No association of *EGF* polymorphism with schizophrenia in a Japanese population

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Epidermal growth factor (EGF) signal regulates the development of dopaminergic neurons and monoamine metabolism. It is suggested that EGF protein levels are decreased in the brain and blood of patients with schizophrenia. A recent study has reported that a polymorphism in *EGF* gene (rs4444903) is associated with schizophrenia in Finnish men. To confirm this association for another population in larger samples, we conducted a case–control association study on a Japanese population (337 cases and 421 controls). No significant difference was observed in both the allelic and genotype distribution between cases and controls in women, men and total samples. Our results suggest that the polymorphism in *EGF* gene might not confer increased susceptibility for schizophrenia in a Japanese population. *NeuroReport* 16:403–405 © 2005 Lippincott Williams & Wilkins.

Key words: Association study; Cytokine; Epidermal growth factor; Schizophrenia; Single nucleotide polymorphism

INTRODUCTION

Schizophrenia is a complex genetic disorder that affects approximately 1% of the population worldwide. Cytokines might be implicated in the etiology or pathology of schizophrenia [1], although the pathogenesis of schizophrenia is still unclear. Epidermal growth factor (EGF) has several effects on dopaminergic neurons: stimulating neurite outgrowth, increasing dopamine uptake and enhancing long-term survival in cultured dopaminergic neurons [2,3]. Subchronic peripheral EGF administration into neonatal rats resulted in abnormal dopamine metabolism in the striatum and brain stem of adult rats [4]. In parallel, rats treated with EGF during the neonatal period displayed various behavioral abnormalities in adulthood [4,5]. Some of these behavioral abnormalities, which are also present in an animal model of schizophrenia, were ameliorated by subchronic treatment with antipsychotics [4]. In the post-mortem brain and fresh serum of patients with schizophrenia, EGF and its receptor protein levels were altered [6]. Shahbazi *et al.* [7] identified a G to A polymorphism at position 61 in the 5′ untranslated region of *EGF* gene, located on chromosome 4q25–q27. Interestingly, blood mononuclear cell cultures from individuals with G/G or A/G genotypes produced significantly more EGF than cells from individuals with A/A genotype, suggesting that this polymorphism might be functional [7]. Recently, Anttila *et al.* [8] reported that the G allele was associated with schizophrenia in Finnish men. To assess whether this functional polymorphism in *EGF* gene could be implicated in vulnerability to schizophrenia, we performed a case–control association study in Japanese samples.

MATERIALS AND METHODS

Participants: Participants included 337 patients with schizophrenia (158 women and 179 men) and 421 controls (207 women and 214 men). The mean ages and SD of the cases and controls were 42.0±15.0 and 38.3±10.5 years, respectively. All the participants were unrelated Japanese living in Niigata. Patients meeting the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria for schizophrenia were recruited from nine hospitals: Kohdo Hospital, Matsuhama Hospital, Minamihama Hospital, Niigata Prefectural Psychiatric Center, Niigata University Hospital, Ohjima Hospital, Sado General Hospital, Shirone-Kensei Hospital and Shirone-Midorigaoka Hospital. The controls were healthy volunteers with no history of psychiatric disorders. Written informed consent was obtained from all patients before their participation in this study. This study was approved by the Ethics Committee on Genetics of Niigata University School of Medicine.

Genotyping: Genomic DNA was extracted from peripheral blood by the standard phenol/chloroform method. A single nucleotide polymorphism at position 61 of *EGF* gene (rs4444903) was genotyped by the TaqMan 5′-exonuclease assay. The primer and probe set was designed and synthesized by Applied Biosystems (Foster City, California, USA). We carried out polymerase chain reaction amplification using TaqMan 2 × Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng of DNA, 0.9 μM of each primer and 200 nM of each probe in a total volume of 5 μl. Each 96-well plate contained 94 samples and two no-DNA template controls. Thermal cycler conditions were 95°C for

Table I. Allele and genotype frequencies of *EGF* gene polymorphism in cases and controls.

