Pharmacogenetics of Antipsychotics: Recent Progress and Methodological Issues

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Abstract

Importance of the field—Antipsychotic drug is the mainstay of treatment for schizophrenia, and there are large inter-individual differences in clinical response and side effects. Pharmacogenetics provides a valuable tool to fulfill the promise of personalized medicine by tailoring treatment based on one’s genetic markers.

Areas covered in this review—This article reviews the recent progress in pharmacogenetic research of antipsychotic drugs since 2010, focusing on two areas: antipsychotic-induced weight gain and clozapine-induced agranulocytosis. Important methodological issues in this area of research are discussed.

What the reader will gain—Readers are expected to learn the up-to-date evidence in pharmacogenetic research, and to gain familiarity to the issues and challenges facing the field.

Take home message—Pharmacogenetic studies of antipsychotic drugs are promising despite of many challenges. Recent advances as reviewed in this article push the field closer to routine clinical utilization of pharmacogenetic testing. Progress in genomic technology and bioinformatics, larger sample sizes, better phenotype characterization, and careful consideration of study design issues will help to elevate antipsychotic pharmacogenetics to its next level.

1. Introduction

Pharmacogenetics and pharmacogenomics aim at taking advantages of the rapid growth in DNA sequencing technology to fulfill the promise of personalized medicine by incorporating genetic makers into prediction of drug response. Antipsychotic drugs are the main treatment for schizophrenia and other psychotic spectrum disorders. Despite their overall efficacy in alleviating psychotic symptoms, treatment responses are variable and these drugs carry significant side effects. The pharmacogenetics of antipsychotics has been an active area of research since early 1990s, and has been summarized in the previous literature review. This article will provide an update on pharmacogenetic research of antipsychotics and discuss some issues that affect clinical utilization of pharmacogenetic findings. There have not been recent significant findings in genetic markers of antipsychotic efficacy, partly due to variable reliability of symptom ratings as well as lack of attention to medication adherence, issues that will be discussed fully later. Two areas of significant progress have been in genetic markers of drug-induced adverse events, namely, antipsychotic-induced weight gain and clozapine-induced agranulocytosis (CIA), which are reviewed below.
2. Antipsychotic-induced weight gain

Weight gain and associated metabolic syndrome is the most prominent side effect associated with the second generation antipsychotics (SGAs), especially clozapine, olanzapine, and quetiapine, and many first generation antipsychotics (FGAs) can also cause significant weight gain. No clear clinical predictors of antipsychotic-induced weight gain have been identified, and the pathophysiological mechanisms of weight gain remain poorly understood. Food intake, energy utilization, metabolism, and body weight are regulated by complex interactions between multiple neurotransmitter systems in multiple brain regions, all of which are targeted by antipsychotic drugs to some extent.

As a phenotype, weight gain has certain advantages in pharmacogenetic studies of antipsychotic drugs. In contrast to clinical symptom ratings, weight gain can be defined with better accuracy and reliability. Furthermore, related phenotypic measures may be even less likely to be influenced by extrinsic factors and thus provide more statistical power for genetic studies. These include body mass index (BMI) and total fat mass, which can be measured reliably and inexpensively in large samples, as well as other biological correlates of weight gain such as fasting glucose levels, insulin levels and circulating levels of key neuropeptides such as leptin, adiponectin and/or ghrelin. These measures also offer the advantage of being readily amenable to quantitative trait analyses and thus may be more informative than the arbitrary responder/non-responder criteria commonly used in antipsychotic pharmacogenetic studies. Finally, 40–70% of the variation in obesity-related phenotypes such as BMI, fat mass, and leptin levels is accounted for by genetic factors, and recent GWAS studies provided strong evidence for several genes including FTO, MC4R, TMEM18 and others in obesity and obesity-related phenotypes.

