Pharmacogenomics in schizophrenia: the quest for individualized therapy

Vincenzo S. Basile1, Mario Masellis1, Steven G. Potkin2 and James L. Kennedy1,*

1Neurogenetics Section, Centre for Addiction and Mental Health (CAMH), Clarke Division, University of Toronto, Toronto, Canada and 2Department of Psychiatry, University of California, Irvine, USA

Received August 7, 2002; Revised and Accepted August 22, 2002

There is strong evidence to suggest that genetic variation plays an important role in inter-individual differences in medication response and toxicity. The rapidly evolving disciplines of pharmacogenetics and pharmacogenomics seek to uncover this genetic variation in order to predict treatment outcomes. The goal is to be able to select the drugs with the greatest likelihood of benefit and the least likelihood of harm in individual patients, based on their genetic make-up—individualized therapy. Pharmacogenomic studies utilize genomic technologies to identify chromosomal areas of interest and novel putative drug targets, while pharmacogenetic strategies rely on studying sequence variations in candidate genes suspected of affecting drug response or toxicity. The candidate gene variants that affect function of the gene or its protein product have the highest priority for investigation. This review will provide demonstrative examples of functional candidate gene variants studied in a variety of antipsychotic response phenotypes in the treatment of schizophrenia. Serotonin and dopamine receptor gene variants in clozapine response will be examined, and in the process the need for sub-phenotypes will be pointed out. Our recent pharmacogenetic studies of the subphenotype of neurocognitive functioning following clozapine treatment and the dopamine D1 receptor gene (DRD1) will be presented, highlighting our novel neuroimaging data via [18F]fluoro-2-deoxy-D-glucose (FDG) metabolism position emission tomography (PET) that demonstrates hypofunctioning of several brain regions in patients with specific dopamine D1 genotype. Preliminary candidate gene studies investigating the side-effect of clozapine-induced weight gain are also presented. The antipsychotic adverse reaction of tardive dyskinesia and its association with the dopamine D3 receptor will be critically examined, as well as the added influence of antipsychotic metabolism via the cytochrome P450 1A2 gene (CYP1A2). Results that delineate the putative gene–gene interaction between DRD3 and CYP1A2 are also presented. We have also utilized FDG–PET subphenotyping to demonstrate increased brain region activity in patients who have the dopamine D3 genotype that confers increased risk for antipsychotic induced tardive dyskinesia. The merits and weaknesses of neuroimaging technologies as applied to pharmacogenetic analyses are discussed. To the extent that the above data become more widely verified and replicated, the field of psychiatry will move closer to clinically meaningful tests that will be useful in deciding the best drug for each individual patient.

Schizophrenia is a paradigmatic disease in which pharmacogenomic and pharmacogenetic research can and has been applied. A devastating psychiatric disorder that affects ~1% of the population, schizophrenia has been treated with an extensive pharmacopeia. Treatment with specific antipsychotic medications often proceeds by trial and error in order to determine the optimal medication and dose that maximize response and minimize toxicity. In spite of the wide array of medicines available, 10–20% of patients do not initially respond to treatment with antipsychotic drug therapy. An additional 20–30% who do respond early on eventually relapse on their maintenance programs, and some develop serious side-effects that cause them to discontinue the medication. With the introduction of chlorpromazine in 1952, patients suffering from psychosis were able to be de-institutionalized. Chlorpromazine and other ‘typical’ antipsychotics (e.g. haloperidol) demonstrate high in vitro binding affinities for the dopamine D2 receptor. Specifically, their binding potential for D2 correlates well with their clinical potencies (1). The reintroduction of clozapine, the prototype of ‘atypical’ antipsychotic, in the late 1980s has led to significant advances in the pharmacological management of schizophrenia. The more diverse binding
profile of clozapine across several central nervous system (CNS) receptors (e.g. serotonergic, dopaminergic, histaminergic, adrenergic and cholinergic) is thought to be responsible for these therapeutic advantages. Since then, there has been a rapid development of novel ‘atypical’ antipsychotic agents that have been pharmacologically modeled, to a certain extent, after their predecessor clozapine. Although both classes of antipsychotics, typical and atypical, offer some degree of efficacy, it is clear that they do not accommodate all symptoms of the disease. As with all drugs, there is variability among individuals in clinical responses to antipsychotics. The inter-individual variation in antipsychotic drug response creates a clinical dilemma most often addressed by empirical drug trials. Poor response to antipsychotic drug treatment and/or the development of adverse side-effects can lead to patient non-compliance, psychosocial disturbances and poor outcome. Unfortunately, efforts to identify biological or clinical predictors of patient response and adverse side-effect profile during antipsychotic drug treatment have been largely unsuccessful. Pharmacogenetics can provide a novel foundation for understanding this inter-individual variability in antipsychotic response, and may provide an avenue for predicting patient propensity to respond and to develop antipsychotic side-effects a priori.

