CHAPTER

7

Pharmacogenomics in Psychiatric Disorders

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OBJECTIVES

1. Discuss the utility of *CYP* genotyping in psychopharmacology
2. List and define the rationale of different drug targets for psychopharmacogenomic investigations
3. Discuss alternative approaches to pharmacogenomic studies in psychopharmacology
4. Describe how pharmacogenomics may play a role in minimizing adverse effects of antipsychotics

INTRODUCTION

Evaluations and prediction of treatment response and potentials of adverse drug reactions in psychiatric patients have in the past been partially limited by the patients' subjective reports and the subjective elements in clinicians' assessments. Despite the availability of different clinical rating scales, there remains no reliable biological marker of response. Since the completion of the Human Genome Project in 2003 and in an effort to improve outcome, the implications of pharmacogenomics in psychiatry have been increasingly evaluated. Many candidate genes have been identified, with the hope that they can be utilized to improve patient outcome. However, so far, applications of pharmacogenomics have been primarily more successful in predicting adverse drug reactions than treatment response. This chapter will review the pharmacogenetic findings, discuss the evidence and challenges of genotyping biomarkers in psychopharmacotherapeutics, and address the future potentials of applying pharmacogenomics in psychopharmacology. Because a comprehensive review of all research in psychiatric pharmacogenomics is beyond the scope of this chapter, affective disorder and schizophrenia will be the focus to highlight the principles and issues in this emerging field.

POLYMORPHISMS IN PROTEINS THAT AFFECT DRUG CONCENTRATIONS

Genes Encoding Drug-Metabolizing Enzymes

Antidepressants

Several polymorphic cytochrome P450 isoenzymes, notably CYP2D6 and CYP2C19, are involved in metabolism and elimination of tricyclic antidepressants (TCAs) and the selective serotonin reuptake inhibitors (SSRIs) (Table 7.1) [1,2]. The lack of therapeutic response even with standard-dosage regimens of the TCA nortriptyline provided one of the earliest clinical examples of how altered expression of *CYP2D6* can impact drug response in patients who have multiple copies of the *CYP2D6**2 allele. The original clinical observation [3] was followed up with additional studies that elucidated the molecular basis [4] and the gene–dose relationship in nortriptyline pharmacokinetics [5] in patients with the ultrarapid metabolizer (UM) phenotype for CYP2D6. In their report of antidepressant dose recommendations based on pharmacokinetics and pharmacogenetics relationships, Kirchheiner et al. [6] suggested increased dose requirement in UMs receiving nortriptyline (up to 230% of

TABLE 7.1 Major CYP Isoenzymes and Transporters Responsible for Metabolism and Efflux of Selected Psychotropics

	Antidepressants	Antipsychotics
CYP1A2		Olanzapine Clozapine
CYP2C9	Not a major isoenzyme, but provides the only secondary pathway for fluoxetine	
CYP2C19	Amitriptyline, citalopram, clomipramine, doxepine, escitalopram, imipramine, nortriptyline, sertraline	Clozapine
CYP2D6	Amitriptyline, desipramine, doxepin, duloxetine, fluoxetine, imipramine, mirtazapine, nortriptyline, olanzapine, paroxetine, trazodone, venlafaxine	Aripiprazole, chlorpromazine, clozapine, haloperidol, iloperidone, olanzapine, perphenazine, pimozide, risperidone, thioridazine
ABCB1	Amitriptyline, nortriptyline, paroxetine, venlafaxine	Risperidone

the usual dose), desipramine (up to 260%), and mianserin (up to 300%). The number of literature reports of lower efficacy in UMs is significantly less for the SSRIs, which is expected given their flatter dose–response curve. Kawanishi et al. [7] showed in a small pilot study a preponderance of UMs (10%) in 81 nonresponders who received at least 4 weeks of standard recommended-dosage regimens of TCAs and SSRIs that are CYP2D6 substrates. In addition to potential impact of metabolic polymorphism on efficacy, Penas–Lideo et al. also suggested an association between discontinuance of amitriptyline and fluoxetine with the CYP2D6 phenotype. In their study of 100 patients with major depressive disorder, all four UMs discontinued drug treatment within the first 4 weeks, whereas no PMs did so after 12 weeks of therapy [8].

The clinical use of SSRIs has expanded over the years to include other psychiatric conditions such as obsessive–compulsive disorders and generalized anxiety disorder. Although *CYP2D6* polymorphism has been shown to influence the plasma fluoxetine-to-norfluoxetine concentration ratio [9,10], the correlation with clinical response has been less robust in patients with major depressive, panic, and anxiety disorders. This may be due to the additional contribution from the polymorphic *ABCB1*, which influences the extent of SSRI entry via the blood–brain barrier (discussed in a later section) [9], as well as that from polymorphisms in the genes encoding the serotonin transporter [11,12] and the serotonin 2A receptor [13] (both are discussed in a later section).

Both citalopram and escitalopram are metabolized significantly by the polymorphic CYP2C19. As expected, a recent study in 2,087 patients showed that the *CYP2C19* genotypes significantly affect escitalopram exposure. Compared to patients with *CYP2C19 *1/*1* genotype, the serum drug concentrations in homozygous and heterozygous carriers of the null allele (defined as *2, *3, *4) were significantly increased by 3.3-fold and 1.4–1.6-fold, respectively. Homozygous and heterozygous carriers of the *CYP2C19*17* allele showed 20% and 10% decreases in concentrations, respectively. This is, by far, one of the largest studies to document the relationship between metabolic genotypes and systemic drug exposure. In addition, therapeutic failure (defined as switching from escitalopram to another antidepressant within 1 year after serum drug-concentration monitoring) were 3.3, 1.6, and 3.0 times more frequent among patients with *CYP2C19 null/null* genotype, patients with *CYP2C19 *1/*17* genotype, and patients with *CYP2C19 *17/*17* genotype, respectively. Switching, presumably due to either insufficient pharmacologic response or occurrence of adverse drug reactions, occurred

in a larger proportion of patients with extreme genotypes (*CYP2C19 null/null* and *CYP2C19 *17/*17*) [14]. Therapeutic failure to citalopram has also been described in a case report [15].

Mrazek et al. studied a cohort of 1,074 citalopram-treated patients and reported CYP2C19 PMs tolerated citalopram less so than other patients [16]. On the other hand, Serretti et al. reported that the CYP metabolic genotypes have no correlation with either response to antidepressants or remission of depression, although most of the 197 nonresponders received antidepressants that depend on multiple CYP enzymes for metabolism [17]. Another report also reported a lack of association between *CYP2C19* genotype or citalopram concentration and treatment response in 223 citalopram-treated patients [18]. These negative association data concur with reports by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group [19], and results of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial [20].

Despite evidence that *CYP2D6* and *CYP2C19* polymorphisms are correlated with the pharmacokinetics of several antidepressants, the effect of genetics on CYP2D6 and CYP2C19 enzyme activity, or the pharmacokinetics of their respective substrates, can be further modulated by other variables such as drug dose [21,22], treatment duration [23,24], patient-specific factors such as presence of concurrent CYP2D6 or CYP2C19 inhibitors (the phenoconversion phenomenon) [25], smoking status [26,27], diet, medication adherence, and ethnicity. The effect of *CYP2D6* variants (**5* and **10*) on fluvoxamine and paroxetine pharmacokinetics are shown only in patients treated with the lower doses of 50 and 10 mg/day, respectively. This is most likely a result of CYP2D6 being a low-capacity enzyme, with saturation of its metabolic capacity occurring with higher-dosage regimens: 100–200 mg/day of fluvoxamine and 20–40 mg/day of paroxetine, respectively [21,22], thus effectively diminishing the impact of the genetic polymorphism at higher dosages.

Difference in treatment-response phenotypes was reported to be evident during the second to fourth week, but not during the eighth week, of antidepressant therapy [23]. A similar finding of better early-treatment response to escitalopram in CYP2C19 PM was also reported in a prospective, open-label observational study of Chinese patients with panic disorder [24]. Because fluvoxamine is a CYP1A2 substrate, the effect of the *CYP2D6* genotype on fluvoxamine pharmacokinetics is further modulated by smoking, which together accounted for 23% of the variance in fluvoxamine concentrations for patients treated with the low-dose regimen of 50 mg/day [26]. Tsai et al. reported polymorphisms in *CYP2D6* and *CYP2C19* impact on the therapeutic outcome and serum concentrations of S-citalopram [28]. Because multiple *CYP2D6* and *CYP2C19* alleles occur at variable frequencies among different ethnic groups, and the patient population in the STAR*D trial [20] included primarily Caucasians (78.1%) and African Americans (16.1%), the conflicting study results [20,28] underscore the importance of heterogeneity of not only study design, but as importantly, study populations and the need of defining ethnicity in pharmacogenomic research. This issue of ethnicity is further discussed in relevant sections of different chapters throughout the book.

Although the use of the TCAs has declined over the years, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has released guideline recommendations for these drugs in patients with different *CYP2C19* and *CYP2D6* genotypes [1]. Even though dosage adjustment recommendations appear in some package inserts, no clear guidance exists on dosing from the Food and Drug Administration. In addition, little evidence exists that prior dosing recommendations [29,30] for CYP-dependent psychotropic medications are widely implemented. This finding is consistent with a report from the Dutch Pharmacogenetics Working Group [30].

In summary, current evidence suggests that the utility of CYP-based pharmacogenetic testing for antidepressants lies more in

anticipating adverse drug effects than in predicting their therapeutic efficacy. In a study of 1,198 elderly patients treated with antidepressants, PMs of CYP2D6 were five times more likely to show significant adverse effects with the use of CYP2D6-dependent TCAs [31]. Rau et al. also reported a predominance of PMs in 28 patients who were treated with CYP2D6-dependent TCAs and SSRIs [32], and patients with the intermediate metabolizer (IM) phenotype for CYP2D6 were found unable to tolerate venlafaxine doses larger than 75 mg [33]. These earlier study results were replicated in a more recent study conducted in an acute psychiatric unit, in which longer hospitalization associated with greater side effects were reported in CYP2D6 PMs compared with other genotypes [34]. With implementation of *CYP2D6* and *CYP2Ç19* genotypes in clinical practice, Muller et al. also confirmed usefulness of genotyping primarily for PMs and IMs [35]. Similar to the results of Penas–Lideo et al. [8], a recent study also reported an almost four times higher antidepressant discontinuance in pregnant women who are PMs or IMs of CYP2D6, suggesting that knowledge of the *CYP2D6* genotype might help identify pregnant patients at risk for antidepressant discontinuance [36].

Antipsychotics

Although literature data provide good evidence that polymorphic *CYP2D6* plays a role in determining pharmacokinetic profiles of different antipsychotics (primarily risperidone, aripiprazole, chlorpromazine, haloperidol, perphenazine, and thioridazine; and to a lesser extent clozapine, olanzapine, and quetiapine, Table 7.1), little evidence exists for a role of *CYP2D6* genotypes in determining antipsychotic efficacy. In a prospective study, Pollock et al. reported no significant differences in improvement of psychotic symptoms between five CYP2D6 PMs and 40 extensive metabolizers (EMs) treated with perphenazine for 17 days [37]. Even though a trend of lower haloperidol efficacy in UMs and higher efficacy in the PMs was shown in the study of Brockmoller et al. [38], the significant overlap in the haloperidol daily doses among the four metabolic groups: with 14 ± 10 mg in UMs versus 13 ± 9 mg in the PMs, preclude the possibility of any useful genotype-based dose recommendation. In 235 patients with schizophrenia or schizoaffective disorder who failed to respond to typical antipsychotics, subsequent *CYP2D6* genotyping showed the presence of the UM phenotype in less than 1% of the patients, suggesting that the UM genotype was not a major contributing factor to the therapeutic failure [39]. In the Clinical Antipsychotics Trials of Intervention Effectiveness (CATIE), Grossman et al. reported little evidence of difference in efficacy of either perphenazine or risperidone in patients with different *CYP2D6* genotypes, although there were no UMs included in the study [40]. Likewise, two other studies with risperidone showed that *CYP2D6* genotypes did not predict clinical improvement [41,42]. Another issue is that very few published studies separated UMs from EMs, which likely would negate any possible difference in efficacy between the UM and other *CYP2D6* genotypes. Finally, although Kim et al. suggested genetic polymorphism of *CYP3A5*3* is associated with the pharmacokinetics of quetiapine [43], there is little evidence that CYP3A polymorphism impacts the dosing of this atypical antipsychotic.