	Women		Men		Total	
	Cases (n=158)	Controls (n=207)	Cases (n=179)	Controls (n=214)	Cases (n=337)	Controls (n=421)
Allele						
A	96 (30.4%)	117 (28.3%)	117 (32.7%)	138 (32.2%)	213 (31.6%)	255 (30.0%)
G	220 (69.6%)	297 (71.7%)	241 (67.3%)	290 (67.8%)	461 (68.4%)	587 (70.0%)
Genotype						
A/A	10 (6.3%)	18 (8.7%)	19 (10.6%)	22 (10.3%)	29 (8.6%)	40 (9.5%)
A/G	76 (48.1%)	81 (39.1%)	79 (44.1%)	94 (44.0%)	155 (46.0%)	175 (41.5%)
G/G	72 (45.6%)	108 (52.2%)	81 (45.3%)	98 (45.7%)	153 (45.4%)	206 (49.0%)

10 min, 40 cycles of 92°C for 15 s and 60°C for 1 min. Fluorescence and allelic discrimination were measured with an ABI PRISM 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems).

Statistical analysis: Deviation of Hardy–Weinberg equilibrium was tested by using the χ^2 test for goodness of fit. The allele and genotype frequencies between cases and controls were compared using the χ^2 test. A probability level of $p<0.05$ was considered to be statistically significant.

RESULTS

The genotype distributions in cases and controls had no deviation from Hardy–Weinberg equilibrium (χ^2=1.4, df=2, p=0.50 for cases; χ^2=0.1, df=2, p=0.95 for controls). Allele and genotype frequencies of *EGF* gene polymorphism (rs4444903) are presented in Table 1. No significant difference was observed in the allelic distribution between cases and controls in women, men and total samples (χ^2=0.3, df=1, p=0.59; χ^2=0.003, df=1, p=0.96 and χ^2=0.2, df=1, p=0.62, respectively). Also, no significant difference was observed in the genotype distribution between cases and controls in women, men and total samples (χ^2=3.1, df=2, p=0.21; χ^2=0.02, df=2, p=0.99 and χ^2=1.5, df=2, p=0.47, respectively).

DISCUSSION

Anttila *et al.* [8] found that the functional polymorphism in *EGF* gene was associated with schizophrenia in Finnish men. In contrast, we failed to confirm this association in our Japanese samples, suggesting that this polymorphism does not play a major role in conferring susceptibility to schizophrenia. This discrepancy might stem from an ethnic heterogeneity in the functional polymorphism in *EGF* gene as in the promoter variants of *tumor necrosis factor* α gene [9] in schizophrenia. Our sample size (337 cases and 421 controls) is larger than that of Anttila *et al.* (94 cases and 98 controls). Using the Genetic Power Calculation [10], our sample has a power of 0.77 to detect a significant association between the G allele and schizophrenia with an α of 0.05, assuming a disease prevalence of 0.01, a risk allele frequency of 0.7, and a genotypic relative risk of 2.0 for G/G and of 1.5 for A/G. Accordingly, the likelihood of type II error with our sample size appears to be considerably low. However, additional studies of the functional polymorphism in *EGF* gene to evaluate across other ethnic populations are needed to draw a conclusion.

Futamura *et al.* [6] reported that EGF protein levels were decreased in the prefrontal cortex and striatum of patients with schizophrenia in Japanese samples. Conversely, EGF receptor expression was significantly elevated in the prefrontal cortex [6]. Serum EGF protein levels were also decreased in patients, even in young drug-free patients [6]. In experimental animals, EGF treatment during the neonatal period transiently increased tyrosine hydroxylase expression and resulted in abnormal dopamine metabolism in adulthood [4]. The neonatally EGF-treated rats displayed schizophrenia-like behavioral impairments such as hyperactivity, decreased prepulse inhibition, decreased social interaction and hypersensitization to psychostimulants in adulthood [4,5]. These observations suggest that EGF signaling might be implicated in the etiology or pathology of schizophrenia. Although we found no association between the functional polymorphism in *EGF* gene and schizophrenia in our Japanese samples, there is the possibility that the genes of other EGF signaling molecules such as EGF family cytokines and their receptors are associated with schizophrenia [11]. In addition, our result also does not imply the exclusion of the contribution of other polymorphisms in *EGF* gene to the pathogenesis of schizophrenia, because *EGF* gene is a very large gene spanning over 110 kbp.