A significant drawback to the treatment of schizophrenia with antipsychotics is the occurrence of adverse reactions. Typical antipsychotics can cause sexual dysfunction and induce movement side-effects, including extrapyramidal symptoms (EPS) and tardive dyskinesia (TD). Novel atypical antipsychotics offer a number of tolerability benefits over the traditional typical antipsychotics, principally regarding EPS. However, the differential binding profile between the two classes, both in terms of the variety of receptors that the antipsychotics have affinity for and in the range of affinities for each receptor, contribute to differences demonstrated with respect to the side-effect profile. Despite an increase in the popularity of atypical use in recent years, typical antipsychotics are commonly prescribed. Although atypical antipsychotics have a lower incidence of motor side-effects, their use is hindered by other adverse events such as weight gain and sedation. A particularly pressing issue has become the prevalent side-effect of weight gain. The significance of this side-effect is often underestimated because it is associated with a common and ‘normal’ presentation as compared with other antipsychotic side-effects. However, it is clear that weight gain can undermine compliance, inclining patients to relapse, and may also lead to significant psychological distress and medical morbidity and mortality.

Pharmacogenomics is defined as the compilation of comprehensive information about genomic sequences using techniques such as gene mapping, genome scans, sequencing, statistical genetics and expression analysis. This information is then applied to the identification of genomic ‘hot spots’ and subsequently to the discovery of susceptibility loci and novel drug targets contributing to inter-individual variation in drug response and side-effect profiles. Pharmacogenetics seeks to identify genetic polymorphisms in or near the coding region of genes that encode protein structures with which a drug interacts. The candidate gene variants that affect the function of the gene or its protein product have the highest priority for investigation. The identified genetic polymorphisms are then assessed for their putative role in the observed inter-individual variability in the clinical profile of the drug (e.g. its pattern of response and/or side-effects). This paradigm can be applied to predict the variable responsiveness to antipsychotics among individuals, thus minimizing the ‘trial-and-error’ approach that currently exists. The initial pharmacogenetic studies in schizophrenia have focused on determining the role of genetic variation in the antipsychotic efficacy of the atypical antipsychotic agent, clozapine—with an emphasis on genes that code for neurotransmitter receptors such as the serotonergic 5-HT2A and 5-HT2C receptors and the dopaminergic D2 and D4 receptors. The results from these studies have been equivocal—most likely secondary to issues surrounding the clinical phenotype of clozapine response and the limited power of individual studies to identify genes of modest effect. A remedy for this may be found in the definition of more quantitative and less subjective psychiatric phenotypes for use in pharmacogenetic association analyses. The combination of neuroimaging technologies such as positron emission tomography (PET) and magnetic resonance imaging (MRI) with molecular genetics can provide more specific and powerful phenotypes that may help to identify genetic predictors of antipsychotic response and side-effects. Here we provide a brief review of the most salient studies in the area of psychiatric pharmacogenetics of antipsychotic medications, with particular emphasis on clozapine response and medication-induced side-effects such as weight gain induced by atypical antipsychotics and tardive dyskinesia induced by typical antipsychotics.

CLOZAPINE RESPONSE AND NEUROPSYCHOLOGICAL MEASURES

Schizophrenia is a complex multifactorial disease with both genetic and environmental influences. Current nosology as defined by the DSM-IV (2) divides the symptoms of schizophrenia into two broad clusters: positive and negative symptoms. The positive symptoms involve an excess or distortion of certain normal functions while the negative symptoms are conversely distinguished by a diminution of other normal functions. The positive symptom cluster can be further subdivided into two dimensions: ‘psychotic’ and ‘disorganized’. The ‘psychotic dimension’ encompasses distortions or exaggerations of inferential thinking (delusions) and perception (hallucinations). The ‘disorganized dimension’ includes distortions in language and communication (disorganized speech) and behavioural monitoring (grossly disorganized or catatonic behavior). The negative symptom cluster is characterized primarily by problems in the reduction of range and intensity of emotional expression (affective flattening), fluency and productivity of thought and speech (alogia), and initiation of goal-directed behavior (avolition).

Antipsychotic drugs are the best means available for symptomatically treating individuals suffering from schizophrenia; however, there is significant variability in clinical response to these psychotropic medications. Take, for example, clozapine, the prototype atypical antipsychotic, where only 30–60% of individuals resistant to typical antipsychotics may demonstrate a beneficial clinical response with respect to positive and negative symptomatology (3).
Pharmacogenetic studies, in attempting to elucidate genetic predictors of global response (e.g., combined measures of positive and negative symptoms), have focused on the impact of genetic polymorphism in serotonin (5-hydroxytryptamine) system receptors such as 5-HT$_2A$, 5-HT$_2C$ and 5-HT$_6$ (4–10), as well as dopaminergic receptors such as D$_2$, D$_3$ and D$_4$ (11–13), as they relate to the clinical efficacy of clozapine. In summary, although there are conflicting results, two studies with sufficient statistical power demonstrate a role for the structural HIS452TYR 5-HT$_2A$ receptor gene polymorphism in predicting clozapine response (5,7). Table 1 provides a summary of some of the findings regarding the pharmacogenetics of clozapine response. More comprehensive reviews are available, and we direct the reader to these for a detailed discussion (14,15).

