-wide association study (GWAS) and exome sequencing (to identify rare as well as common genetic variants across the genome) are set to facilitate a new era of pharmacogenomic testing as a guide to personalized treatments.[29]

Barriers to pharmacogenomic testing in TRS are outlined in Table 2, and the characteristics of TRS which attenuate some of these challenges are shown in Table 3.

Pharmacogenomics and clozapine response

One way to use pharmacogenomic testing in TRS would be to identify specific genetic variations that predict a good response to clozapine, or a low risk of adverse events. Prescribing decisions could then be informed by the genetic test findings.

Table 2 Challenges in using pharmacogenomics to predict clozapine effect in TRS

Characterizing/quantifying drug response
Measuring antipsychotic efficacy
Nonadherence
Treatment duration – early benefits with antipsychotic response vs longer duration of effect with clozapine
Concurrent medication use – mood stabilizers; crossover of antipsychotics during switching
Patient characteristics
Differential diagnosis and unclear diagnostic boundaries
Illness course – first-episode psychosis and TRS
Comorbidities – substance misuse; depression
Risk of neutropenia increased in those of African Caribbean ethnicity
Baseline characteristics: eg, lower BMI and greater risk of weight gain with clozapine treatment

Abbreviations: TRS, treatment-resistant schizophrenia; BMI, body mass index.

Table 3 Characteristics of TRS which make it a more useful population in which to conduct pharmacogenomic studies

Increased uniformity in diagnosis
Ability to monitor adherence with plasma clozapine concentrations
Long-term follow-up of TRS patients within clinical services
The existence of registers for clozapine-monitoring services

Abbreviation: TRS, treatment-resistant schizophrenia.

Two meta-analyses confirm the importance of the dopaminergic system,[28,30] and one confirms the impact of the serotonergic system for the antipsychotic effect of clozapine.[25]

Dopaminergic system

A dopamine receptor type 3 functional polymorphism, *Ser-9Gly*, has been associated with clozapine efficacy in several studies.[31,32] The direction of this association was further shown in a meta-analysis, although it was not found to be statistically significant.[30] Genetic variants of the dopamine receptors type 2 and type 1 and dopamine transporter haplotypes have also been found to be associated with clozapine efficacy.[26,33] Overall, these findings suggest that the dopaminergic system plays some part in mediating clozapine response. However, a recent meta-analysis[25] did not identify the previously reported association between dopamine genes *DRD2 rs1799732* and *DRD3 rs6280* and clozapine response.[26,28] This study[25] applied a stricter inclusion criteria than the previous meta-analysis,[26] only including studies where clozapine was investigated alone (rather than with other antipsychotics).

Serotonergic system

Several serotonergic receptor type 2A (*5-HT2A*) gene polymorphisms have been associated with response to clozapine,[34] although these associations have not been universally replicated.[27,35] Studies have demonstrated an association between clozapine efficacy and genetic variants in the serotonin receptor type 2A (*5-HT2A*),[34] type 2C (*5-HT2C*),[36] and type 6 (*5-HT6*)[37] and serotonin transporter (*5-HTT*) genes,[36] although these findings have not been consistently replicated.[26–28] The most recent meta-analysis identified three genetic variants within serotonin genes associated with the response to clozapine: rs6313 and rs6314 within the *5-HTR2A* gene and rs1062613 within the *5-HT3A* gene.[25]

No single polymorphism is predictive of clozapine response. So, attempts have been made to combine polymorphisms in several genes to predict such a treatment response. A landmark study was conducted by Arranz et al, in which one test combined six different polymorphisms in neurotransmitter receptor-related genes (the included polymorphisms were *5-HT2A 102-T/C* and *His452Tyr*, *5-HT2C 330-GT/244-CT*, and *Cys23Ser*, *5-HTTLPR*, *H2 1018-G/A*). This resulted in a 77% success in the prediction of clozapine response (P=0.0001).[36] This study was the first demonstration that pharmacogenomics could be used to personalize a psychiatric treatment, and this test was subsequently marketed. However, this finding was not replicated,[38] and the test was withdrawn from the market. However, there were differences in clinical characteristics in the replication sample from the sample in the original study. The replication sample had a shorter period of clozapine use compared to the original study, which assessed long-term clozapine treatment (mean duration >1 year).[36] The shorter duration of clozapine use in the replication sample may have led to some being prematurely categorized as nonresponders,[39] as clozapine response can take up to 12 months.[9]