In their report of no difference in perphenazine efficacy between 40 EMs and 5 PMs, Pollock et al. also found that PMs experienced more severe adverse effects, including oversedation and parkinsonism, than EMs during the first 10 days of treatment, although there were no drug-concentration measurements performed [37]. Several studies have also shown PMs and IMs to have a higher incidence of adverse drug reactions, including extrapyramidal side effects, and drug discontinuance associated with the use of antipsychotic agents [38,44–53], whereas the evidence of a role of CYP2D6 in the etiology of tardive dyskinesia in PMs was less clear, with studies reporting both positive [51,54–57] and negative associations [58–61]. Without good data to suggest a concentration-dependent

relationship, it is not surprising that tardive dyskinesia might not be related to the *CYP2D6* genotype. *CYP2D6*10*, a predominant allele in Asian IMs, had been reported to be associated with weight gain in risperidone-treated Chinese patients [62], although it is not known whether plasma concentration correlates with weight gain. The role of pharmacogenomics in antipsychotic-associated weight gain will be discussed in a later section.

Mood Stabilizers

Despite the common clinical practice of monitoring plasma concentration and an inadequate response rate of <50% in lithium-treated patients, no pharmacogenomic studies exist on lithium pharmacokinetics. Published studies have mainly focused on the genes involved in the signaling and biochemical pathways involved in the mechanism of action of lithium, which will be described in a later section.

Genes Encoding Drug Transporters

The lack of data supporting a primary role for CYP gene polymorphisms in determining psychotropic drug response might be due to the presence of the drug efflux ATP-binding cassette (ABC, and formerly known as multidrug resistance [MDR]) superfamily of transporters residing at the blood–brain barrier (BBB). P-glycoprotein (P-gp) was the first-recognized and the most-studied ABC transporter, and together with other more recently discovered ABC transporters, such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP), plays a significant role in limiting the amount of drug crossing the BBB and reaching the cerebral circulation.

P-gp is encoded by the *ABCB1* gene (also known as the *MDR1* gene) in humans. Over the years, several polymorphisms have been identified in the promoter and exon regions of the *ABCB1* gene. The most studied single-nucleotide polymorphisms (SNPs) are the c.C1236T (rs1128503) polymorphism in exon 12, the c.G2677T (rs2032583) polymorphism in exon 21, and the c.C3435T (rs1045642) polymorphism in exon 26. In a randomized study of the effect of C3435T polymorphism in 54 nortriptyline-treated patients and 72 fluoxetine-treated patients, Roberts et al. found no difference in nortriptyline serum concentrations among the three genotypes (C/C, C/T, and T/T) but observed a higher incidence of postural hypotension for homozygous carriers of the T allele [63]. Fukui et al. [64] showed that the effect of the C3435T polymorphism on fluvoxamine pharmacokinetic is dose dependent, with the TT homozygote showing a significantly higher concentration-to-dose ratio than the CC homozygote only at the highest dose of 200 mg/ day. Therefore, the effect of P-gp polymorphism on drug concentrations could be similar to the dose-dependency effect shown with the *CYP2D6* polymorphism.

Although each of the aforementioned three *ABCB1* SNPs is associated with altered P-gp expression, larger-scale studies investigating their effect on antidepressant response have been conflicting [65–68]. This discrepancy might be due to the presence of strong linkage disequilibrium (LD) between several of these *ABCB1* polymorphisms, although conflicting results have also been reported for haplotype association studies [20,69]. In addition, some negative studies evaluated the association with C3435T polymorphism for too many drugs (9) in too few patients (n = 55) [70], which would pose a problem for statistical power. The choice of drug to be evaluated would also be important, as better remission rate was only demonstrated for patients carrying the C allele for the rs2032583 polymorphism *and* receiving a P-gp substrate (e.g., amitriptyline, citalopram, paroxetine, sertraline, or venlafaxine). Meanwhile, the response prediction associated with *ABCB1* polymorphism disappeared when data from all patients or from patients receiving non-P-gp substrates were analyzed [71,72]. Sarginson et al. confirmed the significance of

this substrate dependency for response association with paroxetine but not with mirtazapine, which is not a P-gp substrate [72]. In the large prospective randomized International Study to Predict Optimized Treatment in Depression, the investigators also reported association between response (remission and side effects) and *ABCB1* SNP association in 576 patients who completed an 8-week regimen of escitalopram, sertraline, or venlafaxine for their major depressive disorder.

Breitenstein et al. recently evaluated the clinical application of *ABCB1* genotyping in 58 depressed inpatient participants of the Munich Antidepressant Response Signature (MARS) trial. In this "head-to-head" comparison of treatment outcomes between pharmacogenomics-guided algorithm versus standard of care, the *ABCB1* gene test results (rs2032583 and rs2235015 SNPs) were incorporated into the treatment-decision process. The investigators reported that pharmacogenomics-guided patients had higher remission rates ($P = .005$) with less severe symptoms ($P = .0195$) upon discharge, compared to patients receiving usual care [73]. Needless to say, this pilot, yet encouraging, result needs confirmation. Unfortunately, the *ABCB1* gene variants were not assessed in some of the recent cost-utilization studies (see later section on Clinical Applications).

POLYMORPHISMS IN PROTEINS THAT MEDIATE DRUG RESPONSE

In addition to polymorphisms in the drug-metabolizing enzymes and the transporters, recent work has revealed that genes encoding drug targets such as receptors, ion channels, and intracellular-signaling proteins also play a significant role in determining drug efficacy and safety in patients. Multiple targets for the psychotropics exist for the neurotransmitter systems, including those that affect synthesis, degradation, or uptake of neurotransmitters, as well as their binding to pre- and postsynaptic receptors; and the cascade of downstream signal-transduction proteins within the synapse. Dysregulation of individual or combination of these targets can play a significant role in the etiology of psychotic diseases (Fig. 7.1). Abundant pharmacogenomic data on target polymorphisms exist for the psychotropics, in particular the antidepressants and the antipsychotics.

Antidepressants

Serotonin Transporter

With the primary role of serotonin (5-HT) in regulating emotions and mood, the serotonin transporter (SERT or 5-HTT) and also known as solute carrier family 6 [neurotransmitter transporter, serotonin], member 4 (SLC6A4), which function to transport serotonin within the synapse back to the presynaptic neurons, is one of the main therapeutic targets for SSRIs and also serotonin norepinephrine reuptake inhibitors (SNRIs). The SERT is encoded by the *SLC6A4* gene with several functional polymorphisms that have been extensively investigated and identified to impart variable therapeutic response in patients with different *SLC6A4* genotypes (Table 7.2). Specifically, a functional polymorphism in the promoter region (rs4795541, serotonin transporter-linked promoter-region polymorphism, or 5HTTLPR) of the *SLC6A4* gene results in the insertion/deletion of a 44-base pair repeat. The long (L) allele of the gene has higher transcriptional activity of the *SLC6A4* gene promoter and, hence, higher 5-HTT basal expression and serotonin uptake than the short (S) allele [74].

In vivo neuroimaging studies reported the contradictory effects of 5HTTLPR on brain 5-HTT availability [75–77], However, given the ability of the SSRIs to downregulate the SERT function, investigators hypothesized that SSRI efficacy could be affected by 5HTTLPR polymorphism. Since then, many studies have shown an association between homozygosity for the S allele and inferior response to the SSRIs, in contrast to

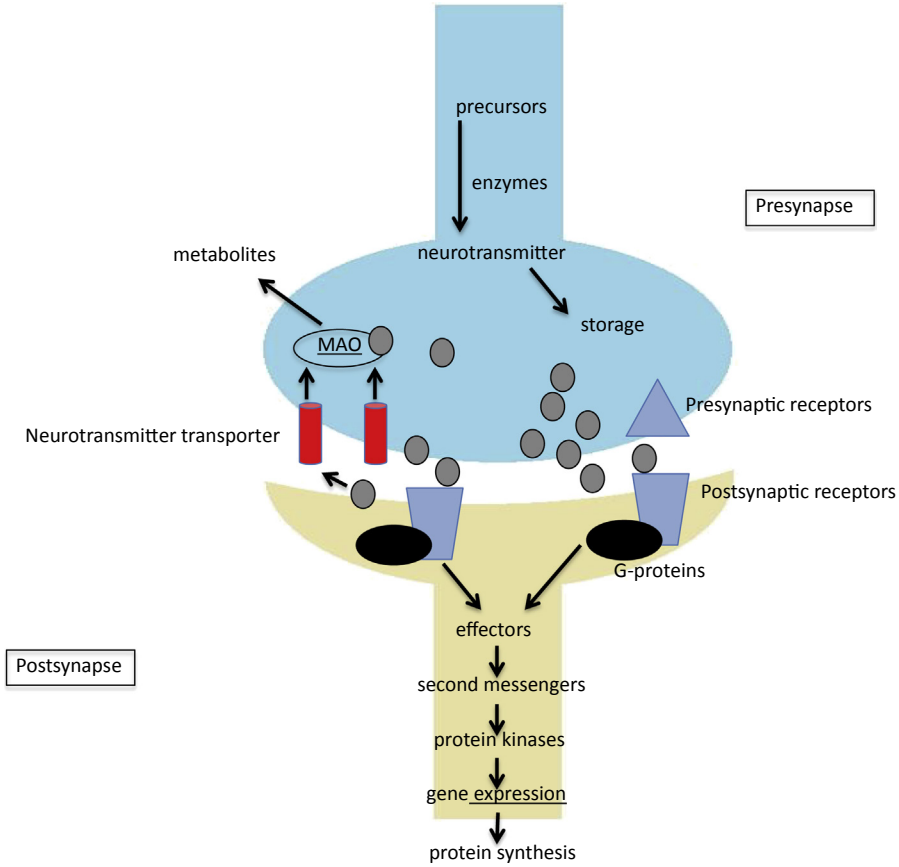

FIGURE 7.1 Schematic representation of psychotropic target proteins. *MAO*, monoamine oxidase.

homo- or heterozygosity for the L allele of the gene, which predicts beneficial outcome with SSRI treatment. Using positron emission tomography (PET) to evaluate the influence of genetic factors on 5-HT_{1A} receptor expression in a living human brain, David et al. observed that the S allele was associated with a reduction in availability of the postsynaptic 5-HT_{1A} receptors in man [78]. This might provide a possible biological basis of the decreased response to SSRIs in carriers of the S allele.

Not only was the therapeutic outcome reported different among patients with different *SLC6A4* genotypes, elderly patients with the *L/L* genotype treated with paroxetine or sertraline had a more rapid response, as early as after 1-week of treatment, than those with the *L/S* and *S/S* genotypes [79,80]. In addition, the lack of similar change in the onset of response in elderly patients treated with nortriptyline suggests that the difference in response is relevant only to antidepressants with a selective effect on serotonin [80]. Based on these findings, a case could be made for a preferential use of SSRI in patients with the *L/L* and *L/S* genotypes versus a TCA or a noradrenergic agent in patients with the *S/S* genotype. In addition, in patients with the *S/S* genotype, augmentation strategy of combining fluvoxamine and pindolol (being a 5-HT_{1A} antagonist as well and accelerating the antidepressant effects of SSRIs) has been shown to reduce the difference in response between carriers of the S and

TABLE 7.2 Summary of Selected Pharmacogenomic Studies With Major Genes Involved in the Serotonergic System

Genes	SNP	Major Findings	References
SLC6A4	5-HTTLPR, rs4795541	Homozygous or heterozygous carriers of 5-HTTLPR L allele showed better and/or faster response to SSRIs, especially in Caucasians Homozygous carriers of S allele showed less response, especially in Caucasians Homozygous carriers of S allele showed better response in patients of Asian descent Addition of pindolol to fluvoxamine improved treatment response in homozygous carriers of S allele	[79] [80] [81] [85] [86] [87,96] [96] [115]
	5-HTTLPR, rs25531	L_G variant (5 HTTLPR L allele with rs25531 allele) functionally similar to 5 HTTLPR S allele No association between treatment outcome and 5HTTLPR alleles or haplotypes. However, lower incidence of adverse effects were reported with the L_A allele of rs25531 Homozygosity for S allele and G alleles of HTR1A gene associated with nonresponse	[92] [91]
	STin2, 12 repeat units	No association with treatment outcome	[94]
	5-HTTLPR, rs25531 STin2	Association between remission and a haplotype consisting of S allele of 5-HTTLPR, L_A allele of rs25531, and 12-repeat allele of STin2	[95]
HTR2A	rs6311 and rs6313	*G* allele and *GG* genotype of rs6311 associated with better response, primarily in patients with Asian descent *C* allele and *CC* genotype of rs6313 associated with better response, primarily in Caucasians No association with response in Non-Hispanic Whites, or African or African American patients	[99,100] [101] [96]

the L allele, resulting in comparable treatment outcomes in all three genotypes [81]. Based on a decision-analytic model of pretreatment genetic testing for *SLC6A4* genotypes, Smits et al. concluded that the testing might result in a greater number of patients achieving remission earlier in the course of the treatment [82].