Pharmacogenetics of antipsychotic-induced weight gain has been a very active research area, and many studies have examined genetic variations in the various neurotransmitter systems in relation to drug-induced weight gain, such as DRD2, HTR2C, and GNB3. Overall, the evidence so far suggests that the C759T SNP in HTR2C may play an important role in antipsychotic-induced weight gain. Only one GWAS study has been published to date, using the CATIE sample. Despite many methodological limitations such as chronic patient samples and multiple treatment arms, several promising signals (p-values < 5*10^-7) were observed even in subsamples of n<300.

A recent study reported that in a pediatric sample of 139 patients who were prescribed antipsychotic drugs (risperidone, aripiprazole, and quetiapine) for the first time, a GWAS of antipsychotic-induced weight gain discovered a single top signal at a marginally genome-wide significant level (p=1.6×10^-7). This was replicated in three other independent samples, indicating the robustness of the finding. The peak signal is located on chromosome 18q21, overlapping a peak identified as a predictor of obesity. The top SNP was rs489693, involving an A to C change in nucleotide sequence. The exact functional consequence of this variant is still unknown, but the locus is approximately 150kb downstream from MC4R, the melanocortin 4 receptor gene, which has long been suspected as a candidate for weight-related phenotypes, including antipsychotic-induced weight gain. Mutations in this gene are linked with extreme obesity in humans, and MC4R knockout mice develop obesity. MC4R-expressing neurons in the ventromedial hypothalamus are regulated by circulating levels of leptin via pathways in the arcuate nucleus. In turn, MC4R regulates 5-HT2C receptors, which is already implicated in weight gain. In the discovery sample, risk allele homozygotes gained twice as much weight as other patients after 12 weeks of treatment, and the genetic effect was not drug specific. The consistency of HTR2C-MC4R findings poses a possibility that a drug may be developed at these targets to treat or prevent antipsychotic-induced weight gain. Another clinical implication is that patients may be genotyped for
rs489693 prior to initiation of antipsychotic drug treatment, and if a patient carries the risk allele for drug-induced weight gain, alternative treatment strategies can be considered including starting antipsychotics that are relatively benign in metabolic side effects and initiating psychosocial and/or behavioral interventions for weight gain\(^7\).

### 3. Clozapine-induced agranulocytosis (CIA)

One area of antipsychotic pharmacogenetics not covered in the previous article\(^1\) is genetic markers of clozapine-induced agranulocytosis, a blood dyscrasia with potentially fatal complications. Clozapine has proven to be the most effective antipsychotic, especially for refractory patients\(^18\text{--}20\). Its use has been limited, however, due to potentially serious drug-induced adverse events such as agranulocytosis. Under current treatment guidelines\(^21\), clozapine should only be used after two failed trials of other antipsychotic agents. CIA is defined as a decrease in absolute neutrophil count (ANC) to less than 500 cells/mm\(^3\). This results in significantly higher risk of infection; about 50 patients on clozapine died of infectious disease in 1970s and the drug had to be withdrawn from the market in 1970s. Re-introduction of clozapine into clinical practice was accompanied by mandated requirement for frequent blood monitoring in many countries. The implementation of the blood-monitoring system has successfully reduced the incidence of CIA to 0.4\% from the 1.3\% incidence observed in patients treated with clozapine in the absence of blood-monitoring\(^22\).

The pathophysiology of CIA is poorly understood, although it has been suggested that some clozapine metabolites may be toxic to neutrophils\(^23\) and that CIA may involve immune-related mechanism\(^24\). Genetic factors may play an important role in the development of CIA because it is primarily biologically determined. Conceivably, identification of sensitive and reliable genetic markers may potentially lift the burden of weekly blood testing associating with clozapine.