Metabolism of clozapine – CYP1A2 enzyme

Pharmacokinetic research in schizophrenia has largely focused on the cytochrome P450 (CYP) family. It was hoped that genotyping for CYP enzyme deficiencies could offer a relatively simple solution for optimizing dosing and predicting response to clozapine. However, this has not proved consistent in practice. The genes coding for these enzymes are highly polymorphic, and the effects of many of the genetic differences contribute to differential metabolism of psychotropic agents. Patient phenotypes can be grouped into three categories – poor metabolizers, extensive metabolizers (corresponding to normal CYP activity), and ultrarapid metabolizers.[40]

Therapeutic drug monitoring of plasma clozapine and of its major plasma metabolite *N*-desmethylclozapine

(norclozapine) in a predose sample can help to track adherence, and to adjust dose to minimize toxicity. For therapeutic effectiveness, a minimum threshold plasma clozapine level has been identified at 0.35 mg/L.[41–45] Monitoring plasma levels of clozapine can identify the following:

- Patients who do not reach therapeutic plasma concentrations at expected therapeutic doses
- Those who have reached an adequate dose (giving plasma clozapine concentrations of 0.35–0.5 mg/L)
- Those who need prophylactic treatment with antiepileptic drugs to support a trial of a higher plasma clozapine concentration (>0.6 mg/L)
- Partial responders who may benefit from drug augmentation
- Those who have plasma toxicity

The CYP1A2 enzyme is primarily responsible for clozapine metabolism. The genetic studies of clozapine drug response are supported by the identification of multiple functional variants in CYP1A2[44,45] with well-defined effects on clozapine metabolism. A number of studies have provided evidence to suggest that the *CYP1A2*1F* allele is associated with clozapine response,[46–48] with the response being linked to plasma concentration levels in the study by Eap et al. Further studies have failed to identify an association between the *CYP1A2* polymorphism and plasma clozapine concentrations, when controlling for clozapine dose and body weight.[49] CYP1A2*1F polymorphisms were associated with a super-refractory schizophrenia group of patients, compared to controls, thus replicating previous work, and identifying this polymorphism as a moderator of clozapine response.[50] However, as is the case in clozapine pharmacodynamic pharmacogenetic research, other studies have not replicated these findings in relation to *CYP1A2* polymorphism and clozapine response.[51,52] Ethnic variation between the study populations has been suggested as a cause of this, with there being a higher frequency of the *CYP1A2*1F* allele in those of European ancestry.[27]

Individuals with increased activity of CYP1A2 enzymes are likely to have reduced levels of medication metabolized by that pathway. Case series have reported ultrarapid metabolizers of clozapine presenting as resistant to treatment, and conversely with increased plasma clozapine concentrations occurring with the concurrent use of fluvoxamine, a CYP1A2 inhibitor.[53] There is substantial individual variability in plasma clozapine concentrations, with higher concentrations in men and lower concentrations in those who smoke.[54] A mutation (*CYP1A2*1F*) in intron 1, which confers a high inducibility of CYP1A2 in smokers, is a suggested explanation for this rapid CYP1A2 activity.[55] However, pharmacogenetic testing for CYP1A2 variations in relation to clozapine metabolism remains at an early stage. Larger studies are needed in clozapine-treated TRS patients, which clearly identify concurrent inducers (such as smoking), in order to clarify the relationship between faulty CYP1A2 alleles and plasma clozapine concentrations.

The inconsistent findings in relation to CYP1A2 and clozapine response were highlighted in a recent systematic review, which identified only a single-nucleotide polymorphism (SNP) in *ABCD1* (3435TT (rs1045642)) to be predictive of plasma clozapine concentrations and response to clozapine,[56] but further longitudinal studies are required to clarify the role of ABCD1.

Pharmacogenomic testing has been done with warfarin-dosing algorithms, where both genetic and nongenetic factors are used to tailor warfarin dosing.[57] However, the translation of pharmacogenomic testing in predicting clozapine response has not, so far, been successful. Much of the research has focused on the CYP system, but at the moment, the use of CYP testing to guide the prescribing and dosing of clozapine cannot be justified.