A meta-analysis of the literature supports a modest association between *SLC6A4* L allele and SSRI efficacy primarily in Caucasians but not in Asians [83]. Whether the more heterogeneous results within Asian populations could partially account for the lack of association between 5HTTLPR polymorphism and SSRI response reported in another meta-analysis that included more studies (n = 28) and patients (n = 5,408) is not known, because the investigators did not analyze the result separately in Caucasians and Asians [84]. This is especially important because opposite yet comparable associations (S allele conferring good therapeutic response) have been reported in Korean and Japanese populations, possibly at least partially related to either ethnic-related difference in the 5HTTLPR S allele frequency, being higher in Asians (74%–80%) than in Caucasians (40%) [85–87], interaction with other functional gene variants, or gene–environment interaction. In addition, a study showed that 5HTTLPR is not a simple insertion/deletion of a 44-base pair repeat, but a complex and highly polymorphic structure consisting of 14 kinds of alleles in different populations, including the Japanese and Caucasian, with variable distribution frequency [88].

The highly polymorphic nature of the *SLC6A4* gene is illustrated with the discovery of rs25531, an SNP located just upstream of the 5HTTLPR, from genetic analysis of the STAR*D sample. A

functional A>G variation in the L allele (but not S allele) of 5HTTLPR, known as the L_G allele, reduces mRNA expression of the *SLC6A4* to a level comparable to that of the S allele, and therefore changes the functional significance of the L allele of 5HTTLPR. On the other hand, the L_A allele increases *SLC6A4* mRNA expression, resulting in a "higher function" phenotype [89,90]. Therefore, by changing the expression of the L allele, this previously unrecognized L_G allele would further modulate the SSRI response predictive value of the *SLC6A4 L/S* and *L/L* genotypes. In essence, within SLC6A4, there would be two promoter polymorphisms and three alleles of functional importance: the high-expression L_A allele, and the low-expression S and L_G alleles. Because the S and L_G alleles are very comparable in SERT expression, the possible genotypes based on this L_A and L_G difference would be no L_A allele (either *S/S*, *S/*L_G, *or* L_G/L_G), one L_A allele (*S/*L_A or L_G/L_A), and two L_A alleles (L_A/L_A). Among the haplotypes constructed, only L_A/L_A is associated with high *SLC6A4* transcription [89]. This is consistent with the findings of carriers of L_A allele having favorable response to SSRI compared to carriers of the L_G allele [91].

In a second study of the STAR*D samples, Hu et al. reported an association between the L_A allele and citalopram adverse effects in all 1,655 subjects (Caucasians, Africans or African Americans, and mixed race). Lower adverse effects were associated with L_A/L_A genotype ($P = .004$) and L_A allele ($P < .001$) in all subjects, and a lesser association in a subset of 1,131 Caucasian subjects ($P = .03$ and $P = .007$, respectively). The adverse-effect association was also evident for the entire study population even when the L_A and L_G alleles were combined in the analysis. On the other hand, association for the Caucasian subject was present only with differentiation of the L allele into L_A and L_G alleles. There was no association between treatment outcome and 5HTTLPR alleles or genotypes in the Caucasian subjects [92].

In addition to 5HTTLPR, additional polymorphisms of the *SERT* gene have been identified with potential roles in modulating the response to SSRIs. Ogilvie et al. discovered a 17-base pair, variable number of tandem repeats (VNTR) polymorphism within intron 2 (STin2) of *SLC6A4*, resulting in three alleles containing 9, 10, and 12 copies of the VNTR element [93]. However, similar to 5HTTLPR polymorphism, both positive [85] and negative [94] associations have been reported. What is interesting is that, despite the lack of association between 5HTTLPR alleles or haplotypes and citalopram response [92], re-analysis of the same dataset from the STAR*D study revealed an association between remission and a haplotype that consists of 5HTTLPR, rs25531, and STin2 (haplotype S-L_A-12) [95].

Therefore, even for *SLC6A4*, a candidate gene with an obvious relevance to the therapeutic effect of antidepressants, especially the SSRIs, it is clear that predicting response in a patient solely with any one SNP would likely yield misleading and conflicting results. Together with the usual heterogeneous study limitations (ethnicity, outcome assessment, study design, sample characteristics, and sample size), significant work remains for appropriate pharmacogenomic study findings related to 5HTTLPR polymorphism to be translated from the bench to the bedside. In this respect, confirmatory findings in a naturalistic setting was reported by Staeker et al., who recently conducted a naturalistic study to evaluate the association between polymorphisms in serotonergic pathways (*SLC6A4*/rs25531, VNTR, and a 5-*HTR2A* intron 2 SNP [see later section on 5-HT_{2A} receptor]) and response (as assessed by Clinical Global Impression [CGI] Scale) and side effects (as assessed by the Dosage Record and Treatment Emergent Symptoms [DOTES] Scale) in psychiatric inpatients. They found significant associations between *SLC6A4*/rs25531 S/L_G alleles and response to SSRI treatment in patients ($P = .037$, CGI ≤ 2, 0% versus 19%, and $P = .0005$, DOTES cluster c, 0.76 vs. 0.19). In addition, there was significant association between *SLC6A4* VNTR 12/12 with adverse effects ($P = .0001$, side

effect rates 51% versus 19%). They also found significant association between the 5-*HTR2A* intron 2 SNP and side effects (to be described in later section) [96]. Additional recent investigations to lay the groundwork for broader-scale implementation have started to appear in the literature, and will be discussed in the later Clinical Application section.

5-Hydroxytryptamine Receptors

5-HYDROXYTRYPTAMINE 2A (5-HT_{2A}) RECEPTOR

The postsynaptic 5-HT_{2A} receptor represents another serotonin-related target for psychotropics. Antidepressants, typical, and atypical antipsychotics all act as antagonists toward and downregulate the receptor [97], reportedly overexpressed in patients with depression [98]. In humans, the polymorphic 5-hydroxytryptamine receptor 2A (*HTR2A*) gene encodes the 5-HT_{2A} receptor, and several polymorphisms had been investigated, including a c.-1438 A/G (rs6311) promoter polymorphism, and two coding region polymorphisms: the silent c.T102C (rs6313) polymorphism in exon 1, and the c.C1354T (rs6314) polymorphism resulting in a p.His452Tyr amino acid substitution. Two of these (rs6311 and rs6313) are in LD and had been associated with antidepressant response (Table 7.3) [99–101]. Although a specific allele, e.g., the C variant of the C102T polymorphism [101] and the G allele of the −1438 A/G polymorphism [99,100] were reported to be associated with antidepressant response, the findings are conflicting and not supported by the large-scale association study of 68 candidate genes in the STAR*D sample.

In the STAR*D study, a single synonymous variant of *HTR2A*, IVS2 A/G (rs7997012) within intron 2, emerged as the only SNP with sufficient predictive value for response to citalopram in a Caucasian population. Homozygous carriers of the A allele have better response (18% reduction in absolute risk of treatment failure) than homozygous carriers of the G allele. In addition, analysis of the genetic data showed that Africans or African Americans had a higher frequency of the "non-responding" allele [102], which might partially account for the findings of poorer response among citalopram-treated African or African American patients in the clinical STAR*D study [103]. Lucae et al. provided the first replicate confirmation of the role of rs7997012 shown in the genetic STAR*D study. In evaluating 637 German Caucasian patients with a major depressive episode, the SNP rs7997012 was significantly associated with remission of depression after 5-weeks treatment with a variety of antidepressants. However, the association (A allele conferred impaired treatment response) was inverse to that of the genetic STAR*D study [104]. Ethnic differences in patient samples (Caucasians versus a more heterogeneous population comprising Caucasians, Africans or African Americans, and mixed races in the genetic STAR*D study) and time of evaluation of treatment response (after 5-week treatment versus at study exit, regardless of length of duration since study entry for the genetic STAR*D study) can complicate interpretation of results. In addition, smaller sample size (Table 7.2) in the study of Lucae et al. could confound the result, limit the comparability between study results, and require additional studies to ascertain the direction of the association.

A more recent meta-analysis found significant association of rs6313 and rs7997012 SNPs with good treatment response to SSRIs in Caucasians but not in Asians [105], which may reflect the two SNPs being more common in Caucasians (about 54% and 36% for rs6313 and rs7997012, respectively) than in Asians (about 49% and 22% for rs6313 and rs7997012, respectively). As mentioned earlier, Staeker et al. studied the impact of serotonergic polymorphisms at the transporter and receptor level on response to SSRI. Although they did not report an impact of the 5-*HTR2A* intron 2 SNP on response, a significant association was found between *A/A* genotype of rs7997012 SNP and side effects ($P = .020$, side effect rates 43% versus 11%). The investigators

TABLE 7.3 Summary of Selected Pharmacogenomics Studies With Major Genes Involved in the Dopaminergic and Serotonergic System for Antipsychotic Response and Toxicity

Genes	SNP	Antipsychotics	Main Findings	References
COMT	c.472 G>A V158M rs4680	Clozapine, olanzapine	Homozygous carriers of Met allele have increased clinical response	[193,194]
DRD1	−48 A>G (rs4532), rs5326, rs265975	Haloperidol, chlorpromazine, sulpiride, flupenthixol, zuclopenthixol	CGC haplotype of the three SNPs associated with tardive dyskinesia (TD) risk	[206]
	rs4532	First-Generation Antipsychotic (FGA) and Second-Generation Antipsychotic (SGA)	No association with TD	[207,208]
DRD2	−141C del/ins, rs1799732	Chlorpromazine, bromperidol, nemonapride, risperidone	Del allele associated with less response	[154–156,159]
	Taq1A, rs1800497, also associated with *ANKK1* gene	Haloperidol, nemonapride, risperidone	A1 allele, A1/A1 genotype, Ins-A2/Del-A1 diplotype associated with better response	[165,166,168,169]
		Risperidone, chlorpromazine	No association with response	[159,170]
		Antipsychotics	A2 carriers at risk for TD	[204]
		Haloperidol, perphenazine, levomepromazine, fluphenazine, chlorpromazine, thioridazine, zuclopenthixol	A1 carriers associated with EPS	[202]
		Bromperidol, nemonapride	No association of A1 allele with EPS	[203]
		Nemonapride, olanzapine quetiapine, risperidone	A1 carriers associated with increased prolactin level	[209–211]
	Ser311Cys	Risperidone	Better response with Ser/Cys genotype	[171]
DRD3	Ser9Gly	Risperidone, chlorpromazine	Gly allele and Gly/Gly genotype associated with less response	[170,192]
		Antipsychotics	Gly allele associated with TD	[213–215]
DRD4	VNTR	Clozapine	No association with response	[178,179,184]
SLC6A4	44 bp del/ins	FGA	No association with response	[180]
HTR2A	102-T/C	Clozapine	T allele associated with better response	[182,184]
		Risperidone	Better response with CC genotype	[191]
		Antipsychotics	C allele associated with TD risk	[217]
	His425Tyr	Clozapine	Better response with His allele	[182,184]
		Antipsychotics	No association with TD	[217]
HTR2C	−759C/T (rs3813929)	Risperidone, chlorpromazine	Less response with C allele, C/C genotype	[170]
		Atypical antipsychotics	Weight gain associated with T allele	[218,219]

noted that all of the response and side effects associations were strong enough to be detectable in a naturalistic clinical setting [96].

5-HYDROXYTRYPTAMINE 1A (5-HT$_{1A}$) RECEPTOR

The 5-HT$_{1A}$ receptor is encoded by the 5-hydroxytryptamine receptor 1A (*HTR1A*) gene. Desensitization (or downregulation) of the somatodendritic 5-HT$_{1A}$ receptor by chronic SSRI treatment results in enhanced serotonergic neurotransmission [106,107]. In addition, antagonism of the 5-HT$_{1A}$ receptor has also been suggested to be associated with antidepressant effects [108,109]. Therefore, genetic variation of the *HTR1A* might change the functional properties of the 5-HT$_{1A}$ receptor, resulting in differences in antidepressant response.