Several models have been proposed to explain the development of positive and negative symptoms in schizophrenia, and it is clear from the majority of them that deficits in cognition are involved (16). Neuropsychological research in schizophrenia has shown that there are profound deficits in cognitive processes such as verbal and working memory, attention, and executive function (17). Thus, understanding the nature of cognitive deficits in schizophrenia may help to elucidate the basic neural mechanisms underlying its overall clinical presentation.

There is a body of accumulating evidence suggesting that clozapine may ameliorate the underlying cognitive deficits of schizophrenia (reviewed in 18). As presented at the First Annual Pharmacogenetics in Psychiatry meeting in New York (19), our group has been involved in identifying genetic predictors of clinical variability in response to clozapine, particularly with respect to cognitive dysfunction. Several lines of converging evidence were presented and represent a novel approach to dissecting out individual genetic components contributing to inter-individual variability in cognitive response to clozapine. The mesocorticolimbic system represents an important anatomical and physiological pathway with respect to cognitive dysfunction in schizophrenia. Dopaminergic projections from the ventral tegmental area in the brainstem ascending to the limbic areas and to the dorsolateral prefrontal cortex (mesocorticolimbic paths) are disrupted (20,21). Specifically, the cognitive deficits observed in schizophrenia are related to reduced dopaminergic innervation of the dorsolateral prefrontal cortex, as suggested by neuroimaging and postmortem studies (22–24).

Dopamine D$_1$ receptors are located in high concentrations in the dorsolateral prefrontal cortex, and are thought to play an important role in modulating mesocorticolimbic circuitry and thereby cognitive functioning in schizophrenia. Furthermore, clozapine is a potent antagonist of dopamine D$_1$ receptors, and this is hypothesized to be important in its unique clinical response profile (25). As such, the dopamine D$_1$ receptor gene is a high-priority candidate gene to assess in predicting response to clozapine with respect to cognition in schizophrenia.

In a pilot study of 35 schizophrenia patients, who were involved in a randomized, prospective clinical trial of clozapine, we observed a significant association between an upstream D$_1$ receptor gene polymorphism and change in scores

<table>
<thead>
<tr>
<th>Gene Studied</th>
<th>Authors/Year (REF.)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D$_2$ receptor (DRD2)</td>
<td>Arranz et al. (1998) (94)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Dopamine D$_3$ receptor (DRD3)</td>
<td>Shaikh et al. (1995) (95)</td>
<td>No significant association</td>
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<tr>
<td></td>
<td>Malhotra et al. (1998) (11)</td>
<td>No significant association</td>
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<tr>
<td></td>
<td>Scharfetter et al. (1999) (96)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td>Dopamine D$_4$ receptor (DRD4)</td>
<td>Rao et al. (1994) (97)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Shaikh et al. (1995) (13)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Rietschel et al. (1996) (98)</td>
<td>No significant association</td>
</tr>
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<td></td>
<td>Kohn et al. (1997) (99)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Ozbek et al. (1997) (12)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Kaiser et al. (2000) (100)</td>
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</tr>
<tr>
<td>Serotonin 2A receptor, 5-HT$_2A$ (HTR2A)</td>
<td>Arranz et al. (1995) (4)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Masellis et al. (1995) (6)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Nothen et al. (1995) (101)</td>
<td>No significant association</td>
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<tr>
<td></td>
<td>Arranz et al. (1996) (102)</td>
<td>Non-significant trend</td>
</tr>
<tr>
<td></td>
<td>Malhotra et al. (1996) (9)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Arranz et al. (1998) (5)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Masellis et al. (1998) (7)</td>
<td>No significant association for one polymorphism; borderline significant association for another</td>
</tr>
<tr>
<td>Serotonin 2C receptor 5-HT$_2C$ (HTR2C)</td>
<td>Lin et al. (1999) (103)</td>
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</tr>
<tr>
<td></td>
<td>Sodhi et al. (1995) (104)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Malhotra et al. (1996) (10)</td>
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<td>Rietschel et al. (1997) (105)</td>
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<td>No significant association</td>
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<td></td>
<td>Yu et al. (1999) (106)</td>
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<td>Masellis et al. (2001) (8)</td>
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<td>Serotonin 7 receptor, 5-HT$_7$ (HTR7)</td>
<td>Masellis et al. (2001) (8)</td>
<td>No significant association</td>
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<tr>
<td>Serotonin transporter, 5-HTT</td>
<td>Tsai et al. (2000) (107)</td>
<td>No significant association</td>
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on the Wisconsin Card Sort Test, which measures working memory, attention and executive function, assessed before and after treatment with clozapine \((F[2,34]= 7.929, \ P = 0.002)\) (19). Interestingly, we have also found evidence suggesting that this upstream dopamine \(D_1\) receptor polymorphism is associated with modulation of dorsolateral prefrontal cortex metabolic activity, as assessed by \([^{18}\text{F}]\text{fluoro-2-deoxy-D-glucose (FDG)}\) PET after clozapine treatment, and that this is predictive of measures of clinical response \((P < 0.1)\) (Fig. 1) (26).