Clozapine and the glutamate system

Even though clozapine is our only evidence-based treatment in TRS, we still do not understand how it works. This limits our ability to generate hypotheses as to which pharmacogenomic tests might be relevant. It has been suggested that the glutamate system may mediate response to clozapine,[58] and this had been investigated more recently.[59] Both preclinical and human studies have suggested that clozapine augmentation of glutamatergic neurotransmission leads to reductions in central glutamate levels. This has been suggested as a possible mechanism mediating clozapine response.[60–62]

The role of glutamate in the pathogenesis of schizophrenia is supported by recent findings from the Psychiatric Genomics Consortium and other studies. Genome-wide significant associations with schizophrenia have been observed in glutamate system genes *GRM3*, *GRIN2A*, *GRIA1*, and *GRIN2B*.[63,64] Neuroimaging studies have provided evidence that there are biological differences in glutamatergic neurotransmission between treatment-resistant and treatment-responsive schizophrenia.[5,6] TRS patients have demonstrated higher glutamate levels in the anterior cingulate cortex,[6] with relatively normal dopamine functioning, in comparison to treatment-responsive patients.[5] These observations indicate that the persistence of symptoms in TRS may be associated

with elevated anterior cingulate glutamate levels. In TRS, a handful of studies have investigated glutamate system genes in relation to clozapine response. These have largely focused on variants in *GRIN2B*, which codes for the 2B subunit of the glutamate *N*-methyl-d-aspartate receptor.[65] Further work is needed to clarify any potential role for the glutamate system in the pathophysiology of TRS and clozapine response. We need to investigate a wider variety of glutamate genetic variants, using prospective study samples, to link the genetic findings to neuroimaging, and phenotypic characteristics, including plasma clozapine concentrations.

Pharmacogenomics and clozapine adverse events

Clozapine and agranulocytosis

One of the main reasons for the underuse of clozapine is patient and clinician fears about sudden clozapine-induced agranulocytosis (CIA).[66] This occurs with an incidence of 0.8% at 1 year after starting clozapine treatment.[67] The highest incidence is at 6–18 weeks.[68] The mechanism of this idiosyncratic event is unclear, though it is certainly multifactorial, with some evidence for genetic variance increasing the susceptibility to CIA.[69]

The best genetic evidence is for dysfunction in the human leukocyte antigen (HLA) system, composed of genes that are important in immune system modulation. A recent GWAS and exome-sequencing analysis reported significant associations between genetic variants in HLA and CIA. This included genetic variants involved in the *HLA-DQB1* locus (a single amino acid at HLA-DQB1 (126Q) and an amino acid change in the extracellular binding pocket of *HLA-B(158T)*).[69] A case–control study found that the odds of developing CIA were 16.9 times higher in patients carrying a cytosine instead of the usual guanine *DQB1* genotype.[70] This SNP was incorporated into a commercially available test, with a sensitivity of 21.5% and a specificity of 98.4% for detecting the haplotype, indicating a 5.1% risk of developing agranulocytosis if the haplotype is present.[70] Although the *HLA-DQB1* locus may be implicated in CIA development, for the test to be clinically useful, it requires high sensitivity and specificity, and thus, the low sensitivity of the test has limited its use in clinical settings.[71] This test gives a 1% (0.05×0.22) chance of identifying patients at risk of developing CIA, which is not very different from the risk for all patients treated with clozapine (0.8%).[71,72]

So far, a dose-dependent link between plasma clozapine concentrations and the risk of neutropenia and agranulocytosis has not been consistently demonstrated.[73,74] However, a recent GWAS meta-analysis identified a novel genome-wide significant association with clozapine-associated neutropenia and rs149104283, intronic to transcripts of *SLCO1B3* and *SLCO1B7*, members of a family of hepatic transporter genes involved in drug uptake.[75] This study[75] offers a tantalizing suggestion that CIA may be related to plasma concentrations while offering a novel link between clozapine pharmacokinetics and bioavailability and the genetic risk of neutropenia/agranulocytosis.