Of the 10 polymorphisms identified in the *HTR1A* gene, the most investigated ones are c.-1019C/G (rs6295) located in the promoter region, p.Gly22Ser (rs1799920), and p.Ile28Val (rs1799921). The G allele of the rs6295 polymorphism has been associated with up-regulation of 5-HT$_{1A}$ receptor expression [110] and response prediction with antidepressant treatment [111]. In 118 patients treated with fluoxetine or nefazodone augmented with pindolol, or monotherapy with flibanserin (a 5-HT$_{1A}$ agonist), the homozygous *G/G* genotype was more prevalent in nonresponders than the homozygous *C/C* genotype ($P = .0497$ for the augmentation group and $P = .039$ for the monotherapy group) [111]. However, other investigators reported positive association being evident only for females [112] or in patients with specific depressive manifestation [113]. In a retrospective study, Levin et al. found no association between seven *HTR1A* polymorphisms, including rs6295, and SSRI response in 100 responders and 33 nonresponders [114]. As additional evidence that response to antidepressants likely is influenced by more than one gene, Arias et al. reported in 130 subjects treated with citalopram that homozygosity for both the G allele of the *HTR1A* polymorphism and S allele of the *SLC6A4* polymorphism predict nonresponse to SSRI treatment ($P = .009$) [115]. Differences in ethnic and allele distributions in study subjects could partially account for the conflicting results in replication studies. As an example, with very low frequencies of the Gly22Ser and Ile28Val polymorphisms in Japanese populations, the effect of the more common Gly272Asp polymorphism of the *HTR1A* on clinical response to fluvoxamine was studied in 65 depressed Japanese patients. Subjects with the Asp allele had a significantly higher % reduction in score of the 17-item Hamilton Rating Scale for Depression (HAMD-17) than homozygous carrier of the Gly allele at week 2 ($P = .009$), week 6 ($P = .036$) and at week 12 ($P = .031$) [116].

Antidepressant-associated side effects are well-known contributory factors to lower medication adherence, poor-health outcomes, and premature discontinuance of treatment. In a randomized placebo-controlled trial of 12-week treatment of escitalopram in patients 60 and older, Garfield et al. reported that side effects (increased sleep duration, dry mouth, diarrhea, and decreased sexual desire) are associated with genetic polymorphisms affecting *SLC6A4*, *HTR1A*, and *HTR2A*. Decreased sexual desire was experienced more in patients with high-expressing genotypes of the three serotonergic components, whereas higher incidence of dry mouth and diarrhea are associated more frequently with patients with the low-expression genotypes for *SLC6A4* polymorphism and low-transcription genotype for the *HTR1A* polymorphism, respectively. In contrast, there was no relationship between the three genetic polymorphisms and drug concentration [117].

Glutamate Receptor

With glutamate as the primary excitatory neurotransmitter in the brain, the glutamatergic system has also been investigated in pharmacogenomic studies of antidepressant response. Glutamate receptors selectively bind to glutamate to modulate excitatory neurotransmission, and increased glutamate levels have been observed in patients with depression [118]. Chronic use of

SSRIs such as citalopram was shown to attenuate glutaminergic transmission and reduce excitatory glutamate activity [119]. The STAR*D study has identified significant association between antidepressant response and a C/T SNP (rs1954787) residing in the first intron of the ionotropic kainite 4 gene (*GRIK4*) that encodes a kainic acid-type glutamate receptor. The C allele was associated with better outcome and suggested that the glutamate system could have a significant role in antidepressant response. In addition, homozygous carriers of both the A allele of *HTR2A* and the C allele of *GRIK4* were twice as likely to be associated with better response to citalopram than patients who did not carry either of these two outcome-related alleles [120]. The association of the C allele and *C/C* genotype with response has been confirmed in a meta-analysis [121]. In contrast, Perlis et al. reported they could not replicate the rs1954787 association in 250 Caucasian patients with nonpsychotic major depressive disorder and treated with daily regimens of duloxetine 60 mg/day for 6 weeks. In addition, to smaller sample size and difference in study population, one additional reason for the discrepancy could be related to the differential mechanisms of action of duloxetine (a serotonin–norepinephrine reuptake inhibitor) versus SSRIs (inhibiting selectively the reuptake of serotonin). It is also noteworthy that the investigators also reported their failure to replicate previously reported associations with rs25531, 5HTTLPR, and the 17-base pair VNTR polymorphism in intron 2 (STin2) for *SLC6A4*. Negative associations were also shown in the same study for four SNPs for *ABCB1*, six SNPs for four genes coding for phosphodiesterases, and a single SNP for *OPRM1* coding for the opioid receptor μ 1 [122].

Genome-Wide Association Studies

In contrast to candidate-gene studies involving, for example, the 5-HTTLPR, advances in sequencing technology have enabled the interrogation of many millions of SNPs within the entire genome and elucidation of molecular pathways involved in disease etiologies and drug actions through genome-wide association studies (GWASs). Unfortunately, the three major GWASs in patients with major depression, namely the Genome-Based Therapeutic Drugs for Depression (GENDEP) [123], MARS [124], and the STAR*D [125], have not identified any individual gene with convincing replication results in a sufficiently large sample size. In an effort to identify SNPs most likely associated with antidepressant response, investigators conducted several meta-analyses of GWASs, which partially overlap in dataset. The first meta-analysis included data from three large response cohorts in United Kingdom, Germany and the United States: the GENDEP, the MARS, and the STAR*D, respectively. Together, the three studies, which included 2,256 subjects of Northern European descent with major depressive disorder, were deemed to have statistical power to detect individual variants accounting for one to 2% of variation in antidepressant response. However, no individual variants were found to meet the genome-wide significance [66]. The second meta-analysis, Novel Methods Leading to New Medications in Depression and Schizophrenia (NEWMEDs), included additional cohort to that of the GENDEP, and also found no association with efficacy [126]. O'Dushlaine et al. conducted a meta-analysis of two GWASs: STAR*D cohort and another cohort drawn from electronic health records of a large health system (i2b2 cohort) that together comprised 1,263 Caucasians with major depressive disorder. Initial treatment responders were contrasted with those with treatment-resistant depression (TRD), defined as no symptomatic remission despite two antidepressant treatment trials. Copy number variants (deletions and duplications) were derived from 778 subjects (including 300 with TRD) in the i2b2 cohort and 485 subjects from the STAR*D cohort (including 152 with TRD). They reported a modest contribution of rare copy number variants to treatment-resistant phenotypes, both individually and in aggregate, but no associations survived genome-wide correction [127].

These meta-analysis results are not unexpected given the results of Tansey et al., in which data from two large major depression studies (NEWMEDs and STAR*D) in about 4,100 patients were analyzed. Using genome-wide complex trait analysis [128], the investigators reported additive effects of common genetic polymorphisms across the human genome accounting for about 42% of individual variation in antidepressant response [129]. Not only do the results confirm the highly polygenic basis of antidepressant response that involve many variants, but as importantly, none of the variants have large effects, despite collectively accounting for a substantial portion of the variation. It remains to be determined which pharmacogenomic markers for drug disposition and/or response could account for a large portion of the variability.

In summary, despite significant progress in antidepressant pharmacogenomic research over the years, the lack of consistent findings among all studies of different neurotransmitter receptors and transporters, including single candidate-gene association studies, GWASs, and meta-analyses, make it difficult to identify definitive association that can be used to predict antidepressant response in clinical setting. Differences in study design, disease phenotypes, patient population, response definition and assessment, and sample size all contribute to the conflicting results. In addition, it is also unclear how many pharmacogenomic studies measure medication adherence, which acts as a confounding variable that affects treatment outcome. Nevertheless, studies that employ pathway analysis of gene variants involved in fluoxetine pharmacodynamics have shown some potential utility in identifying important gene variants with significant contributions to treatment response with fluoxetine [130].

Potential Role of Other Molecular Pathways

Research over several decades suggest that increased monoaminergic neurotransmission is important for antidepressant action [131]. Although studies reviewed in previous sections mostly demonstrate the essential function of the serotonergic system (transporter and receptor) and the impact of their regulating genes for antidepressant response, Nicket et al. reported that both paroxetine and the serotonin reuptake *enhancer* tianeptine are effective antidepressants [132]. In addition, a meta-analysis also showed that the effects of monoamine depletion are conflicting, and depletion does not induce depression in healthy subjects [133]. Hence, additional biological pathways, including those identified recently [134–137], could possibly serve as biomarkers for treatment response. The following section illustrates how studies of molecular pathways associated with neuronal plasticity over the last few years provide insight of additional antidepressant targets.

The neuroplasticity hypothesis suggests that antidepressant action is partially related to proliferation of neuronal stem cells, and that the slow onset of antidepressant action is a result of neuroplasticity changes mediated by such proliferative effects in the hippocampus [138]. Hence, other investigators have proposed an entirely different approach to search for SSRI-response biomarkers, which is based on reports of genome-wide expression profiling in human lymphoblastoid cell lines (LCLs) previously demonstrated for anticancer drugs [139–141]. They first identified and demonstrated the existence of LCLs with variable SERT functional expression and hence high or low sensitivities to different SSRIs [142]. The investigators then screened 80 LCLs for growth inhibition by paroxetine. A 6.4-fold difference in expression between the two paroxetine-sensitivity phenotypes was demonstrated for the cell adhesion molecule with homology to L1 cell-adhesion molecule (L1CAM) gene (close homolog of L1 [CHL1]) encoding a neuronal cell-adhesion protein that is implicated in correct brain circuitry, and *CHL1* was identified as a tentative transcriptome biomarker of paroxetine. In addition to *CHL1*, 12 additional genes implicated

in brain function or psychiatric disorders also showed more than 1.5-fold difference in expression between the two phenotypic groups [143]. In a follow-up study, the effect of fluoxetine on cell proliferation and gene expression in LCLs derived from patients with documented treatment response outcome was investigated. The investigators identified multiple genes with different expression before and after ex vivo incubation with fluoxetine [144].

Although one can argue that the discovery of yet another set of genes for predicting SSRI response does not necessarily translate to definitive and clinically relevant biomarkers, comparison of gene-expression levels from patients with major depression could be further investigated to identify targets for antidepressant therapy. In a study of 58 patients selected from the MARS study, investigators showed an association between response (better remission with antidepressants) and basal expression of *CHL1* and another gene, integrin beta 3 (*ITGB3*). After 5 weeks of antidepressant treatment, homozygous carriers of the T allele of the *CHL1* SNP (rs1516338) had significantly better response than homozygous carriers of the C allele, further suggesting that CHL1 expression in patient-derived LCLs correlated with clinical outcome [145]. Another group of investigators analyzed genes associated with outcomes from the STAR*D GWAS and confirmed the potential roles of *CHL1* and *ITGB3* [146].

Antipsychotics

Dopamine Receptors

The catecholamine neurotransmitter dopamine controls a variety of central nervous system functions including cognition, emotion, endocrine system regulation, food intake, and locomotor activity. The five dopamine receptors are grouped into the D_1-like receptors (DRD1 and DRD5) generally associated with stimulatory functions, and the D_2-like receptors (DRD2, DRD3, and DRD4), which are more associated with inhibitory functions. All antipsychotic agents, especially the first-generation antipsychotics, are dopamine D_2 receptor (DRD2) blockers [147]. Functional brain-imaging studies suggest, and pooled analyses and meta-analyses confirmed, that a threshold level (60%–65%) of D_2 receptor binding by antipsychotic agents in the mesolimbic pathway is needed for sustained therapeutic effect, and excessive blockade (≥78%–80%) in the nigrostriatal pathway is associated with extrapyramidal side effects [148–151].

Of the five subtypes of dopamine receptors, D_2, D_3, and D_4 receptors are the most studied for pharmacogenetic evaluation of antipsychotic efficacy. Several polymorphisms of the D_2 receptor gene (*DRD2*) have been identified: the −141-C ins/del polymorphism (rs1799732) with deletion of a cytosine in the promoter region at position −141, the Taq1A polymorphism (rs1800497), and the p.Ser311Cys polymorphism (rs1801028) within the coding region. The del allele of the −141-C ins/del polymorphism is associated with not only lower expression of the D_2 receptor in vitro [152], but also higher striatal D_2 receptor density in vivo [153]. Studies that evaluated the functional effects of the polymorphism have yielded mixed results. Several investigations and meta-analysis have also shown that the del allele predicts less beneficial response from antipsychotics (Table 7.3). [154–159], Interestingly, a recent report showed that carriers of the del allele have higher rates of improvement in depressive symptoms severity during treatment with olanzapine, perazine, and ziprasidone [160]. In addition, even though the del allele was associated with lesser clinical improvement in risperidone-treated patients. [159], Wang et al. reported no association with *DRD2* polymorphisms in patients treated with paliperidone, the active metabolite of risperidone [161]. Thus, in addition, to replication challenges such as different study designs and outcome measurements, the issue of whether response association with the candidate-gene

approach is limited to an individual psychotropic drug versus applicable to a wide range of antipsychotic medications would need to be addressed as well.