**CLOZAPINE-INDUCED WEIGHT GAIN**

Clozapine, the prototype atypical antipsychotic, remains the most effective agent for the treatment of refractory schizophrenia and over recent years has gained much popularity as a first-line treatment; however, among the atypicals, clozapine appears to have the greatest weight gain liability (27). Some patients may gain as much as 50 kg over a 1-year treatment period. Reviewing the literature, Leadbetter et al. (28) found that 13–85% of patients treated with clozapine had an associated increase in weight. Umbricht et al. (29) found that the cumulative incidence of all patients reaching \(\geq 20\%\) overweight, representing a significant long-term health risk, was \(\geq 50\%\). This side-effect can undermine compliance, leading to relapse, and may also cause significant psychological and medical morbidity. Considerable weight gain may also lead to increases in obesity-related comorbidities and health risks such as type II diabetes mellitus, hypertension, cardiovascular disease, respiratory dysfunction and some types of cancer, which are all associated with significant mortality (30). There appears to be considerable variability among individuals with respect to the ability of an antipsychotic to induce weight gain, i.e. not all patients treated with clozapine gain weight. Thus, the side-effect of weight gain occurs in only a proportion of treated patients who are predisposed to this side-effect. It is likely that this variability in patient propensity to gain weight is determined by a combination of genetic and environmental factors. The genetic factors may include pharmacokinetic (i.e. factors involved in the metabolism and elimination of the drug from the body) as well as pharmacodynamic (i.e. factors at the direct site of action of the drug within the body) elements. Genetic variation in pharmacodynamic factors such as brain receptors may subject some patients to have receptors with higher affinity for the medication and may allow prediction of those patients who are most likely to respond or develop side-effects. Genetic differences in pharmacokinetic factors such as drug-metabolizing enzymes may subject some patients to less active enzymatic forms resulting in higher plasma levels of the medication, and this may also allow prediction of good response and propensity to side-effects. A genetic predisposition to clozapine-induced weight gain has been suggested (29,31), and ample evidence exists demonstrating that body weight and feeding behavior are influenced by genetic factors (32,33).

Weight gain induced by atypical antipsychotics is likely to be due to a combination of disturbances and alterations in satiety control mechanisms, energy expenditure, metabolism and lipogenesis, although there is a limited amount of research seeking to uncover the precise mechanisms. Figure 2 provides a schematic representation of both the central hypothalamic weight regulation and peripheral thermogenic pathways, highlighting putative areas where clozapine may disrupt these pathways to cause weight gain in predisposed patients (34). Collectively, data from several research paradigms converge and suggest that weight gain induced by atypical antipsychotics and obesity result from multiple neurotransmitter/receptor interactions, with resultant changes in appetite and feeding behavior. Patients treated with clozapine generally complain that they have an inability to control their appetite even after eating a full meal. Satiety signals arise in a variety of areas, including the olfactory and gustatory tracts, esophagus, stomach, liver, and intestines, and are processed in the hypothalamus, which contributes to the regulation and maintenance of an individual's homeostatic body weight. Therefore, it is possible that some antipsychotics may disturb satiety processing in the hypothalamus by binding to receptors involved in weight and satiety regulation. As such, genetic differences in these candidate receptors that have affinity for clozapine and are expressed in the hypothalamus are prime candidates for investigation when trying to uncover genetic determinants of clozapine-induced weight gain.

A large body of evidence supports a role for the serotonin system in regulating feeding behavior (reviewed in 32). Studies in both animals and humans have shown that increasing serotonin results in decreased feeding, and decreasing serotonin increases feeding (35–37). Interestingly, it has been shown that agonists of the 5-HT\(_1\) family of receptors caused hyperphagia, and conversely agonists of the 5-HT\(_2\) family of receptors caused hypophagia (reviewed in 38). More specifically, rat studies have shown that 5-HT\(_{1A}\) agonists as well as 5-HT\(_{2C}\) antagonists cause a marked increase in feeding (39). It is interesting to note that clozapine is a potent 5-HT\(_{2C}\) antagonist and 5-HT\(_{1A}\) agonist. Autoradiographic studies have shown that both 5-HT\(_{2C}\) and 5-HT\(_{1A}\) receptors are localized with high density in the medial hypothalamus, within this established satiety control center (40,41). Perhaps the most compelling evidence supporting a role for the 5-HT\(_{2C}\) receptor in feeding behavior is from a study of knockout mice lacking 5-HT\(_{2C}\) receptors (42). The knockouts were overweight compared with wild-type mice, and, based on paired feeding analysis, this appeared to be due to increased feeding as opposed to metabolic changes in these animals. For a more comprehensive characterization of the factors to be considered in clozapine-induced weight gain, see Figure 2 and a more detailed description of this figure in our recent review (34). In addition to clozapine, several other atypical antipsychotics induce weight gain. The common factors among these antipsychotics are that they are all 5-HT\(_{2C}\) antagonists and 5-HT\(_{1A}\) agonists, as well as histamine H\(_1\) receptor antagonists (reviewed in 43). These receptors are therefore again prime candidates for investigation when trying to elucidate genetic determinants of clozapine-induced weight gain.