An initial study primarily comprised of Ashkenazi Jewish subjects in 1990 reported that the HLA-B38 marker was present in 83\% of 6 CIA cases, compared to only 20\% of 25 controls\(^24\). The human leukocyte antigens (HLA) are coded by multiple genes in the major histocompatibility complex (MHC) genomic region, located on chromosome 6p21, which was recently shown to be related to the development of schizophrenia in several GWAS reports\(^25, 26\). Further analysis showed that a haplotype of HLA-B38, HLA-DR4, and HLA-DQ3 was associated with CIA in 83\% of the cases (100\% in Ashkenazi Jews), as opposed to 8\% of the controls (12\% in Ashkenazi Jews). It is worth noting that this study used traditional blood typing method to define HLA markers. Later studies utilized restriction fragment length polymorphism (RFLP), then more modern method to genotype patients in HLA and MHC regions. The same group later collected a larger sample with 31 CIA patients and 52 controls, confirming the findings from the previous study, but it also found different HLA haplotypes in association with CIA in Jewish subjects as compared to non-Jewish subjects\(^27\). The authors suggested that genes of the MHC other than class I and class II might be responsible for CIA, particularly the heat-shock protein 70–2 (HSP70-2) or tumor necrosis factor (TNF)\(^28, 29\). As shown in Table 1, studies also examined the role of genes coding for enzymes that are responsible for metabolizing clozapine such as cytochrome P450 2D6 (CYP2D6), Myeloperoxidase (MPO), NADPH-oxidase genes, and dihydronicotinamide riboside quinone oxidoreductase 2 gene (NQO2), but findings were not consistent\(^30\text{--}32\). Due to the rarity of CIA, most studies had small sample sizes and all studies were conducted by four or five research groups. There were also overlaps in samples among studies. Nevertheless, HLA markers in association with CIA have emerged as a consistent finding across studies.
The most recent study of genetic factors of CIA \(^{33}\) examined 74 candidate genes in a sample of 33 cases and 54 controls, then attempted to replicate significant findings in a second independent sample of 49 cases and 78 controls. Although five genes were significant in cohort I, including \(HLA-DQB1\), \(CSF2Rh\), \(DRD1\), \(NTSR1\), and \(HLA-C\), only \(HLA-DQB1\) was significant in Cohort II. After sequencing the gene in both samples, a SNP in \(HLA-DQB1\), \(6672G>C\), was significantly associated with CIA in the combined sample, \(p<0.001\), with an odds ratio (OR) of 16.9, which is a very strong effect and not commonly seen in psychiatric pharmacogenetics. Unfortunately, the sensitivity of the marker to predict CIA is only 21.5\%, indicating that a majority of individuals who develop CIA are not carriers of the risk allele, and presumably have other genetic risk factors. Thus, a more comprehensive risk profile would be necessary in order to obviate the need for invasive monitoring. However, the marker may be clinically useful due to its high specificity (98.4\%), suggesting that a patient carrying this marker is at a much higher risk of developing CIA., and therefore the risks of clozapine use should be carefully weighed against its considerable benefits. More studies and larger sample sizes are needed to discover other genetic factors involved in CIA.

4. Expert Opinion

In spite of recent progress of pharmacogenetics of antipsychotic drugs, clinical utilization of these findings has been sparse. Lack of replication in most pharmacogenetic findings, low sensitivity and specificity in single-marker genetic tests, small effect sizes of genetic markers in most studies contributed to obstacles in clinical applications of pharmacogenetics in schizophrenia treatment. To date, there is no randomized clinical trial that provide solid support for using genetic testing to guide drug treatment in psychiatry. Most retrospective studies have small sample sizes limiting statistical power. Although most registration trials have collected blood samples that can potentially be used for genotyping, pharmaceutical companies have been reluctant in publishing pharmacogenetic studies (with a few exceptions\(^{41}\)), perhaps due to fear of market limitation and compromised return on investment. In addition, pharmacogenetic testing may be expensive and takes long time to get results, rendering its clinical application less practical. Furthermore, risks and benefits analysis should be carefully considered for each case even if a patient carries the risk allele for a significant drug-induced side effect. Add-on medication can be used to help manage side effects if an antipsychotic drug is deemed to be necessary. Nevertheless, pharmacogenetic provides a useful tool for the risk and benefits analysis that is essential for evidenced-based personalized medicine. Several issues should be focused on in pharmacogenetic studies of antipsychotic drugs in order to advance the field.