The Taq1A polymorphism (rs1800497), now also associated with *ANKK1* gene [162], is located downstream of *DRD2* and has two variants: A1 and A2, with lower striatal D_2 receptor density reported in carriers of the A1 allele [163]. The A1 allele is in LD with two *DRD2* intronic variants (rs1076560 and rs2283265) that affect DRD2 splicing [164], The *A1/A1* genotype had been reported to be associated with better response (greater improvement in positive symptoms) to aripiprazole, haloperidol, nemonapride, and risperidone [165–168], whereas the *Ins-A2/Del-A1* diplotype was reported to be associated with better response to risperidone [169]. In contrast, lack of association have been reported, primarily in patients of Asian descent with first-episode schizophrenia [170] or drug-naïve schizophrenic patients [159]. These negative study results are in agreement with the lack of association reported in a meta-analysis [157].

The rs1801028 polymorphism represents a C>G SNP in exon 7 that changes the codon 311 from the more common Ser to the less common Cys, with the Cys311 variant associated with lower affinity for dopamine. In 123 Chinese patients treated with risperidone for up to 42 days, patients with the *Ser/Cys* genotype of *DRD2* polymorphism showed greater absolute score reduction and greater percent change in negative symptoms than patients with the *Ser/Ser* genotype. However, there were only 12 subjects with the *Ser/Cys* genotype and no patient had the homozygous *Cys/Cys* genotype [171]. Nevertheless, a meta-anlaysis by Hwang et al. also showed a trend for lesser response in carriers of the Ser allele [172]. In summary, although most studies of the *DRD2* polymorphisms have been associated with treatment outcome, the effect of individual polymorphism has not been consistent across different studies.

Dopamine binds to the D_2 receptor and inhibits its prolactin secretion, and the Taq1A genotype has been associated with hyperprolactinemia [173]. Fukuri et al. hypothesized that basal prolactin level accurately reflects DRD2 function, and investigated the association of the basal prolactin levels of 140 healthy Japanese subjects with *DRD2* "tagging" SNPs that covered the *DRD2* gene, as well as with the Taq1A, Ser311Cys, and −141C Ins/Del polymorphisms. Significant associations were found between two *DRD2* variants (rs7131056 and rs4648317) in intron 1 and serum prolactin levels, but only in the female subjects, which is consistent with the known gender difference in prolactin concentration [174]. These preliminary data suggest that the two new polymorphisms can be considered as candidate functional *DRD2* polymorphisms, and should be further investigated in future studies.

Antipsychotic agents also show affinity for the dopamine D_3 receptor (DRD3), with increased receptor expression after treatment [175]. The *DRD3* gene contains an SNP that results in a serine to glycine amino acid substitution (rs6280). The p.Ser9Gly polymorphism had been implicated with conflicting results showing lesser [172] versus greater [176] antipsychotic response in carriers of the Gly allele. Literature data also evaluated its association with development of tardive dyskinesia, which will be discussed in latter sections of this chapter.

The ten-fold higher affinity of the atypical antipsychotic agent clozapine for the D_4 receptor than for the D_2 and D_3 receptors results in a lower risk of inducing extrapyramidal side effects. The *DRD4* gene is highly polymorphic, with a tandem duplication of 120 base pairs (120-bp duplication) in its promoter region, resulting in reduced DRD4 expression in vitro and lower gene transcription. Despite the earlier report of this tandem-repeat polymorphism linked to clozapine efficacy with a better response in carriers of the long allele (240 base pair) [177], subsequent studies were not able to detect significant association [178,179].

Serotonergic System

Although no differences in response to typical antipsychotic agents were reported in 684 patients with different *SLC6A4* genotypes [180], the pharmacological action of the atypical antipsychotic agents partially involves the serotonergic system, with single-photon emission computed tomography evidence of high occupancy of the 5-HT_{2C} receptor by clozapine and risperidone [181], making it a logical candidate gene for evaluation of response association. Based on clozapine's high affinities for the 5-HT_{2A} and 5-HT_{2C} receptors, several polymorphisms of the *HTR2A* gene (c.-1438-G/A and c.102-T/C in the promoter region and p.His425Tyr in the coding region) and the *HTR2C* gene (c.-759-T/C [rs3813929] in the promoter region and p.Cys23Ser [rs6318] in the coding region) have been extensively investigated in the literature for response prediction. Meta-analyses of literature data reported association between 102-T/C and His425Tyr polymorphisms and response [182]; Although a significant association was found between the Ser allele of the Cys23Ser polymorphism of the *HTR2C* gene [183], subsequent studies were not able to replicate the results.

Recognizing the limitation of evaluating single SNPs in a single gene, Arranz et al. evaluated 19 genetic polymorphisms that affect the different pharmacological targets of clozapine. Based on association studies of these polymorphisms in 133 responders and 67 nonresponders, the investigators reported a combination of six different polymorphisms across different loci. These six (the −1438-G/A and 102-T/C polymorphisms that are in LD; the His425Tyr polymorphism of the *HTR2A* gene; the Cys23Ser and −330-GT/-244-CT polymorphisms of the HTR2C gene; the 5HTTLPR polymorphism of the *SLC6A4* gene; and the −1018-G/A polymorphism for the histamine-2 receptor) resulted in a 76.7% success in predicting response to clozapine. In addition, about 50% of the patients are homozygous carriers of the T allele of the 102-T/C polymorphism and the His allele of the His425Tyr polymorphism of the *HTR2A* gene, and good response was evident in 80% of this patient subgroup. Interestingly, despite the high affinity of clozapine for the D_4 receptor, no association was found with response [184]. Nevertheless, despite the appeal of this polymorphism-combination approach to more accurately predict clozapine response, the result was not replicated in another study [185]. To date, no studies replicate the primary findings of Arranz et al. [184].

The dopamine and serotonin receptors targeted by the antipsychotics are G-protein-coupled receptors (GPCRs) and signal to effector proteins through intracellular G-protein subunits. Regulators of G-protein signaling shorten the time period of neurotransmitter signaling through the GPCRs. The regulator of G-protein signaling 4 (RGS4) is one such regulator, and it regulates the activity of the GPCRs. The gene that encodes RGS4 had been identified as a vulnerability gene for schizophrenia [186,187], and variants of *RGS4* have been studied as predictors for antipsychotic treatment response. Conflicting reports of treatment response association with three SNPs (rs951439, rs2842030, rs2661319) of *RGS4* have been reported in three ethnic groups (patients of African descent, European descent, and Chinese descent) for different antipsychotics (perphenazine, ziprasidone, quetiapine, and risperidone) [188,189]. These data with *RGS4* polymorphisms underscore the importance of stratification of patient population by ethnicity in pharmacogenomic investigations, which is further evidenced by the lack of association reported in another study of 482 unrelated schizophrenia patients of South Indian origin [190]. It is noteworthy that the investigators of the Chinese study [189] also had reported in several different studies that polymorphisms affecting the D_2 receptor (Ser311Cys), D_3 receptor (Ser9Gly), and 5-HT2A receptor (102-T/C) predict treatment response to risperidone [171,191,192]. Whether a combination of

polymorphism approach similar to that for clozapine could result in better response prediction remains to be investigated.

Catechol-O-MethylTransferase

Dopamine level in the frontal lobe of the brain is essential for executive function. The catechol-O-methyltransferase (COMT) mediates the degradation of dopamine and terminates its action, especially in the frontal cortex. Although not studied as extensively, polymorphism in *COMT* encoding the enzyme may modulate antipsychotic effect. The Val108Met polymorphism (rs4680 with G to A transition at codon 158 of the membrane-bound form of COMT, which corresponds to codon 108 of the soluble form of the enzyme) results in 3–4-fold lower COMT activity in homozygous carrier of the Met allele when compared to those with the *Val/Val* genotype. Studies have shown carriers of the Met allele (with less dopamine degradation and, hence, more dopamine in the synapse) have improved cognitive function after treatment with clozapine and olanzapine [193,194]. A recent meta-analysis of studies with a total of 1,416 patients confirmed the association between the *Met/Met* genotype and antipsychotic efficacy [195]. Interestingly, although the independent effect of the DRD4 120-base pair duplication for predicting clozapine response [177] has not been duplicated [178,179], a more recent study by Rajagopal et al. demonstrated a gene–gene interaction between the *DRD4* and *COMT* for clozapine response in 93 patients. A carrier of the Met variant who also is a homozygous or heterozygous carrier of the *DRD4* 120-base pair allele showed better clinical response to clozapine than those without these alleles. A carrier of the Met allele and the *DRD4 240/240* genotype showed no additive clinical response, whereas poor response was associated with both the *DRD4 120/120* and *120/240* genotypes in the presence of the *COMT Val/Val* genotype [196]. Although the mechanism for the additive response interaction between the *COMT* Met and the *DRD4* 120 allele is not known and this result needs confirmation, this study highlights the need of evaluating interaction among target genes in pharmacogenomic research.

Additional Regulatory and Development Genes

In addition to the genes involved in the dopaminergic and serotonergic system, there are additional genes of interest that, although not as extensively studied as dopaminergic and serotonergic genes, could contribute to antipsychotic response, probably via their influence on neuronal function and neurotransmitter signaling. Although an extensive review of investigations of all these SNPs is outside the scope of this chapter, these include the glutamatergic system [197], specifically two SNPs in the glutamate-receptor delta 2 (GRID2) gene involved in glutamate signaling, as abnormal glutamatergic function could modulate dopaminergic function in psychosis [198], rs13025959 (E1647D) in *MYO7B* encoding myosin VIIB, which plays a role in brain development, and rs10380 (H622Y0 in *MTRR* encoding 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, which might play a role in determining antipsychotic response similar to that of methylene-tetrahydrofolate reductase (MTHFR), which is encoded by the *MTHFR* gene [199].

In summary, compared to the antidepressants, the research data for antipsychotic pharmacogenomic studies are very limited. Among the literature studies, some association studies with individual candidate genes encoding their respective targets showed positive findings with overall antipsychotic response prediction with genes involved in the dopaminergic system, and improved negative symptoms with genes involved in the serotonergic system. Currently, the *DRD2* -141-C Ins/Del and the Taq1A polymorphisms are included in some pharmacogenomic test panels. However, the data are far from convincing, and there are just about as many negative associations reported

in the literature. Although it is obvious that a combination of different genes would account for a greater portion of the response variance than individual genes, analysis of how genetic variants influence improvement in positive or negative symptoms as well as cognitive function would likely yield more useful insight than improvement in overall symptomatology.

As with antidepressants, GWASs over the years have identified additional SNPs, including rs17390445 on chromosome 4p15 from the CATIE study, to be associated with treatment response, even though the study itself was not designed as a pharmacogenomic study [200]. However, the SNP is located in an intergenic region with unknown functional significance of the associated variants. Additional SNPs in the ankyrin repeat and sterile alpha motif domain-containing protein 1B gene (ANKS1B) and in the contactin-associated protein-like 5 gene (CNTNAP5), which play a role in modulating neuronal cell proliferation and differentiation, as well as communication among neurons within the brain, have also been shown in the same study to approach genome-wide significance. However, how these borderline significant results could affect antipsychotic response remains unknown. Although GWAS results could have implications in identifying new molecular pathways and targets that warrant additional investigations, currently the practical utility of the SNP results from GWASs of the CATIE trial for practitioners is minimal.

Adverse Drug Reaction with Antipsychotics

Antipsychotic use is associated with a variety of adverse effects, with extrapyramidal symptoms (EPS) and weight gain being the most commonly reported and also the focus of much of the pharmacogenetic studies of psychotropic-induced adverse-drug reactions. Among the different EPS, tardive dyskinesia (TD) is a debilitating and irreversible movement disorder that develops in up to 30% of patients after long-term antipsychotic treatment. As indicated earlier, excessive blockade of D_2 receptor is associated with extrapyramidal side effects, although primarily a problem for the typical antipsychotics [149,201].

Both positive [202] and negative [203] associations with EPS had been reported in A1 carriers of the Taq1A polymorphism of the *DRD2* gene, whereas a meta-analysis found a risk-increasing effect for TD in carriers of the A2 allele [204]. Because an imbalance between D_1 and D_2 receptors had been suggested to result in TD [205], the conflicting results reported for association between *DRD2* polymorphism and EPS as well as the risk of TD [202–204] could be related to genetic variants in *DRD1* as well. In a recent study involving 220 Chinese patients with TD and 162 Chinese patients without TD treated with stable dosage regimens of typical antipsychotics for at least 6 months, the SNP rs4532 (also known as −48 A>G) in *DRD1* was significantly associated with TD risk in the schizophrenic patients. The positive association was also evident in haplotype analyses involving two additional SNPs: rs5326 and rs265975, specifically the haplotype CGC (rs5326-rs4532-rs265975) [206]. The study result contrasted with the negative association reported by two studies [207,208], which could be related to ethnic differences in allele frequency of rs4532 (18% frequency for the G allele in Chinese versus 39% in Caucasians [207]) and contribution of *DRD1* to TD, as well as the inclusion of patients treated with atypical antipsychotics in the two negative studies.