The peptide that exerts the most significant effects on feeding and weight regulation is leptin. Leptin is secreted by adipocytes in direct proportion to the amount of fat stored within that cell. It is believed to act at the level of the hypothalamus, where it initiates a cascade of events that lead to the regulation of appetite, energy expenditure and satiation. In a recent study by
Morimoto et al. (44), central injection of leptin into mouse brain caused a marked decrease in feeding in wild-type control mice, but not in genetically altered mice lacking the histamine H1 receptor, suggesting that leptin may operate directly through a histamine H1 receptor-mediated pathway. It is well established that histamine H1 receptor antagonism causes increased feeding and weight gain (43,45,46). Autoradiographic studies have shown that histamine H1 receptors are localized with high density in the ventromedial nucleus and the paraventricular nucleus in the hypothalamus (47).

Wirshing et al. (48) noted an exponential relationship between the maximum amount of weight gained while being treated with an antipsychotic and that particular antipsychotic’s affinity for the histamine H1 receptor. Those antipsychotics with the maximum weight-gain liabilities (i.e., clozapine and olanzapine) had the greatest affinities for the histamine H1 receptor. Atypical antipsychotics such as clozapine bind to histamine H1 receptors as well as to 5-HT2C and 5-HT1A receptors, and the subsequent change in the action of these receptors within the hypothalamus may disrupt satiety control mechanisms, in turn resulting in weight gain. Genetic differences in these central brain receptors may predict patient propensity to gain weight while being treated with clozapine.

It is important to note that clozapine not only may disrupt central hypothalamic weight regulation and satiety control mechanisms, but also may interfere with peripheral thermogenic, lipogenic, lipolytic and adipocyte-regulating pathways to cause weight gain. Agonism at peripheral β3 and α1 adrenergic receptors located on white and brown adipocytes, as well as on muscle cells, is known to stimulate intracellular lipolysis and to increase basal metabolic rate by increasing the expression of the mitochondrial uncoupling proteins (UCP1, 2 and 3). In a highly uncoupled state, fuels are oxidized unrelated to the performance of work and the usable potential energy is lost as heat (Fig. 2) (49). Transgenic mice that overexpress UCP3 are lean when compared with wild-type control mice, despite being...
hyperphagic (50). Consequently, individuals with higher levels of UCPs are not as efficient at generating ATP, and as such must consume more energy substrates (e.g. glucose and fat) than would an individual with lower levels of UCPs. As a result, these differences contribute to inter-individual variations in body weight. Clozapine antagonism of peripheral $\beta_3$ and $\alpha_1$ adrenergic receptors may inhibit these mechanisms, making patients more efficient with their energy intake, which can cause significant weight gain in those who are predisposed.

We tested hypotheses for 10 genetic polymorphisms across nine candidate genes from both central hypothalamic weight regulation and peripheral thermogenic pathways in a recent paper (34) that provides a comprehensive review of the current obesity and weight regulation research, while also speculating on how atypical antipsychotics may disrupt these pathways to cause weight gain in those patients who are predisposed. The candidate genes investigated were the 5-HT2C, 5-HT2A and 5-HT1A receptor genes (HTR2C, HTR2A and HTR1A) the histamine H1 and H2 receptor genes (H1R and H2R) the cytochrome P450 1A2 gene (CYP1A2), the $\beta_3$ and $\alpha_1$a adrenergic receptor genes (ADRB3 and ADRA1A), and the tumor necrosis factor $\alpha$ gene (TNF-$\alpha$). We obtained prospective weight gain data for 80 patients with schizophrenia who had completed a structured clozapine trial. During clozapine treatment, weight in kilograms was assessed at baseline and 6 weeks. ANCOVA analyses correcting for covariates (sex,
ethnicity, baseline weight and response status) were utilized to
detect differences among genotypes at candidate gene loci for
mean change in weight while on clozapine. Non-significant
trends were observed for ADRB3 ($F = 2.29$, $P = 0.10$),
ADRA1A ($F = 1.58$, $P = 0.22$), TNF-α ($F = 1.94$, $P = 0.15$)
and HTR2C ($F = 0.63$, $P = 0.54$); however, replication in
larger, independent samples is required (34). Recently,
Reynolds et al. (51) demonstrated an association between
antipsychotic-drug-induced weight gain and a novel promotor
region polymorphism (C759T) that may alter 5-HT2C gene
expression (52). This finding was not replicated in our
prospective sample of clozapine-treated patients (53). Further
investigation is required to assess whether this polymorphism
may have a predictive role for clozapine induced weight gain
that is clinically relevant.