CONCLUSION

We conclude that the functional polymorphism in *EGF* gene (rs4444903) does not play a major role in conferring susceptibility to schizophrenia in a Japanese population. However, several studies indicate that EGF signaling might be implicated in the etiology or pathology of schizophrenia. It will be necessary to evaluate EGF signaling molecules and other polymorphisms in *EGF* gene in several ethnic populations for association with schizophrenia.

Electronic-Database Information

URLs for data presented herein are as follows:

Genetic Power Calculator, http://wbiomed.curtin.edu.au/genepop/

National Center for Biotechnology Information, Single Nucleotide Polymorphism Database, http://www.ncbi.nlm.nih.gov/SNP/ (for reference identification numbers for SNP).

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Regular Article

Linkage disequilibrium in *aquaporin 4* gene and association study with schizophrenia

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Abstract Aquaporin 4 (AQP4) has an important role in water homeostasis of human brain and a dysfunction of AQP4 could induce pathological conditions in neuronal activity. Several genome scan studies for schizophrenia found a suggestive linkage on 18q, where human *AQP4* (18q11.2–12.1) is located nearby. A case-control study was performed which comprised 261 schizophrenia subjects and 278 controls from the Japanese population with four SNP markers. We found strong linkage disequilibrium (LD) and an LD block in the *AQP4* gene but found no association between *AQP4* and schizophrenia, both single SNP and haplotype analyses. The present study shows that *AQP4* is not directly associated with schizophrenia in these Japanese patients.

Key words aquaporin, case-control study, haplotype, schizophrenia, single nucleotide polymorphism.

INTRODUCTION

Water homeostasis in the brain is important in both physiological and pathological conditions. Neuronal activity and ion water homeostasis are coupled. Water channel proteins, aquaporins, are membrane proteins that mediate the selective efficient movement of water across the cell membrane. In 11 isoforms of the aquaporin family, aquaporin 4 (AQP4) is most highly expressed in brain, including ependymal cells, pial cells, paraventricular hypothalamic nucleus, hippocampus, and cortex of the cerebrum.[1–5] Since AQP4 is supposed to play an important role in potassium buffering in a neuronal environment,[6,7] a dysfunction of AQP4 could induce pathological conditions in neuronal activities.

Schizophrenia is a complex genetic disorder with high heritability (approximately 80%) and shows a wide variety of behavioral and cognitive symptoms caused by brain dysfunctions. Although genetic factors of schizophrenia still remain to be elucidated, several genome scan studies for schizophrenia revealed that a suggestive linkage is on chromosome 18q,[8,9] which is in the vicinity of human *AQP4* (18q11.2–12.1). Williams *et al.* found a multipoint MLS of 1.62 near D18S450 which is located on 18q12,[8] and Paunio *et al.* also found a Z_{max} score of 2.37 at D18S877 on 18q12 in Finnish population.[9] Hence, we examined whether AQP4 is involved in susceptibility to schizophrenia performing an association study between the *AQP4* gene and schizophrenia.

MATERIALS AND METHODS

The subjects in the present study were 261 schizophrenic patients (137 males and 124 females) and 278 of control subjects (149 males and 129 females). All the subjects were Japanese and the controls were healthy volunteers with no history of psychiatric disorders confirmed by clinical interviews. The mean ages of the cases and controls were 45.7 ± 14.1 and 41.7 ± 10.6 years, respectively. All patients were diagnosed as having schizophrenia according to the DSM-IV criteria.[10] No patient or control was related. All subjects were residents of the Niigata area, in northern Japan. Written informed consent was obtained from all subjects prior to their participation in this study. The study was approved by the ethics committee on genetics of the Niigata University School of Medicine.

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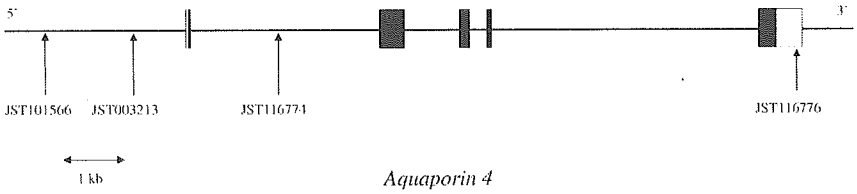

Figure 1. Locations of the SNPs used in this study. *Aquaporin 4* gene has five exons (rectangles) and spans more than 10 kb. JST101566 and JST003213 is 2.2 kb and 0.8 kb away from translation initiation site of *AQP4*, respectively. JST116774 is located on the first intron and JST116776 is located on the 3′-non-coding region of *AQP4*. Solid rectangles indicate coding regions.