4.1 The importance of studying early phases of schizophrenia

Most pharmacogenetic data in psychiatry to date are derived from ongoing clinical trials. Although it is convenient to use data from already conducted studies, there are many limitations to this approach. The patients in these studies tend to be chronically ill with lengthy prior treatment histories and exposure to multiple antipsychotics. Highly responsive and stable patients may not present for these trials, as they do not seek changes to treatment if adequate response has already been attained. Hence, study samples drawn from trials in chronic subjects may be systematically biased towards inclusion of less responsive and more severe patients, and/or patients who are nonadherent with treatment, and therefore not represent the full spectrum of treatment outcomes\(^{42}\). Chronically ill cohorts are also marked by increased duration of psychotic symptoms, substance abuse, and functional/social disabilities, all of which may influence drug response rates and introduce increased variance into data analyses. For these reasons, studies that concentrate on early phase, or first episode patients, may provide enhanced power for pharmacogenetic studies of drug efficacy. For example, a meta-analysis of pharmacogenetic studies of antipsychotic drug efficacy demonstrated a 50% greater effect size for the \(DRD2\) promoter polymorphism, \(-141C Ins/
Del, in studies containing first episode patients compared to studies of chronic patients. Another example is from the studies on HTR2C and antipsychotic-induced weight gain. A recent meta-analysis of 12 cohorts found that the C-759T polymorphism in HTR2C is significantly associated with antipsychotic-induced weight gain, with the C allele conveying a 2.42 folds increase in risk of gaining >7% body weight after being treated with an antipsychotic drug. However, further inspection of the data revealed that the finding is mostly driven by first episode samples (Figure 2). In fact, the pooled OR for the four first episode samples is 5.40, p=0.001, while the chronic samples produced a pooled OR of 1.64, p>.20. The difference in pooled ORs between the two groups of samples approaches statistical significance, p=0.06. These data suggest that focusing on early phases or first episode schizophrenia represents a unique advantage in studying pharmacogenetics of antipsychotic drug response, which may yield findings that have real clinical implications.

### 4.2 Medication adherence and statistical power

Clinical researchers have long recognized that treatment adherence is poor among psychiatric patients. A recent review demonstrated that the rates of nonadherence are 20% to 72% in patients with schizophrenia. In the CATIE trial, three quarters of patients stopped their initially assigned drugs within 18 months, and almost one third stopped medication due to “patient’s decision”, which strongly suggests treatment nonadherence. In addition to the fact that medication nonadherence can result in symptom relapse, it also leads to weakened signals and lower statistical power in pharmacogenetic studies of drug response. However, this important issue has often been overlooked in the field, and the effect of nonadherence on statistical power has not been quantified. Conceptually, if a substantial proportion of subjects are nonadherent with treatment, it would be very difficult to detect a significant genotype-phenotype (i.e., response to an administered drug) relationship regardless of the strength of the effect of the genetic marker. A pharmacogenetic study that failed to formally assess medication adherence would misclassify non-adherent subjects as non-response or lack of a particular side effect.

A recent computer simulation study attempted to quantify the effect of nonadherence on statistical power. In a typical scenario in pharmacogenetic studies (i.e., moderate effect size, 20% frequency of risk genotype), as the nonadherence rate increases, statistical power for detecting a significant signal drops down rapidly. Even with a sample size of 400, which would be a large sample size in pharmacogenetic studies, power drops below 0.70 when the nonadherence rate reaches 50%, which is not uncommon in a clinical trial. However, by reducing the nonadherence rate from 50% to 10%, a sample size of 200 is adequately powered (>0.80). Therefore, by maximizing medication adherence, total sample sizes can be cut in half with maintenance of adequate study power. In the above-mentioned GWAS of antipsychotic-induced weight gain in a pediatric sample, plasma antipsychotic drug levels were collected at each study visit and subjects with undetectable levels were excluded from the genetic analysis. With ensured medication adherence, a relatively small sample of 139 patients was sufficient to detect a near genome-wide significant result, which was subsequently replicated in three additional samples also with monitored adherence. These data suggest that careful attention to adherence could markedly enhance study power at lower overall cost than additional recruitment of larger sample sizes.