TYPICAL ANTIPSYCHOTIC-INDUCED
TARDIVE DYSKINESIA

Tardive dyskinesia is an important side-effect of typical
antipsychotics and has been the focus of a number of
pharmacogenetic studies. TD is an involuntary movement
disorder of the orofacial musculature and may involve the trunk
and extremities as well. It occurs in 20–30% of patients either
after chronic administration of antipsychotics or after with-
drawal of these agents and is potentially irreversible once it
arises. The reasons for the development of TD in some patients
and not in others are unknown, but there is suggestive evidence
that susceptibility has a genetic component. Animal models
and family studies indicate that genetic factors importantly
influence the risk for TD following treatment (53–56).
Although the precise mechanism(s) of TD is not well
understood, overactivity of the dopaminergic neurotransmission
in the basal ganglia and upregulation of the dopamine D2-like
receptors (D2, D3 and D4) have been postulated to play a role in
its pathophysiology (57). Consequently, genetic association
studies on the pharmacodynamic component of the TD
phenotype have primarily focused on the dopamine system
genes. Several risk factors for TD have been identified, such as
age, female gender, African-American ethnicity, duration of
antipsychotic exposure and organic brain abnormalities (58–
61). These factors predict only a minor portion of the variance
in the TD phenotype, and therefore a prominent pharmacoge-
netic component may contribute to this variance and help in
predicting those patients who will develop TD. The phenotype
of TD has the advantage of being objectively visible and is
relatively amenable to scaled scoring in terms of the degree of
severity. In contrast to genetic studies of clozapine response,
the genetic studies of TD have yielded quite promising and
replicable results. Pharmacogenetic studies of TD have
investigated both pharmacodynamic and pharmacokinetic
aspects of typical antipsychotics. The most interesting and
consistent findings regarding candidate gene studies of TD
have focused on the dopamine D3 receptor gene (DRD3).

DRD3 mRNA and protein have been localized to the ventral
striatum and the ventral putamen in the basal ganglia, a brain
region implicated in motor control (62). Pharmacological
studies provide evidence that the D3 receptor exerts an
inhibitory effect on locomotor activity. Kling-Petersen et al.
(63) found that 7-OH-DPAT, a D3-selective agonist, inhibits
locomotion when injected into the nucleus accumbens of rat
brain (63). Conversely, D3 antagonists increase locomotor
activity (63). Consistent with this, DRD3 knockout mice
exhibit hyperactivity (64). A postmortem study in patients with
schizophrenia who were previously treated with typical
antipsychotics illustrated a 45–56% increase in the number of
D3 receptors in the basal ganglia as compared with controls
(65). These data collectively provide evidence that the D3
receptor may play a role in motor control. The DRD3 gene
exhibits a single-nucleotide polymorphism (SNP) that results in
a serine-to-glycine amino acid substitution (Ser9Gly) in the
N-terminal extracellular domain of the D3 receptor (66).
A functional study of this Ser9Gly polymorphism in CHO
cells revealed allelic differences in affinity for dopamine (67).
Specifically, a significantly higher affinity for dopamine was
shown for cells homozygous for glycine than heterozygotes
and serine/homozygous (67). It is likely that the
substitution of a polar serine residue by a non-polar glycine
residue might have altered the tertiary structure of the receptor,
thus affecting its binding affinity for dopamine.

Several groups, including our own, have independently
shown that the Ser9Gly DRD3 gene polymorphism is
associated with risk for TD (68–74). Each group found that
either the glycine/glycine genotype or the glycine allele
conferred elevated risk for TD. However, this result was not
replicated in studies from Inada et al. (75), Rietschel et al. (76)
and Garcia-Barcelo et al. (77). The discrepancies among these
studies may be due to study population differences and
differences in study designs. This finding is promising in that
it has been replicated in several independent studies from
samples of various ethnic origins. A recent collaborative
effort by numerous groups (nine centers), including our own,
confirmed the initial finding of association between the
Ser9Gly DRD3 receptor gene polymorphism and TD (78).
Another interesting pharmacodynamic candidate is the 5-HT2A
receptor gene (HTA2A), which was found to be associated with
TD in two independent studies (79,80), although we could not
replicate this finding in a relatively large prospective study (81).
Table 2 provides a summary of the various candidate gene
association studies that have been undertaken for the phenotype
of TD.

In light of the replicating DRD3 genotype finding for TD, we
have used FDG–PET and MRI in a subset of our patients to
determine if there are any differences in regional brain activity/
metabolism following haloperidol treatment in patients who are
glycine/glycine homozygous when compared with other
patients. We found that following haloperidol treatment,
patients who were glycine/glycine homozygotes had increased
FDG metabolism within the caudate nucleus and the putamen
when compared with serine/homozygotes and serine/ glycine
heterozygotes (82). These brain regions are known to
mediate the control of movement. Interestingly, those patients
that exhibited the increased brain activity in these regions also
had the most severe TD (Fig. 3).

Under the assumption of the involvement of multiple genes
in the phenotype of TD, our group then examined the role of
pharmacokinetic aspects by investigating the cytochrome P450
genomes CYP2D6 and CYP1A2, both of which are known to be
involved in the metabolism of typical antipsychotics.
Differences in the metabolism and elimination of the anti-psychotic could lead to increased risk for TD in those who are genetically poor metabolizers. The CYP2D6 gene variation investigated did not predict risk for TD; however, the CYP1A2 polymorphism did show a significant association. Patients who were C-allele homozygotes for CYP1A2, which correlates with a less easily inducible form of the enzyme (83), had much more severe TD as measured by mean AIMS scale score (84). However, a recent study in a German sample could not replicate this finding (85).