Dopamine binds to D_2 receptor and inhibits its prolactin secretion; therefore, use of antipsychotic agents results in increased prolactin level, although the effect is less with the atypical antipsychotics. Several studies showed that hyperprolactinemia is related to the Taq1A polymorphism, with the A1 allele associated with elevated prolactin level [209–211], as well as being drug specific, with the effect being more prominent with risperidone and olanzapine than with quetiapine [211]. However, no such

association was reported in a subsequent study of 47 younger patients with autism-spectrum disorders [212].

Brain-imaging studies also showed that haloperidol-treated patients with the *Gly/Gly* genotype for the Ser9Gly polymorphism of *DRD3* gene (rs6290) had greater fluorodeoxyglucose metabolism in the anterior striatum than patients who were either heterozygous or homozygous carrier of the Ser allele. The increased brain activity observed in the patients correlated with the presence of the most severe TD symptoms [213]. In a meta-analysis of data from 317 patients with TD and 463 patients without TD, patients with the Gly allele were found to experience a higher incidence of TD ($P = .04$). In addition, patients who were homozygous carrier for the Gly allele had higher abnormal involuntary movement scores than heterozygotes ($P = .006$) and homozygotes for the Ser allele ($P < .0001$). The effect of the Gly allele, though significant, was modest with an odds ratio of 1.33 [214]. Nevertheless, the role of the Ser9Gly polymorphism in TD was confirmed in another meta-analysis, which also suggested that the association was related to ethnicity, with a stronger association in non-Asians versus Asians [215]. In contrast, both the CATIE trial and a more-recent meta-analysis of 13 studies reported no association between *DRD3* rs6280 polymorphism and prevalence of TD [61,216]. Finally, a pooled analysis of 256 patients with TD and 379 patients without TD showed a positive association for the C allele of the 102-T/C polymorphism of *HTR2A*, especially in the elderly. This suggests that 5-HT receptors can also be involved in etiology of TD [217].

Although the atypical antipsychotic agents have lower propensity to produce extrapyramidal side effects, their use is associated with a higher incidence of weight gain than the typical antipsychotics. Given the deleterious effects of weight gain on the cardiovascular system as well as lipid and glucose metabolism, identification of potential markers for weight gain in at-risk patients treated with psychotropics would be beneficial. Among the various neurotransmitters involved in etiology of schizophrenia and/or mechanism of antipsychotic drug action, the involvement of the 5-HT_{2C} receptor is the most convincing with evidence converging on the –759C/T (rs3813929) polymorphism in the promoter region of the *HTR2C* gene as a predictor of risk of weight gain associated with atypical antipsychotic use [62,218–223], despite conflicting report of the functional significance of the C versus the T allele [224,225]. Nevertheless, most study results showed the C allele was significantly associated with weight gain. In contrast to studies that showed positive association of weight gain with the T allele, atypical antipsychotic treatment duration (less than 3 months) and ethnicity (European Americans and not African Americans or Asians) are variables that are found to be more prominent in studies with positive association of the C allele as a risk for weight gain.

More recently, research has also focused on the leptin–melanocortin system. The melanocortin 4 receptor (MC4R) is primarily located in the hypothalamus and mutations in the *MC4R* gene encoding MC4R are the most common genetic cause of obesity [226]. In a GWAS of pediatric patients, Malhotra and colleagues reported that SNPs in *MC4R* showed the strongest association after 12-weeks of second generation antipsychotic treatment in an initial discovery cohort of the 139 pediatric patients. Similar results were replicated for rs489693 in three additional cohorts comprising a total of 205 adult schizophrenic patients [227]. Subsequent replication studies showing lesser magnitude of association in autistic pediatric patients after 8-week risperidone treatment [228] and in adult patients after 4-weeks of second-generation antipsychotic treatment [229] suggests the association might be related to other factors related to the chronic nature of the illness and/or the duration of treatment.

Leptin is a peptide hormone secreted by the adipose tissue, with a proportional correlation

between the adipose-tissue amount and leptin level. Leptin activates secondary signals associated with food-intake inhibition and increased energy expenditure and high serum leptin level results in appetite suppression and energy storage. A functional −2548 A/G (rs7799039) polymorphism occurs in the promoter region of the gene coding the leptin protein (LEP), with the G allele implicated as the risk allele for weight gain [230,231], However, this SNP was not one of the four *LEP* SNPs identified in a more recent study [232]. Another SNP, a 223 Gln/Arg (rs1137101) polymorphism of the gene coding the leptin receptor (LEPR) have also been reported as risk predictors for weight gain [230,231]. Nevertheless, a report of negative association [222] makes it difficult to assess the clinical significance of these SNPs.

Mood Stabilizer

Response to Lithium

Even though therapeutic efficacy of lithium as a mood stabilizer has been shown to be associated with *SLC6A4* genotypes, with better outcome for patients with the *L/L* or *L/S* genotypes [233], most of the published pharmacogenomic studies of lithium primarily focused on the inositol turnover signaling pathway and the inhibition of glycogen synthase kinase 3-β (GSK3B). Patients with bipolar disorder are reported to have hyperactive signaling in the inositol turnover signaling pathway, and lithium use inhibits the activity of inositol polyphosphate-1-phosphatase (INPP1) and inositol monophosphotases (IMPA1 and IMPA2), resulting in reduced amount of free inositol available for signaling activity [234]. When comparing responders and nonresponders, an SNP (rs2067421) in the *INPPI* gene had been reported to be associated with lithium response [235], and Bremer et al. reported that the association is likely dependent on clinical subtype [236]. Benedetti et al. reported an association between *GSK3B* polymorphism and lithium response [237]. In the study by Bremer et al., the SNP (rs2199503) for *GSK3B* also was shown to be associated with lithium response in patients with posttraumatic stress disorder [236]. Failure to differentiate clinical comorbidity in past association studies might contribute to the conflicting results with *INPPI* and *GSK3B* polymorphisms in the literature. The potential role of *INPP1* and *GSK3B* polymorphisms has also been confirmed in a more recent study [238].

More recently, Hou and colleagues conducted a GWAS of lithium response in 2,563 patients worldwide and reported a single locus of four-linked SNPs on chromosome 21 detected genome-wide significance for response association. However, the same study did not report any association between lithium response and any of the previously reported SNPs [239]. Hopefully, the pending results from the multicenter prospective Pharmacogenomics of Bipolar Disorder (PGBD) study (ClinicalTrials.govNCT01272531) would provide additional insight and clarification on the genetic factors that influences clinical response to lithium [240].

Adverse Drug Reaction to Carbamazepine

One of the most useful applications of pharmacogenomics in psychiatry relates to the use of the anticonvulsant carbamazepine as a mood stabilizer. Despite its usefulness for patients with bipolar disorder, carbamazepine use is associated with severe adverse effects such as aplastic anemia and life-threatening cutaneous drug reactions such as Stevens–Johnson syndrome/toxic epidermal necrosis (SJS/TEN). The highly polymorphic Human Leukocyte Antigen Class 1 (HLA-1) genes encode proteins that bind and present antigens to immune cells. Abundant literature data support that the major histocompatibility complex *HLA-B*15:02* is a strong predictor of carbamazepine-induced Stevens–Johnson syndrome, primarily in patients with Asian descent [241]. The presence of *HLA-B*15:02* was documented in all 44 Taiwanese Chinese of Han descent with SJS/TEN.

Another study reported a positive association with *HLA-B*15:02* in 98% of 60 Han Chinese patients with the adverse drug reaction compared to 4% of patients who did not have the reaction [242,243]. A subsequent study confirmed the positive association in 94% of Han Chinese patients SJS/TEN compared to 9.5% of carbamazepine-tolerant patients, and 9% of healthy control subjects [244]. Similar associations have been reported for other Asian populations, despite variability in the frequency of *HLA-B*15:02* in those populations (Fig. 7.2) [245–249]. A black-box warning regarding this association in specific populations of susceptible individuals carrying the *HLA-B*15:02* allele was issued by the FDA in 2007, with a recommendation that regardless of their countries of origin, all patients of Asian descent should be screened for *HLA-B*15:02* prior to initiation of carbamazepine therapy, and alternative agent to be used in patients who are tested positive for the allele. However, it should be noted that (1) phenytoin also causes SJS/TEN [250] and is not a suitable alternative agent for carbamazepine in patients with the *HLA-B*15:02* variant, and (2) *HLA-B*15:02* is rare in both Japanese and Korean patients. Instead, other more common HLA alleles such as *HLA-B*15:11* and *HLA-B*31:01* are associated with carbamazepine-induced SJS/TEN in these two Asian populations

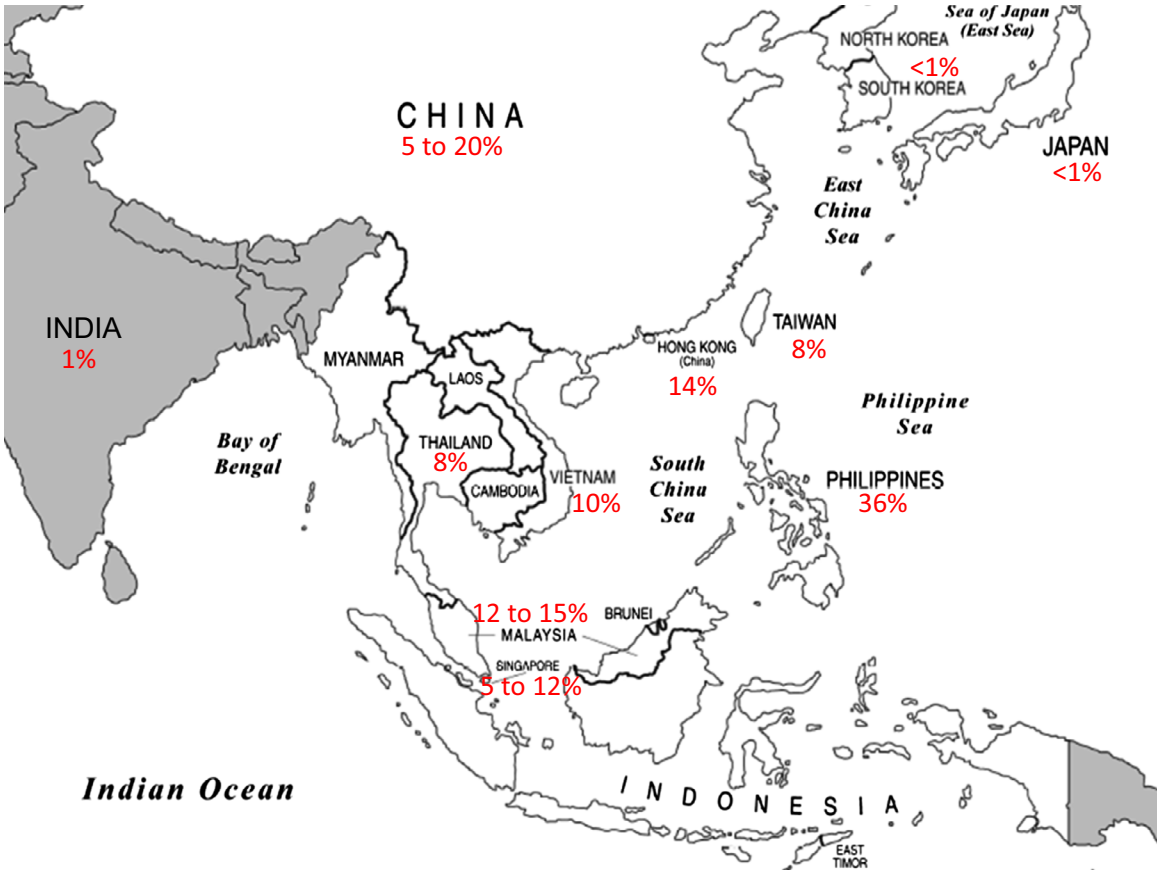

FIGURE 7.2 Ethnic differences in *HLA-B*15:02* in Asian populations.

TABLE 7.4 Challenges for Psychopharmacogenomic Evaluation and Implementation

Genomic studies mostly based on *post hoc analyses* of DNA samples collected from clinical trials that are not initially designed for pharmacogenomic evaluations
Heterogeneity of study populations with respect to
• Allele frequencies
• Ethnicity
• Patient-specific variables (gender, age, concurrent drug)
• Disease phenotypes
• Prior drug use
Study design with differences in
• Prospective versus retrospective versus naturalistic study
• Gene(s) investigated
• Selection of genetic biomarkers: single SNP versus haplotypes
• Treatment duration
• Response definition
• Response assessment
Inadequate sample size
Few prospective trials of pharmacogenomic-based clinical practice versus standard of care
Small incremental value in current quality and evidence-based driven clinical environment

[245,251,252] as well as in Caucasians [253,254]. A detailed clinical guideline for using HLA genotype in conjunction with carbamazepine or oxcarbazepine is included in the recently published 2017 update [255].