The replication of an association between CIA and genetic variants involved in the *HLA-DQB1* locus[69,76–78] is promising, but the majority of those who develop a CIA are not carriers of the risk alleles. So, none of these pharmacogenomic tests are yet clinically useful.[72]

Clozapine and metabolic disturbance – candidate gene studies

Schizophrenia is associated with increased rates of cardiovascular morbidity[79–82] and associated excess premature death, which translates to a 15- to 20-year shortened life expectancy for those with schizophrenia.[83,84] There is an increased prevalence of weight gain, dyslipidemia, and type 2 diabetes seen with both clozapine and olanzapine.[80,85] Clozapine is especially associated with weight gain, which can occur early in the course of treatment, before plateauing as the treatment continues.[86–88]

For clozapine-associated weight gain, the only consistently replicated genetic variant is the 759T/C polymorphism in the promoter region of the *HTR2C* gene (rs3813929).[89–91] Those clozapine patients homozygous for the *HTR2C* gene 759C polymorphism have been shown to have increased obesity rates at 6 months of clozapine treatment.[92] Conversely, those with the T allele for this polymorphism have shown less weight gain over the course of the first 6 months of clozapine treatment.[93,94] However, as is typical of candidate gene studies, these results have not been consistently replicated, with frequent findings of no associations between the –759C/T polymorphism of the *HTR2C* gene and clozapine-associated weight gain and obesity.[95–97] Further, in a recent review, a meta-analysis could not be performed on genes associated with weight gain due to a lack of genetic data from studies in which clozapine was analyzed separately to other antipsychotics.[25]

Other candidate genes identified to be associated with clozapine-induced weight gain include *LG*,[98–100] *TNF*α,[101] *CNR1*,[102] *ADRA*$_{2A}$,[103,104] *MC4R*,[105,106] and *BDNF* genes.[107]

Metabolic syndrome associated with clozapine use has been associated with polymorphisms in *HTR2C*,[108–111] LG (G allele of the *–2548A/G LG* promoter polymorphism),[100] the *INSIG2*

(*INSIG2 rs11123469* C allele), which encodes a protein which mediates feedback control of lipid metabolism, with the C allele significantly overrepresented in those with metabolic syndrome,[112] and *MTHFR* genes (*MTTP* rs1800591 T allele).[113,114]

An increased risk of dyslipidemia secondary to clozapine use is associated with a polymorphism in the *PRKAR2B* gene, detected in a GWAS of participants from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study.[115] *ApoC3* (TG haplotype) and *ApoA5* (CG haplotype) genes were associated with decreased serum triglyceride and serum cholesterol levels, respectively, in clozapine-treated patients. The *ApoC3 CC* haplotype was associated with increased serum triglyceride levels.[116]

Table 4 summarizes significant findings from candidate gene studies of clozapine efficacy and side effe cts.[32–37,92–94,96,100,106,108,109,117–136]

Epigenetics

Studies of genetic associations with schizophrenia are characterized by nonreplication and significant heterogeneity. The heterogeneous course of schizophrenia makes it difficult to apply traditional gene–environment-based approaches[137] and has led to speculation that epigenetic factors may mediate susceptibility and account for the "missing heritability".[138,139] Epigenetic influences on disease phenotypes may explain the effect of early life stressors on risk of psychosis in later life.

Epigenetics and antipsychotic use/development

The dynamic nature of the epigenome means that, unlike pathogenic DNA sequence mutations, epigenetic disruption is potentially reversible, and thus a realistic target for pharmacological intervention. Methylation of a promoter CpG island located ~30 kb upstream of the gene encoding MEK1 was significantly correlated with lifetime antipsychotic use in postmortem frontal cortex brain samples.[140] Epigenetic changes on GABAergic and glutamatergic gene promoters have been suggested as explanations for the therapeutic action of clozapine.[141–143] This occurs at least in part due to increased GABAergic activity mediated by histone methylation and

Table 4 Positive and negative candidate gene studies of clozapine treatment response and clozapine-induced metabolic disturbance in schizophrenia