Table 1. Genotype and allele frequencies in cases and controls

	Genotype								Statistics			
	Case ($n = 261$)				Control ($n = 278$)				Genotype		Allele 1 versus Allele 2	
SNP	11	12	22	Minor allele freq.	11	12	22	Minor allele freq.	χ^2	P (d.f. = 2)	χ^2	P (d.f. = 1)
JST101566 (rs162004) Allele 1; C, Allele 2; G	125	109	27	0.312	138	116	24	0.295	0.50	0.78	0.30	0.58
JST003213 (rs162006) Allele 1; G, Allele 2; A	112	115	34	0.351	120	123	35	0.347	0.02	0.99	0.003	0.97
JST116774 (rs162009) Allele 1; C, Allele 2; T	115	111	35	0.347	124	121	33	0.336	0.29	0.86	0.09	0.77
JST116776 (rs3763043) Allele 1; G, Allele 2; A	94	120	47	0.410	89	145	44	0.419	2.06	0.36	0.06	0.81

We selected four SNPs, JST101566 (dbSNP ID; rs162004), JST003213 (rs162006), JST116774 (rs162009) and JST116776 (rs3763043) from the JSNP database.[11] Their order and physical location are shown in Fig. 1. SNPs were genotyped by the TaqMan assay. The probe sets were designed and synthesized by Applied Biosystems, Foster City, CA, USA. We carried out polymerase chain reaction using TaqMan Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng DNA from leukocytes of the subjects, 0.9 μM each primer and 200 nM each probe in total volume of 5 μL. Each 96-well plate contained 94 samples and two no-DNA template controls. Thermal cycler conditions were 95°C for 10 min, 40 cycles of 92°C for 15 s and 60°C for 1 min. Measuring the fluorescence and allelic discrimination were performed on an ABI PRISM 7900HT Sequence Detection System with SDS 2.0 software (Applied Biosystems).

We analyzed association and Hardy–Weinberg equilibrium using the χ^2 test. Haplotype frequencies were estimated using the expectation maximization algorithm. Pairwise linkage disequilibrium (LD) indices, D′ and r^2, were calculated in the control subjects.[12,13] We identified a strong LD region from JST101566 to JST116776, therefore we assessed haplotype association between cases and controls. In this analysis, rare haplotypes with frequencies less than 2% were not assessed. To detect a haplotype block, we used Haploview program (http://www.broad.mit.edu/mpg/haploview/).[14] The level of significance was 0.05.

RESULTS

The genotype frequencies of all SNPs in both groups were not significantly different from the values expected from Hardy–Weinberg equilibrium (all $P > 0.50$, d.f. = 2). Table 1 shows the allele and genotype frequencies for each SNP. The allele and genotype frequencies of the patients with schizophrenia did not differ from those in the controls. Table 2 shows strong LD among three SNPs, JST003213, JST116774 and JST116776. A LD block comprises of three SNPs, from JST003213 to JST116776, which was showed by Haploview, contains almost all the region of *AQP4* gene except for more than 1 kb 5′ upstream. These findings may indicate that 5′ regulatory region of the gene and all exons of *AQP4* are on different LD blocks. Next, we analyzed the haplotype structures constituted with

Table 2. Linkage disequilibrium indices (D′ and r^2, above and below diagonal, respectively) in control subjects

SNP and the distance between adjacent SNPs	JST101566	JST003213	JST116774	JST116776
JST101566 (rs162004)	–	0.674	0.685	0.008
1.4 kb				
JST003213 (rs162006)	0.101	–	0.966	0.882
2.3 kb				
JST116774 (rs162009)	0.099	0.883	–	0.982
8.4 kb				
JST116776 (rs3763043)	0.000	0.299	0.350	–

Table 3. Haplotype structures of *AQP4* and their frequencies

Haplotype	SNP JST003213	SNP JST116774	SNP JST116776	Haplotype frequency Case	Haplotype frequency Control	Permutation *P*
Haplotype A	1	1	2	0.406	0.414	0.802
Haplotype B	2	2	1	0.348	0.334	0.657
Haplotype C	1	1	1	0.246	0.252	0.830

1 and 2 denote major and minor alleles, respectively.

these three SNPs. Three haplotypes were estimated to be more than 2% frequency in both cases and controls. No difference was observed in these haplotype frequencies between two groups (Table 3).