In view of these two genetic associations with DRD3 and CYP1A2, each contributing to risk for TD, a gene–gene interaction analysis was conducted by our group using methods modified from those of Frankel and Schork (86). First, the various models of gene–gene interaction have been delineated in matrices representing combinations of dominant, recessive, additive, heterogeneity and epistatic effects. The DRD3 and CYP1A2 genetic data were then fitted to each of these models. Thus far in the analyses, the best fit is an additive, co-recessive glycine/C-allele model of DRD3 and CYP1A2 interaction (F = 17.36, P = 0.00007). It was found that those patients who exhibited the risk genotype at both DRD3 (glycine/glycine) and at CYP1A2 (C/C) had the most severe TD, whereas those who had only one risk genotype (glycine/glycine or CC) demonstrated intermediate severity of TD. Those patients who did not have any of the risk genotypes at either locus demonstrated the lowest mean TD severity AIMS scale scores (Fig. 4). The overall model now accounts for >50% of the variance in risk for TD in our sample. This promising investigation of gene–gene interaction is thus paving the way, starting with two genes, toward a multigene, demographic and environmental variable model that may account for most of the variance in risk TD. The application of artificial neural network modelling techniques may help decipher the complex interactions among genetic, demographic and environmental variables, to elicit a predictive model or diagnostic kit for TD. If replicated, this model would have significant clinical value in the day-to-day practise of psychiatrists who treat chronically psychotic patients, and may help to predict those patients who are most and least likely to develop TD following treatment with typical antipsychotics.

**CONCLUSIONS AND FUTURE DIRECTIONS**

Based on our review of the pharmacogenetics of antipsychotic-drug-induced side-effects and response, several recommendations can be made. It is apparent that a great deal of

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**Table 2. Association studies of tardive dyskinesia**

<table>
<thead>
<tr>
<th>Gene Studied</th>
<th>Authors/Year (Ref.)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine D2 receptor (DRD2)</td>
<td>Badri et al. (1996) (68)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Chen et al. (1997) (108)</td>
<td>Borderline significant association</td>
</tr>
<tr>
<td></td>
<td>Inada et al. (1997) (75)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Hori et al. (2001) (109)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Dopamine D3 receptor (DRD3)</td>
<td>Badri et al. (1996) (68)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Steen et al. (1997) (69)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Inada et al. (1997) (75)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Basile et al. (1998) (70)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Segman et al. (1999) (71)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Rietschel et al. (2000) (76)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Lovlie et al. (2000) (73)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Liao et al. (2001) (72)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Garcia-Barcelo et al. (2001) (77)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Woo et al. (2002) (74)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Lerer et al. (2002) (78)</td>
<td>Comprehensive analysis of most of the above-mentioned data sets found a statistically significant association</td>
</tr>
<tr>
<td>Serotonin 2A receptor, 5-HT2A (HTR2A)</td>
<td>Segman et al. (2001) (79)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Tan et al. (2001) (80)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Basile et al. (2001) (81)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Serotonin 2C receptor, 5-HT2C (HTR2C)</td>
<td>Segman et al. (2000) (110)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Basile et al. (unpublished data)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Cytochrome P450 2D6 (CYP2D6)</td>
<td>Arthur et al. (1995) (111)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Badri et al. (1996) (68)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Armstrong et al. (1997) (112)</td>
<td>Non-significant trend</td>
</tr>
<tr>
<td></td>
<td>Andreassen et al. (1997) (113)</td>
<td>No-significant trend</td>
</tr>
<tr>
<td></td>
<td>Kapitany et al. (1998) (114)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Ohmori et al. (1998) (115)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Ohmori et al. (1999) (116)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Cytochrome P450 1A2 (CYP1A2)</td>
<td>Basile et al. (2000) (84)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Schulze et al. (2001) (85)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Manganese superoxide dismutase, MnSOD</td>
<td>Hori et al. (2000) (117)</td>
<td>Statistically significant association</td>
</tr>
<tr>
<td></td>
<td>Zhang et al. (2002) (118)</td>
<td>No significant association</td>
</tr>
<tr>
<td></td>
<td>Basile et al. (unpublished data)</td>
<td>No significant association</td>
</tr>
<tr>
<td>Serotonin 6 receptor, (5-HT6)</td>
<td>Ohmori et al. (2002) (119)</td>
<td>No significant association</td>
</tr>
</tbody>
</table>
methodological heterogeneity exists among these pharmacogenetic studies. This is expected, given the relatively early stage of the field. However, in order for the field to progress, methodological consistency must be achieved not only to form the basis of comparison among studies, but more importantly to allow for collaborative efforts that combine samples. Efforts to combine studies have been very useful, as evidenced by the recent multicenter collaborative study of DRD3 association with TD (78). Sample-size limitations that decrease the power of analysis can be overcome through these collaborative efforts. Methodological considerations include the establishment of discrete inclusion/exclusion criteria regarding patient diagnosis and sample characterization; consensus regarding the use of particular psychiatric rating instruments should be established a priori (87). Furthermore, studies should be designed specifically for the identification of genetic susceptibility to pharmacogenetic traits. To date, studies have used samples obtained from prospective or retrospective clinical trials, with testing of genetic hypotheses in an opportunistic fashion after the trial is completed.