Gene	Variant	Reported association	Nonsignificant associations (references)
Dopamine			
DAT	Multiple[33]	Improved psychotic symptoms	121
D1	Multiple[118,119]	Improved psychotic symptoms	–
D2	Taq A/B[120]	Improved psychotic symptoms	35,122
D3	Ser9Gly[32]	Increased efficacy in those with Ser allele	36,123
Serotonin			
5-HT2A	102 T/C[34]	C/C genotype: with improved response	124,127
	–1438 G/A[35]	G/G genotype poorer response	128 (group treated with clozapine, amisulpride, olanzapine, or risperidone)
	His 452Tyr[124,125]	Tyr associated with nonresponse	127,129
5-HT2C	Cys23Ser[126]	Ser associated with increased efficacy	124,130
5-HT6	267 T/C[37]	Improved response in T/T genotype	131
5-HT2C	759C/T[92–94]	C allele associated with weight gain and T allele with a protective effect	96
5-HT2C	Cys23Ser-polymorphisms rs518147, rs1414334, and 5-HTR_{2C}:c.1-142948(GT)n[108,109]	Increased risk of metabolic syndrome	
LEP	–2548A/G (GG/GA genotype)[100]	Increased weight gain and risk of metabolic syndrome	132
MC4R	rs17782313 C allele (CC genotype) rs8087522 A allele[106,133]	Increased weight gain	134
D2	rs4436578 C allele[135]	Increased weight gain	
TNFα	308 G>A polymorphism *TNFα* gene (–308 GG genotype)[101]	Increased weight gain	136

Notes: Although numerous studies suggest that clozapine-induced side effects and efficacy are associated with candidate gene polymorphisms, most findings are of modest effect, with inconsistent results to date (ie, multiple negative studies of candidate genes exist). Reproduced with permission of Royal College of Psychiatrists via PLSClear, Lally J, MacCabe JH. Personalised approaches to pharmacotherapy for schizophrenia.[117]

chromatin relaxation, and the targeting of DNA demethylation via GADD45b.[142] Clozapine, but not haloperidol, is associated with the induction of nuclear H3K9 acetylation[144] and an increase of GADD45b mRNA in mice.[142] Epigenetic research into therapeutic mechanisms of clozapine action remains in its infancy, though findings to date suggest the possibility of future tests to predict clozapine responders and of novel therapeutic interventions.

Challenges in applying pharmacogenomics in TRS and future directions

Several factors contribute to the difficulty in implementing pharmacogenomic testing to predict clozapine tolerability and efficacy. Schizophrenia is a heterogeneous disorder, characterized by variability in clinical presentation, which makes it a challenging phenotype to accurately assess. While TRS may represent a distinct and more uniform subtype of schizophrenia,[145] it is likely that remaining heterogeneity continues to obscure true genetic signals. Varying environmental and clinical factors impacting on clozapine response and tolerability further complicate pharmacogenomic research.

Some potential clinical risk factors for TRS have been suggested, such as a young age of illness onset (and specifically onset before the age of 20[146]), an insidious onset, a greater severity of negative symptoms at illness onset, living in less urban environments, comorbid personality disorders, and cumulative effects of lifetime trauma and adversity.[146–153] However, to date, the predictive value of demographic and clinical risk factors for TRS, linked to pharmacogenomic findings, has not been widely investigated. One recent study identified an association between treatment resistance and polygenic risk score, an association which was stronger in those with a younger age of illness onset and with poorer premorbid functioning.[149] A more recent study, comparable in size, failed to identify an association between treatment resistance and polygenic risk score.[153] Given these equivocal findings, the use of common genetic variants to index a polygenic risk score to predict treatment resistance requires further evaluation using larger case–control population samples, and factoring in other clinical and demographic risk factors. This approach could clarify the utility of the polygenic risk score in predicting TRS.

Previous attempts to identify clinical predictors of clozapine response have generally identified few predictors. The most consistently identified predictors of clozapine response include a later age of illness onset,[154–156] more severe positive symptoms,[154,157,158] an earlier use of clozapine in the illness course when treatment resistance emerges,[159] and related to this, a lower number of antipsychotic trials and hospitalizations prior to clozapine use.[160] As demonstrated in this review, candidate gene and genome sequence studies alone have not proved successful in identifying consistent predictors of clozapine response. Future pharmacogenomic studies could benefit from incorporating clinical risk factors for clozapine response, to guide the development of valid and useful clozapine treatment algorithms for predicting response.

Pharmacogenomic studies of clozapine have also been limited by sample size. This is further compounded by the heterogeneity of participants, few measures of clozapine adherence, levels of concurrent medications, lack of controlling for confounding factors such as smoking, and the absence of an agreed response to clozapine by a numerical reduction in scale scores (such as the Positive and Negative Syndrome Scale or the Brief Psychiatric Rating Scale score).