DISCUSSION

Water homeostasis is frequently disturbed in patients with schizophrenia, as in a case of polydipsia (pathological excessive water drinking). A potential function of AQP4 is regulation of the neuronal environment through potassium buffering. In addition, *AQP4* locus is within a suggestive linkage region with schizophrenia. From these points of view, *AQP4* is a candidate gene for susceptibility of schizophrenia. However, we could not find association in either the SNP study nor in the haplotype analysis. Using the Genetic Power Calculation[15] (http://statgen.iop.kcl.ac.uk/gpc/), our sample has powers of 0.97–0.98 to detect a significant association between each minor allele and schizophrenia with an α of 0.05, assuming a disease prevalence of 0.01, each risk allele frequency of each observed minor allele frequency in cases, and a genotypic relative risk of 2.6 for homozygotes of minor allele and of 1.8 for heterozygotes. Accordingly, the likelihood of type II error with our sample size appears to be considerably low.

We found strong LD in the region between JST003213 and JST116776 in the Japanese population, consequently these SNPs that we tested can provide most of the information on the pattern of genetic variation in this region. For that reason, we did not examine any extra SNPs in this study. In the international HapMap project[16] data (http://www.hapmap.org), a similar pattern of strong LD is observed in a region containing whole *AQP4* gene and nearly 1 kb of 5′-upstream region in four different populations (Utah residents with ancestry from northern and western Europe, Han Chinese in Beijing, China, Japanese in Tokyo, Japan and Yoruba in Ibadan, Nigeria).

We found no association between *AQP4* gene and schizophrenia but the possibility that AQP4 is involved in other brain disorders remains to be clarified. Our findings about LD can facilitate further investigations for a molecular genetic study of *AQP4*.

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A Candidate Pathway Strategy for Integration of Pharmacogenomic Components of Variability in Antipsychotic Treatment Outcomes: A Focus on Aripiprazole

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Abstract: Aripiprazole is the first atypical antipsychotic introduced to medical practice with partial dopamine-serotonin agonist properties. Other new molecular entities such as bifeprunox, a partial agonist at the dopamine D2 and serotonin 5-HT_{1A} receptors, are currently being evaluated in early stage drug development as potential antipsychotic agents. As a partial agonist, whether aripiprazole displays an agonist effect or attenuates dopaminergic neurotransmission may depend on regional variations in endogenous dopamine tone. Hence, aripiprazole offers a therapeutic advantage to differentially modulate dopaminergic activity in brain regions in a graded fashion. This mechanism of action is intriguing when considered in the context of the dopamine hypothesis of schizophrenia whereby positive symptoms (e.g. hallucinations and delusions) are associated with increased mesolimbic dopaminergic activity while reduced activity in mesocortical dopaminergic pathways underlies negative symptoms (e.g. avolition and anhedonia) and cognitive deficits. Despite its therapeutic promise, antipsychotic response to aripiprazole is highly variable, and some patients do not respond at all to drug therapy. Treatment-emergent adverse events associated with aripiprazole include insomnia, anxiety, akathisia or worsening of psychosis in some patients. These observations suggest that the underlying mechanism of action of aripiprazole in psychotic disorders is more complex than what would be anticipated solely by simple partial agonist effects at the dopamine D2 receptor. For example, while aripiprazole attenuates dopaminergic hyperactivity it does not increase locomotor activity in reserpinized (hypodopaminergic) rats, which is not fully consistent with a partial agonist mode of action.