Another critical issue is the definition of the pharmacogenetic phenotype. Regarding response to antipsychotics, groups working in this field need to agree upon a standard definition of response that would include multiple criteria of assessment. Furthermore, categorical non-parametric approaches are limited in power and should be supplemented by parametric continuous measure designs. For clozapine response, this would entail using the mean change in clinical rating over time (controlling for baseline) in an analysis of variance (ANOVA) with genotype as the grouping variable. Pharmacogenetic studies of TD have begun to move forward in this direction. Further refinement of the TD phenotype has been attempted through the use of an endophenotype that quantifies the degree of involuntary movements by measuring facial muscle activity using standardized electromyographic techniques (88). Techniques such as these should reduce phenotypic heterogeneity, adding to the power of the analysis (89), in addition to removing the problems associated with inter-rater reliability in the primarily subjective assessment of patient symptoms in psychiatry. Another method of refining the pharmacogenetic phenotype of interest is to use multiple rating instruments (e.g. GAS, BPRS and PANSS for clozapine response; AIMS, SARS and Simpson Dyskinesia scale for TD), and conducting factor analysis in order to extract items from each scale that tend to cluster together (90). The use of more narrowly defined phenotypes such as these increases the power of genetic studies.

Neuroimaging may provide an avenue for measuring more narrowly defined endophenotypes, thus limiting some of the heterogeneity inherent in psychiatric genetic studies. The use of structural brain imaging as an endophenotype for genetic studies is beginning to gain popularity, and an association

Figure 3. Brain [18F]fluoro-2-deoxy-D-glucose (FDG) metabolism PET following haloperidol treatment versus dopamine D3 receptor genotype (N = 14). The top row presents scans from patients who were either serine/serine homozygotes or serine/glycine heterozygotes at DRD3 (N = 9). Within this row and from left to right, we have the mean FDG–PET following 5 weeks of haloperidol treatment, the mean baseline FDG metabolism and the subtraction of the previous two entities overlayed on MRI to indicate the change in FDG metabolism due to treatment. The bottom row depicts the results of the same methodologies in patients who were glycine/glycine homozygotes (N = 5). Note that the subtraction image in these glycine/glycine homozygote patients demonstrates significantly increased metabolic activity in striatal brain regions. Modified from Potkin et al. (82). Reprinted with the permission of Cambridge University Press.
between parietal lobe volume and brain-derived neurotrophic factor (BDNF) alleles has been reported (91). Laruelle et al. (92) failed to find an association between dopamine D2 receptor binding and alleles at the Taq1-A restriction fragment length polymorphism (RFLP) site in the DRD2 gene using single-photon emission computed tomography (SPECT) imaging. A recent and seminal study by Hariri et al. (93) used functional MRI (fMRI) to assess neuronal activity in subjects genotyped for the serotonin transporter promotor polymorphism. They found that individuals with one or two copies of the short allele exhibit greater amygdala neuronal activity in response to fearful stimuli when compared with individuals homozygous for the long allele.

Despite their small sample sizes, these preliminary studies provide intriguing data regarding the ability of brain imaging to serve as an endophenotype in pharmacogenetic investigations. These studies, however, did not examine the relationship between allelic variation, clinical outcomes to pharmacological treatment (response and side-effects), and the intermediate phenotypes as revealed by brain imaging. One advantage of PET imaging is that specific ligands can be radiolabelled in order to measure specific pharmacological targets in the brain. For example, a radioligand specific for the serotonin transporter has been developed and utilized in studies of patients’ response to selective serotonin reuptake inhibitor (SSRI) medications.

The ideal candidate gene to study for this combined neuroimaging plus pharmacogenetic phenotype is the serotonin transporter gene. FDG–PET has the ability to measure regional brain metabolic response while the subject is performing an activation task. PET also has the ability to resolve individual gyri and to distinguish subcortical regions from each other. PET can measure ‘brainwork’ because of the close coupling between glucose utilization and neuronal activity. Unlike fMRI and SPECT, FDG–PET allows for absolute quantification of metabolic activity. However, fMRI provides a relatively safe and repeatable method of generating brain maps. Each of the imaging techniques may provide differential benefits in defining pharmacogenetic endophenotypes. It may be possible to monitor specific gene expression using neuroimaging, thus quantifying in vivo changes as a result of pharmacological challenges. Caveats exist in that neuroimaging endophenotypes may not correlate directly with the behavioral manifestations of pharmacogenetic and neuropsychiatric phenotypes. However, clear strengths exist in using this technology to limit phenotypic heterogeneity and increase the power to detect genetic contributors to pharmacogenetic phenotypes.

Through predictability testing, pharmacogenetic testing kits could break the ‘trial-and-error’ approach to prescribing antipsychotics. Although in its infancy, pharmacogenetics may in future lead to individualized pharmacotherapy based
on the specific genetic, environmental and demographic characteristics of each patient. The pharmacogenetic goal of providing treatment based on these client-centered characteristics in order to maximize efficacy and minimize the risk of adverse events—getting the right medicine in the right dose to the right patient—will inevitably become common in the not-so-distant future. This results in increased patient comfort in terms of both higher initial response rates and reduced propensity to developing debilitating side-effects.

REFERENCES