Probably as a result of such limitations, most positive candidate gene studies have not provided sufficiently robust findings nor been replicated. Meta-analysis has confirmed the need for replication studies of much larger sample sizes to detect real associations.[25,27] Most pharmacogenomic studies in clozapine use have focused on candidate genes coding for specific enzymes believed to be involved in the absorption, distribution, metabolism, and/or excretion of antipsychotic medications.[27,28,89] Studies using the candidate gene approach are inherently limited by the genes chosen, while linkage method studies require families and are impractical for most pharmacogenomic questions. The introduction of GWAS has opened the doors to genetic research which transcends candidate gene studies.[63,69,149,161] GWAS research has developed rapidly, and there is a growing set of GWASs related to phenotypes, including TRS.[145] However, this remains a novel area, and there are but a handful of studies assessing clozapine response using a polygenic risk-scoring method (based on GWAS).[24,161,162] GWAS has been more widely applied in the identification of genetic variants associated with clozapine adverse events, such as agranulocytosis[69] and metabolic disturbance. In the most noteworthy GWAS of clozapine metabolic effects from the CATIE study, positive associations for metabolic disturbance (SNP in *PRKAR2B* gene)[115] were identified, while in other GWASs, associations with the *MC4R* gene and weight gain with clozapine use were seen.[106,163] However, the concurrent use of multiple antipsychotics and the failure to consider clozapine separately preclude meta-analysis of the genetic variants contributing to clozapine metabolic disturbances.[27]

Future directions

Barriers to implementation of pharmacogenomic testing in TRS

The translation of pharmacogenomic research findings into clinical practice has been slow in all medical settings.[164,165] In psychiatry, the limitations of the available tests have prevented the use of pharmacogenomic testing in the clinical use of clozapine and other antipsychotics. The reasons for this include the limitations of the candidate gene approach and study heterogeneity.

Substantial economic barriers also remain.[166] The stratification of patients with psychotic illness into more refined subsets may advance medication development, but it may also reduce the size of the potential market and deter industry investment.[167] Drugs developed in this way may take longer to recoup development expenses. It may also prove less economic to develop drugs to treat rarer genetic subtypes of TRS or genetically determined TRS subtypes which are more prevalent in lower income countries.[118]

On the other hand, as technology advances, genotyping should become cheaper. In the future, the focus will shift from genotyping costs to the interpretation and production of reports for clinical use. The clear communication of pharmacogenomic test results will be a critical part of their effective clinical use.

Pharmacogenomic test results for the individual patient should be available for that patient's lifetime. However, due to the fragmentation of health care services, this genetic test information may be lost as the patient moves from one health care setting to another. This could be overcome by using integrated patient records. Currently, in the UK, pharmacogenomic information might sit best in primary care records, ensuring that it is available for all potential prescribers.

Furthermore, clinicians are not used to using this kind of information to inform their prescribing. This is, perhaps, not surprising when these tests have limited clinical usefulness.[168] For example, in schizophrenia in general, the characterization of CYP genes has not led to the widespread utilization of these tests to predict response and side effects to treatment.[169] Surveys have indicated that when clinicians have used pharmacogenomic tests, they have focused on the assessment of medication intolerance rather than response.[170]

Overcoming barriers

Even when pharmacogenomic tests become more clinically informative, it will still be necessary to ensure that clinicians are fully informed of the ways in which such tests can improve clinical practice. Health service providers will need to be convinced that this technology is cost effective and that it represents an improvement over the current trial-and-error approaches to prescribing. Thorough implementation will require clinician education, the incorporation of pharmacogenomic testing advice into clinical guidelines for TRS, and integrated medical records.[171]

Conclusion

Personalized medicine in the diagnosis and treatment of TRS will involve multiple strands including genetics, neuroimaging, and biomarkers. The use of genomic markers should enable us to create more identifiable homogenous subgroups of schizophrenia patients. In turn, this should mean that we can better identify both those who will respond to clozapine and those who will better tolerate it. Such predictive testing should allow low-risk patients to get the most benefit from clozapine while reducing the risk of adverse events for higher risk patients.

However, given the weakness of the tests currently available, pharmacogenomic testing is not yet at a point where it can effectively inform the clinical use of clozapine. What needs to be done? We need clearly agreed definitions of TRS and standardized measurements of response to treatment. This would allow the construction of large and well-characterized samples which could be subject to prospective assessments. Such studies will need to link genetic data with phenotypic stratification. They will also need to allow for the effects of other factors such as current and historical use of medication, doses and plasma concentrations of clozapine, and lifestyle factors such as smoking.

Disclosure

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