Despite documented substantial variability in psychotropic drug exposure among subjects with different genotypes of the cytochrome P450 (CYP) enzymes and suggested dosage regimens for carriers of the different CYP genotypes [6], there were only few studies that provided clear evidence of association with adverse effects, with even lesser-documented clinical validity based on psychotropic response prediction. Given the current literature data and the emerging role of neurotransmitter receptors and transporters in psychotropic response association, the utility of CYP genotyping in improving drug treatment could ultimately be in reducing side effects and improving medication adherence. Despite this utility, current literature suggests that the serotonergic system, in particular the –759C/T polymorphism affecting the 5-HT_{2C} receptor is much more promising in specifically predicting weight gain associated with the use of antipsychotics. Nevertheless, even with the abundance of research with neurotransmitter receptors and transporters, predicting psychotropic drug response remains a significant challenge (Table 7.4). Similar to CYP genotyping, the same limitations of lack of large-scale, prospective clinical trials, sample size, and ethnic variability need to be overcome. In addition, unlike drug metabolism, drug response is more likely to be mediated by multiple genes, and haplotype analyses would be critical in identifying appropriate association for prediction. Furthermore, disease progression could be impacted by environmental factors [256], which in turn, could impact treatment response and make interpretation of pharmacogenomic study data more difficult.

Application of Pharmacogenomics in Psychiatry

Drug development in psychiatry had made little progress over the last several decades. Although there have been better safety profiles for newer psychotropics, the CATIE study showed that the atypical antipsychotics represent only small improvement over the typical antipsychotics. Among all antidepressants, there were no real advantages of any newer SSRI over their older counterparts. Over the years, there have been many advances in

pharmacogenomics and expectations of what psychopharmacogenenomis could bring to psychiatric practice. Arguments for utilizing genetic information to maximize effectiveness of current drugs have been made by many investigators within the field of psychopharmacogenenomis. Nevertheless, there is still concern among many clinicians of the lack of clear evidence (based on large-scale randomized clinical trials) demonstrating when pharmacogenomic testing would be appropriate.

Clinical Validity and Utility of Psychopharmacogenomics

Genetic differences in psychotropic metabolism, most of which are mediated by the CYP enzyme systems, are well-established, and the frequency of drug-metabolizing enzyme polymorphisms also had been characterized in different ethnic groups. However, the limitation of single *CYP* gene screening is well-recognized [257], and clinical validity of such approach is only demonstrated for a few psychotropics that are significantly metabolized by one CYP isoenzyme. In addition, the small effect size for association between clinical outcomes and most of the *CYP* variants make the clinical significance somewhat questionable. The combinatorial pharmacogenomics approach combines different variant alleles to achieve more complete genomic information related to a drug, and has been advocated as a logical replacement for individual-gene testing [258,259]. Screening of different variant alleles for metabolizing enzymes, including common *CYP2C19* and *CYP2D6* alleles could be achieved with commercially available test panels [260], and is the most common application of pharmacogenomic advances in clinical practice when abnormal metabolic capacity is suspected to contribute to unexpected response [261]. However, even though multigene panels incorporating different genetic variants into a single assay is available [260], there is no standardization as to which pharmacokinetic and pharmacodynamic genetic variants are included in commercially available genetic test panels [262]. This lack of standardization is discussed further with respect to clinical implementation in Chapter 4.

Although current evidence demonstrates that most commercially available pharmacogenetic panels possess high analytic validity with good sensitivity and specificity in *CYP* genotype prediction (similar to the AmpliChip *CYP* genotyping test that was approved in 2004), demonstration of clinical benefit (clinical validity and utility) rests with the practitioners [261]. This not only leads to absence of specific dosing guidance from the regulatory agency for psychotropics, including atomoxetine, but also provides support against reimbursing *CYP* genotyping in psychopharmacotherapeutics. In addition, the availability of some of the gene analysis panels (primarily CYP1A2, CYP2C19, and CYP2D6), and a list of 26 psychotropic medications classified into different categories of recommendations that include "use as directed," "use with caution," and "use with caution with more frequent monitoring,"[263] but with little interpretation and/or guidance might be confusing to the consumers.

Not surprisingly, with very few well-designed clinical trials using patient-specific genotypes to demonstrate the clinical relevance of pharmacogenomic-guided dosing to optimize response rates and/or minimize adverse drug reactions, the utility of pharmacogenomics in clinical practice to influence prescribing pattern and patient outcome is almost nonexistent. Hall–Flavin et al. provided one of the few examples of potential utility and benefit of pharmacogenomic testing in the clinical environment. They first demonstrated in a prospective, proof-of-concept study that utilization of pharmacogenomic testing (*CYP1A2*, *CYP2C19*, and *CYP2D6* genotypes) in an outpatient setting resulted in significantly improved outcome in 44 patients (31.2% reduction in depression scores from baseline for pharmacogenomic-guided study participants compared to 7.2% in non-guided

participants (Quick Inventory of Depressive Symptomatology—Clinician Rated [QIDS-C16], $P = .002$) for different antidepressants and antipsychotics [263]. They then replicated the results in a follow-up prospective open-label study. Antidepressant response and remission rates in 227 patients were compared between genomic-guided prescribing (n = 114) with provision of pharmacogenomic report to clinician for their use, and usual care (n = 113) with no sharing of pharmacogenomicsinformation until completion of study. *CYP1A2*, *CYP2C19*, *CYP2D6*, *SLC6A4*, and *HTR2A* were the five genes available in the multigene test panel. After 8 weeks of therapy, patients receiving antidepressants based on genomic-guided interpretative reports provided to their prescribers had greater response (HAMD-17, $P = .03$; QIDS-C16, $P = .005$) and remission (QIDS-C16, $P = .03$) [264]. Despite the limitations of open-label design and lack of blinding of patients or clinicians that could be problematic with the well-known substantial placebo response to antidepressants, these two studies provide data (improved outcome) and perspective related to real-world application of pharmacogenomic testing. Such approach suggests an opportunity for incorporating pharmacogenomic data into clinical workflow for implementation in practice settings to guide treatment decision.

Cost-Effectiveness of Psychopharmacogenomics

In addition to clinical validity and utility, the issue of cost-saving remains uncertain. Chou and colleagues provided the earliest pilot utilization data in supporting potential cost-effectiveness of pharmacogenetic testing, consisting primarily of *CYP* genotyping at that time. The investigators genotyped 100 patients for *CYP2D6* and followed them over 1 year with assessment of adverse drug reactions, hospital stays, and total cost. They found three trends, including a higher incidence of side effects in patients with IM or PM phenotypes, a longer hospital stay for PMs, and an estimated higher annual cost of US$,4000 to $6,000 when treating patients with the extreme phenotypes (UMs and PMs) [47,265]. These results suggest that proper application of pharmacogenomic information could help reduce adverse drug events and better managing hospitalization duration, with resultant cost reduction. Subsequently, the cost-effectiveness for genetic testing with clozapine was evaluated [266]. Since then, more recent cost-effectiveness studies have also shown some encouraging data of reduced resource utilization and/or decreased average cost associated with pharmacogenomic testing. [34,258,267–272], including cost-effectiveness of pharmacogenomic testing in developed countries [271]. Implications of these study results will be explored further in Chapter 4.

Nevertheless, despite these real-world application results, additional studies with larger sample size are needed to validate the clinical utility of pharmacogenomic testing, including determining whether these multigene panels can shorten the remission time course, sustain duration of clinical remission, and reduce hospitalization and outpatient visits. In addition, such studies should expedite clinicians' decisions on medication choice or dosage adjustment with reasonable turnaround time for result reporting and interpretation [273], especially in patients from a diverse geographical locations and/or ancestral origins. In this regard, perhaps another approach to clinical psychopharmacogenomic investigations would be with a concentration-controlled trial to integrate relevant pharmacokinetic variants with important pharmacodynamic variants and complemented with PET evidence of drug-target occupancy, for example serotonin transporter occupancy for SSRIs [274,275]. Based on PET study, there is evidence of threshold 76%–85% serotonin-transporter occupancy for therapeutic response from different SSRI treatments [275–279]. In a 2001 study that investigated the

relationship between paroxetine concentration and serotonin-transporter occupancy, Meyer et al. showed the plateau occupancy of about 85% occurred when serum concentration of paroxetine exceeded 28 ng/mL [274]. Because paroxetine is metabolized by the polymorphic CYP2D6, the threshold drug concentration in the range of 28 ng/mL would not be achieved in some patients administered the standard dosage regimens, especially the UMs. One could argue that, without sufficient drug exposure at the target site, the relevance of any target polymorphism might be less. Data from this concentration-controlled approach might provide a more pragmatic design as an alternative to randomized controlled clinical trial, and hopefully an alternative perspective to the value of testing panels of genomic variants. For most practicing psychiatrists and clinicians, this may be more useful information than an endless list of potential and almost completely different sets of biomarkers of SSRI efficacy that have been identified by different GWASs [123–125,143].

Challenge Posed by Ethnic Variation in Allele Frequency

In assessing the clinical utility and cost-effectiveness of psychopharmacogenomic testing, the major challenges for drawing appropriate conclusions from drug-disposition and response investigations are undoubtedly related to differences in phenotypes (response definition, clinical presentation, treatment history), sample size (variable and mostly small), and different study designs (non-uniformed protocols and lack of standardization of data collection). In addition, significant variations in genetic background exist among various ethnic groups. Therefore, interpretation of psychopharmacogenomic findings in drug disposition and response among many of the study groups could be further complicated by regional differences in frequencies of known alleles and/or overinterpretation of data for a large region consisting of different racial or ethnic groups. Among people residing in the Pacific region, the frequency of *HLA-B*1502* risk allele for SJS is extremely high for subjects of Chinese heritage, but occur in less than 1% in Koreans and Japanese. Using ethnic variation in allele frequencies for genes encoding drug-metabolizing enzymes (CYP2C19 and CYP2D6) and targets (SLC6A4) that are relevant for antidepressant disposition and response, the following sections will highlight the importance of ethnicity definition and the implications of ancestry for psychopharmacogenomics research.

As described earlier in this chapter, the S-allele for 5-HTTLPR is associated with inferior response to SSRI therapy. However, this association appears to hold true primarily for Caucasian populations, whereas the opposite association (S allele conferring better therapeutic response) is observed in patients within Asian populations [83,87]. This may be partially related to ethnic-related differences in the frequency of the 5-HTTLPR L- and S allele, with the L allele as the predominant allele for Caucasians, whereas the S allele is the predominant variant for the Asian populations (Table 7.5).

The SNP rs25531, located just upstream of the 5-HTTLPR, was also shown to affect *SLC6A4* expression. The SNP results in expression level for the G allele that is comparable to that of the S allele for 5-HTTLPR, and much lower than that of the A allele for rs25531. Therefore, carriers of the L_G allele (G allele of rs25531) would be expected to respond less to SSRI compared to carriers of the L_A allele (A allele of rs25531). This has been shown by the study results of Dreimueller et al. The investigators reported a favorable therapeutic outcome in L_A allele carriers that was correlated with serum SSRI concentration ($P = .001$) but not in patients with the L_G allele ($P = .31$) [91]. Not surprisingly, significant ethnic variations in the triallelic and resulting genotype frequencies exist, as reported by Haberstick et al. The L_G allele for rs25531 is less frequent (<25%) than the L_A allele and the S allele for all studied populations from North America, Southeast Asia, and Africa. Among the different populations, the

TABLE 7.5 Ethnic Differences in Allele Frequencies (%) of 5HTTLPR and STin2 VNTR

Ethnicity	L Allele of 5HTTLPR	S Allele of 5HTTLPR	9-Repeat Allele of VNTR	10-Repeat Allele of VNTR	12-Repeat Allele of VNTR
Caucasians	60	40	1	47	52
African Americans	83	17	1	26	73
EAST ASIANS					
Chinese	26	74	<0.1	8	92
Japanese	20	80	<0.1	2	98
Koreans	23	77	<0.1	10	90
SOUTH ASIANS					
Chinese		64			
Indian		58			
Malay		61			
Hispanics	49				N/A

Pooled data from references S. Porcelli, C. Fabbri, A. Serretti, Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with antidepressant efficacy, European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology 22 (2012) 239–258, T. Niitsu, C. Fabbri, F. Bentini, A. Serretti, Pharmacogenetics in major depression: a comprehensive meta-analysis, Progress in Neuro-psychopharmacology and Biological Psychiatry 45 (2013) 183–194.

frequency of the L_G allele is lower (absence or near absence) for Hispanics, Caucasians, and Native Americans than in Asians and Africans or African Americans [280].