Aripiprazole can induce a diverse range of effects at dopamine D2 receptors (agonism, antagonism, partial agonism) depending on the cellular milieu defined by promiscuous interactions with a host of signaling partners and variability in local G protein complement and concentration. This diversity provides an opportunity to illustrate the importance of integrating data on genetic variation in pharmacokinetic pathways and molecular targets for antipsychotics including biogenic amine receptors and their downstream signaling partners. Theragnostics, a new subspecialty of molecular medicine formed by combination of therapeutics with diagnostics, offers the potential to synthesize different types of biomarkers (DNA and protein-based) in the context of antipsychotic treatment outcomes. Because the dopamine receptor genetic variation is extensively reviewed elsewhere, we discuss the pharmacogenomic significance of variability in genes encoding for the 5-HT_{1A} (*HTR1A*) and 5-HT_{2A} (*HTR2A*) receptors and CYP2D6- and CYP3A4-mediated aripiprazole metabolism. As the field moves toward predictive genetic testing for newer antipsychotics, we emphasize the need for collaboration among pharmacogeneticists, bioethicists and specialists in science and technology studies.

Key Words: Aripiprazole, OPC-14597, pharmacogenomics, atypical antipsychotics, genetic biomarkers, personalized therapeutics, CYP2D6, CYP3A4, HTR1A, HTR2A, bioethics.

1. INTRODUCTION

The discovery of chlorpromazine in 1952 led to development of typical antipsychotics with full antagonistic properties at the dopamine D2 receptor for the treatment of schizophrenia and other psychotic disorders. Over the past two decades, serotonin/dopamine antagonists such as clozapine signaled the development of a second wave of "atypical" antipsychotic compounds that displayed enhanced drug safety profiles, most notably through reduction of risk for extrapyramidal symptoms (EPS), as well as improvements in negative symptoms and cognitive deficits of schizophrenia [Marder *et al.* 2002].

Aripiprazole is the latest atypical antipsychotic introduced to medical practice [Davies *et al.* 2004]. In contrast to

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previous atypical antipsychotics that act as full antagonists at the serotonin and the dopamine receptors, aripiprazole displays partial agonist actions on the dopamine D2, D3 and the serotonin (5-hydroxytryptamine, 5-HT) 5-HT_{1A} receptors and antagonist effects on the 5-HT_{2A} receptor [Aihara *et al.* 2004; Shapiro *et al.* 2003; Jordan *et al.* 2002; Lawler *et al.* 1999]. The renewed optimism for the treatment of schizophrenia, in large part driven by the availability of atypical antipsychotics, has been hampered by the emergence of a new class of side effects typified by excessive weight gain and disturbances in lipid and glucose homeostasis [Nasrallah and Newcomer, 2004]. In addition, similar to conventional antipsychotics, 20% to 30% of patients treated with atypical antipsychotics fail to respond while other patients may be noncompliant to therapy due to weight gain or concerns for drug safety. To this end, it is noteworthy that recent systematic reviews of clinical trials have further reframed the current thinking on aripiprazole and the broader discussions on the effectiveness and safety of atypical antipsychotics [Stip, 2002]. For example, Leucht *et al.* [2003] conducted a meta-analysis of randomized controlled trials where atypical antipsychotics were compared with low-potency (equivalent or less potent than chlorpromazine) typical antipsychotics. They found that mean doses of chlorpromazine at less than 600 mg/day or its equivalent had no higher risk of EPS than new generation drugs [Leucht *et al.* 2003]. An earlier meta-regression analysis by Geddes *et al.* [2000] of more than 12,000 patients drawn from 52 randomized trials comparing atypical (amisulpride, clozapine, olanzapine, quetiapine, risperidone, and sertindole) and typical antipsychotics (e.g. haloperidol or chlorpromazine) suggested that the risk-benefit ratio of typical antipsychotics may approach that observed with newer generation antipsychotics when the former are used at an optimal dose or concentration. Meta-analyses may not, however, able to identify drug effects in 'niche' populations or qualitative measures of therapeutic outcomes expressed by the patients [Kapur and Remington, 2000; Kerwin, 2001]. Nonetheless, these data collectively point toward the importance of developing biomarkers, or predictive tests that can better delineate the patient subpopulations wherein aripiprazole and the new generation atypical antipsychotics may display improved therapeutic efficacy and further differentiation from older typical antipsychotics.