### 4.3 Unique aspects of pharmacogenetic clinical trials

In order to provide useful data for clinical application, prospective pharmacogenetic studies are needed. Studies should match individuals with a particular genotype with a specific effective treatment, i.e., testing the genotype X treatment interaction. In addition, an essential characteristic of a prospective pharmacogenetic clinical trial is randomization by genotype, which will be able to examine whether genotyping patients and using genotype
information to guide treatment selection makes any clinical difference. To optimize pharmacogenetic study design, a number of issues need to be considered. In a hypothetical pharmacogenetic clinical trial, we would examine the efficacy of alternative treatments associated with a hypothetical genetic marker. It is hypothesized that medication treatment A is efficacious for patients with a particular genotype of the marker, but not for patients without the genotype. In contrast, medication treatment B is efficacious for patients without the particular genotype, but not for patients with the genotype. Patients would preferably in their first episode of illness with minimal prior medication exposure, and could genotyped at baseline. Minimal turn-around time for genotyping will be needed, as many patients will require rapid initiation of treatment and randomization cannot be delayed for more than 24–48 hours in many cases. Patients are then randomized into either A or B treatment, stratified on genotypes. Hence, this is a 2×2 randomized factorial design. Medication adherence should be carefully monitored by plasma drug levels, or perhaps by novel techniques such as digestive event markers. This hypothetical study will be able to maximize the statistical power to detect a true effect, and answer the question of whether a particular genetic marker is useful in guiding clinical treatment decisions.

In summary, pharmacogenetic studies of antipsychotic drugs are promising despite of many challenges. Recent advances as reviewed in this article push the field closer to routine clinical utilization of pharmacogenetic testing. Progress in genomic technology and bioinformatics, larger sample sizes, better phenotype characterization, and careful consideration of study design issues will help to elevate antipsychotic pharmacogenetics to its next level.

References

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15**. Malhotra AK, Correll CU, Chowdhury NI, et al. Association Between Common Variants Near the Melanocortin 4 Receptor Gene and Severe Antipsychotic Drug-Induced Weight Gain. Arch Gen Psychiatry. 2012 May 7. This study found that variations in MC4R are associated with antipsychotic-induced weight gain, and the signal reached near genome-wide significance.


Figure 1.
A Manhattan plot from a genome-wide association study of antipsychotic-induced weight gain. Y-axis is the statistical significance levels ($-\log_{10} P$ values) of the association tests for each single nucleotide polymorphisms (SNP) and the phenotype (body mass index). Y-axis is the chromosomal position for all autosomal SNPs. Peak values are observed on chromosome 18, between positions 55.934 and 56.037 megabases (Mb). (Reprint from Malhotra et al.15)
Figure 2.
Meta-analysis of the effect of HTR2C C-759T polymorphism on antipsychotic-induced weight gain. The first eight studies were from chronic patient samples, and the last four studies were from first episode patient samples. (Data derived and re-analyzed from Sicard et al.\textsuperscript{11})
Pharmacogenetic findings of clozapine-induce agranulocytosis.

<table>
<thead>
<tr>
<th>1st Author/year</th>
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<th>Controls (n)</th>
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<td>52</td>
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<td>For non-Jewish patients, haplotype of HLA-DR<em>02, DQB1</em>0502, and DQA1*0102, p=.004, OR=21.</td>
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<td>Athanasiou 2011 33</td>
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