In addition to 5-HTTLPR, additional polymorphisms of the *SERT* gene have been identified with potential roles in modulating SSRI response. This include the extralong allele with high-frequency occurrence in Asians, African Americans, and non-Hispanic Whites reported by Haberstick et al. [280]; 14 novel allele variants in Japanese and Caucasians, all with variable distribution frequency [88]; and the 17-base pair, variable number of tandem repeats (VNTR) polymorphism within intron 2 of *SLC6A4* [93], with higher allelic frequency in Asians compared to Caucasians (Table 7.5) [281]. Therefore, it is clear that even for *SLC6A4*, response prediction would have limited success if evaluating only single SNPs in a given patient, which could be further impacted by the significant ethnic variation in frequency of important alleles, small number of study participants from various ethnic populations, and the use of self-identity for defining ethnicity in pharmacogenomic investigations.

Another issue for considering ethnicity as a variable in associating response with genetic variants is the ancestral origin of the subject cohort. Although it might be commonly perceived that Asian populations in regions of close proximity are relatively "homogeneous," that might not be necessarily true in reality. As an example, the South Indian populations as a group are genetically distinct from the North Indians and East Asians[282]. Similarly, early pharmacogenetic studies reported population differences in distribution of *CYP2C19* and *CYP2D6* variants and genotypes exist among Han, Bai, Wei, Zang, and Mongolian subpopulations in China [283,284]. More recently, Suarez–Kurtz et al. also reported ancestral influence on frequency distributions of different pharmacogenomic genes among three Brazilian populations with different ancestral roots [285].

In this regard, even though there is no unanimous accepted definition of ethnicity and race, and maybe even ancestry, it is important to recognize that "ethnicity" and the usually interchangeable term "race" are not biological terms. Rather, they are sociological terms describing groups of people with common heritage and sharing similarities in culture, beliefs, values, and possibly language as well as cuisine. Ancestry, on the other hand, is a biological term in population genetics, and represents the origin or genetic line of descent of one individual or family. Although outside the scope of this chapter, it is generally accepted that genetic diversity has been "modified" since the first human colonization of Europe more than 40,000 years ago, resulting in admixed populations around the world with variable extent of genetic diversity from African, European, and Native American ancestries. This undoubtedly has an influence on allele frequencies of any gene of interest among global populations, as discussed earlier with the *SLCA64* gene.

One can even further argue that the complicated issues associated with admixed populations and self-identified ethnicity discussed in aforementioned sections are the reasons that data extrapolation from one ethnic/racial group to another should be minimized. Nevertheless, while awaiting future pharmacogenomic studies enrolling large numbers of cohorts from under-represented ethnic minority groups or development of dosing guidelines/algorithms specific to individual ethnic groups or admixed populations, ethnicity-related genetic information may still be useful for practitioners when no genotyping result is available. A good example is carbamazepine. Regardless of the exact reason why *HLA-A*3101* is of high prevalence in Caucasians versus *HLA-B*1502* is of high frequency in Asians, the fact remains that in the absence of genotype information, one could make a rational therapeutic decision and advocate use of other antiepileptic drugs instead of carbamazepine or phenytoin in patients of Chinese heritage.

In summary, the "increased' genetic diversity represented by admixed populations [286] in pharmacogenomic studies presents further challenges to assessment of association between genetic variants and pharmacological responses in studies not properly stratified by ethnic groups. The challenge of interpreting different, sometimes even contrasting, allele frequencies reported from multiple studies of gene variants is further compounded by subject cohorts with self-identified ethnicity. Although financial restraints and "ease-of-use" are legitimate reasons allowing self-identified ethnicity to categorize study subgroups, the absence of allele(s) important for response assessment from pharmacogenomic studies or test panels would complicate interpretation of study results, as illustrated with the case of warfarin and highlighted in Chapter 6.

Clinical Application in Selective Patients

From the perspective of *individualized* therapy, *individual* difference in drug response is attributable to his or her specific genotype for the gene variant of interest. Hence, in this regard, ancestral origin and/or ethnicity (regardless of whether it is well defined or self-identified) of the patient is not necessarily a good predictor of pharmacological response. This is illustrated with the example of James Watson, who, despite being self-identified as a Caucasian, is a homozygous carrier of the *CYP2D6*10* allele [287]. Therefore, despite the rare occurrence of this **10* variant in the overall Caucasian population, Dr. Watson would be expected to metabolize CYP2D6 substrates at a rate similar to that of most Asians, which is slower than most Caucasians. The following sections present how psychopharmacogenomics information could be used in optimizing therapy for individual patients.

Most of the literature focuses on assessing potential improved efficacy and/or reduced toxicity with pharmacogenomic testing, and very few studies evaluate the potential utility

for genetic testing to guide appropriate use of alternative drug therapy. Although there are suggestions that homozygous carriers of S allele of *SLC6A4*, especially Caucasians, would be least likely to benefit from SSRI, few literature data document the clinical outcome, let alone cost-effectiveness, of switching to antidepressants other than SSRIs. Rather than waiting for affirmative studies or consensus guidelines, which might not happen for years to come, perhaps one value of pharmacogenomic testing in clinical psychiatric practice is to help determine the basis for an individual patient's lack of response and/or exhibition of unusual adverse reactions to drug regimens. Leahy described an 18-years-old patient with intermittent explosive disorder who had failed multiple medication regimens, including fluoxetine and escitalopram (produced side effects of restlessness and diarrhea in the patient), as well as risperidone, aripiprazole, and ziprasidone (produced side effects of irritability and weight gain). The patient consented to pharmacogenomic testing, which revealed that he is a heterozygous carrier of the S allele of *SLC6A4* and the risk allele of *DRD2* rs1799732, as well as a homozygous carrier of the C allele of the *5HT2C* rs3813929. This genetic profile provides a biological basis for his poor response to SSRIs and dopamine-2 receptor antagonists, as well as his history of intolerable weight gain associated with the use of the atypical antipsychotic agents. Just as importantly, the pharmacogenomic analysis suggested that the patient is likely not a candidate for drug that targets the SLC6A4, DRD2, or 5HT2C. Based on this information, a trial of lithium was initiated for the patient and titrated to achieve a target concentration of 1 mEq/L, which resulted in decreased outbursts and disappearance of extreme rage. Over a 3-month period after starting lithium, the patient only exhibited two brief anger episodes, both of which were of much less severity and much shorter duration compared to those before initiation of lithium therapy [288].

Another example of using genetic profile to identify appropriate alternate therapy involves variants of *MTHFR*, which encodes methylenetetrahydrofolate (MTHFR). MTHFR is an important enzyme involved in the pathway that produces methylfolate and the mood-regulating monoamine neurotransmitters. The C677T is a *MTHFR* variant associated with decreased MTHFR activity and methylfolate level, ultimately resulting in impaired synthesis of neurotransmitter, increased risk of depression, and reduced response to antidepressants [289,290]. The use of L-methylfolate after identification of the C677T variant was reported in a 69-years-old Caucasian male patient with major depressive disorder. The patient failed duloxetine therapy and partially responded to venlafaxine. Pharmacogenomic analysis revealed the patient as a carrier of the S allele of *SLC6A4*, the C677T variant of *MTHFR*, as well as a homozygous carrier of the Val allele of *COMT* and the C allele of the −759C/T polymorphism. The patient's genetic profile provides an insight into his therapeutic responses. Duloxetine has higher selectivity for serotonin and norepinephrine transporters. The Val allele is associated with higher COMT activity and partially explains his apathy, poor concentration, and lack of motivation. After evaluating his genetic profile, the clinician initiated a trial of 15 mg of L-methylfolate, which resulted in a complete remission of symptoms [291].

Therefore, a potential *practice* model for patient care, especially in primary care settings, could involve using the electronic health record to identify patients who can potentially benefit from pharmacogenomic testing. Clinical pharmacists can then perform comprehensive medication review and strategies for best-candidate genes. Results from testing can then be used to guide any necessary medication changes in patients with suboptimal control of symptomatology, with further patient evaluations using standardized clinical ratings and additional medication

monitoring. Further contribution to advancing the field can involve collecting standardized data from the healthcare professionals regarding the impact of the testing for the providers and the patients. The value from such an approach would be an emphasis on how pharmacogenomic testing results in appropriate drug selection with improvement in outcome and reduction in associated healthcare cost in an individual patient, rather than whether pharmacogenomic testing should be part of the standard of psychiatric care.

Incorporation in Drug Development

Chapter 3 provides several examples of how pharmacogenomic data can be incorporated in drug development. The following section will illustrate another example of pharmacogenomics application.

Vilazodone, approved by the FDA in January 2011, is the first of a new class of antidepressant (the indolealkylamines) with dual action of serotonin reuptake inhibition and partial agonist activity at the 5-HT_{1A} receptor [292]. The initial development of vilazodone in Phase II was discontinued because response was not significantly better than placebo, even though studies incorporating an active comparator also showed failure of comparators demonstrating superiority over placebo [293]. Subsequent development included a clinical trial with patient stratification according to a combination of genetic biomarkers most likely to be associated with therapeutic response to vilazodone but not to other antidepressants [293].

A report described the association of haplotypes of biomarkers involved in neurotransmitter signaling and vilazodone metabolism with clinical response, although the identity of the biomarkers was not revealed. The result indicated that 75.5% of 49 vilazodone-treated patients who also possess one specific biomarker ($M1^+$) responded to therapy (defined as a decrease of at least 50% from the baseline Montgomery–Asberg Depression Rating Scale [MADRS] score after 8-weeks of treatment), whereas only 35.2% of 108 "marker-negative" patients ($M1^-$) treated with vilazodone. Remission (defined as final MADRS score of less than 10) was achieved in 44.9% of "marker-positive" patients and 20.4% of "marker-negative" patients. 57.1% of 14 vilazodone-treated patients with another biomarker ($M2^+$) were reported to have nausea and vomiting compared to 15.5% of patients without the same biomarker [294]. Despite the small number of patients, the study represents an example of early use of biomarkers in drug development. Vilazodone studies listed on ClinicalTrials.gov include one that investigates genetic markers associated with response in major depressive disease. When published, results from this trial and those from ongoing replication studies will provide insight as to whether these biomarkers allow clinicians to predict which patients might respond more fully to vilazodone and who would experience adverse side effects. If confirmed, the unique dual pharmacological action of vilazodone and availability of clinically relevant biomarkers could provide significant contribution to individualized clinical treatment.

CONCLUSIONS

Psychopharmacogenomic research over the last decade or so has attempted to associate treatment response with neuronal circuits upstream and regulatory genes downstream [134–137,144–146]. Despite many findings within the field of psychopharmacogenomics, only a few of the results are ready for translation into clinical practice. Although *CYP* genotyping was previously recommended for incorporation into the therapeutic decision-making process, the current evidence-based approach significantly limits its application in clinical practice. Compared to other therapeutic areas such as cardiovascular disease and

cancer, promising research findings to predict drug response in psychiatric illnesses is still in its infancy. Multiple genetic biomarkers have been identified by either candidate-gene approach or GWAS, and evaluated in clinical studies involving different designs and various ethnic populations. To date, lack of consistent results among the clinical studies does not point to definitive associations for most biomarkers. However, that should not preclude the rational use of psychopharmacogenomic test panels to guide choice of therapy for patients in clinical practice, especially for those who could not respond to, or are intolerant of, evidence-based first-line therapies.

Given the currently available psychotropics and the lack of novel compounds in the foreseeable future, pharmacogenomics hold significant promise in optimizing drug therapy for the mentally ill populations. Further advances in the field would require indepth understanding of mental-disease etiology, developing clear definitions of response phenotypes and outcome measurements, and refining current molecular approaches. Pharmacogenomic results can nevertheless be incorporated into a decision-making model to enable a genetically informed and data-driven approach to optimize therapy for individual patients.

QUESTIONS FOR DISCUSSION

1. How do ethnic variabilities in allele frequencies affect interpretation of study results in psychopharmacogenomics?
2. Are there significant roles for *ABCB1* polymorphism in psychotropic disposition and response?
3. What is the significance of the STAR*D study with respect to 5HTTLPR polymorphism?
4. What are some of the factors that slow translation of pharmacogenomic findings into practice for psychopharmacology, in contrast to other therapeutic areas such as oncology and cardiovascular diseases?

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