It is notable that numerous lead compounds are presently being evaluated in clinical trials as atypical antipsychotic candidates for therapeutic use in schizophrenia, bipolar disorder or other psychotic disorders [Grady *et al.* 2003]. For example, bifeprunox (DU-127090) is another partial agonist at dopamine D2 (K_i = 3.2 nM) and 5-HT_{1A} (K_i = 10.0 nM) receptors but appears to be devoid of activity at the 5-HT_{2A} receptor [Lieberman, 2004]. In this regard, the End-of-Phase 2A (EOP2A) meetings between the regulatory agencies and the pharmaceutical industry are becoming an essential step before critical [go/no-go] decisions are made to proceed with costly confirmatory large-scale phase 3 trials [Ozdemir *et al.* 2005]. Hence, focused phase 1 and phase 2A trials in patients identified with biomarkers that predict a higher likelihood of therapeutic response can markedly facilitate the EOP2A reviews by rational selection (or attrition) of new antipsychotic candidates and drug development timelines [Ozdemir and Lerer, 2005].

Pharmacogenomics is the study of the role of genetics on inter-individual and between population variability in drug effects, using a broad survey of the human genome [Kalow, 2002; Evans and McLeod, 2003; Malhotra, 2003]. According to the definitions provided by the US National Institutes of Health expert working group, a biological marker (biomarker) is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [Biomarkers Definitions Working Group, 2001]. Customization of antipsychotic drug therapy by pharmacogenomic biomarkers is an area of growing interest in clinical psychiatry [Collier, 2003; Lahdelma and Koskimies, 2004; Malhotra, 2004]. Initial investigations in the field of psychiatric pharmacogenomics dealt with cross sectional patient samples based on retrospective study designs and focused on candidate genes concerned primarily with drug metabolism and pharmacokinetic elements. These studies provided an important baseline assessment for the clinical promise of pharmacogenomic research that would lead toward personalized prescribing [Kalow, 1962; Daly, 2004]. Increasingly, genetic variability in a broader array of molecular drug targets (pharmacodynamics) and their relevance for psychotropic drug efficacy and safety are being studied [Masellis *et al.* 1995; Nebert, 2000; Lerer, 2002; Lerer and Macciardi, 2002; Pickar, 2003; Reidenberg, 2003]. Yet despite numerous reports in the literature concerning pharmacogenomic associations with antipsychotic drug response phenotypes, there remains a lamentable gap in pharmacogenomic research at the point of patient care to translate these findings into genetic tests and therapeutic policy or treatment guidelines [Nebert *et al.* 2003; Albers and Ozdemir, 2004]. Moreover, strategies for optimal study design (and the attendant barriers) on how best to integrate pharmacogenomic information on pharmacokinetic and pharmacodynamic variability in the field of psychiatric pharmacogenomics are in need of further evaluation.

Pharmacogenomic investigations of antipsychotic treatment outcomes have focused largely on the prototype atypical antipsychotic clozapine. There is a paucity of clinical pharmacogenomic data with other atypical antipsychotics [de Leon *et al.* 2005]. It is not yet clear whether the genetic biomarker findings that have emerged from studies with clozapine are drug specific or are applicable to other newer antipsychotic agents. The reader is referred to existing comprehensive reviews on pharmacogenomics of clozapine and other serotonin-dopamine antagonist antipsychotics [Correll and Malhotra, 2004; Malhotra *et al.* 2004; Ozaki, 2004; Scharfetter, 2004]. In the present overview, we discuss instead the pharmacological mechanism of action of aripiprazole as an example of a new class of antipsychotic drug with functionally selective effects on dopamine D2 receptors and significant interactions with selected biogenic amine receptors [Shapiro *et al.* 2003]. By examining the case of aripiprazole and its proposed mode of action in psychotic disorders, we review the potential sources of genetic variation in primary pharmacodynamic and pharmacokinetic

candidate pathways that are likely to play a role in therapeutic response to aripiprazole.

2. GENETIC VARIATION IN DRUG TARGETS AND PHARMACOKINETIC PATHWAYS

2.1. Candidate Pathway Approach to Pharmacogenomic Study Design: A Balanced Compromise Between Statistical Power and Scope of Genetic Inquiry

Investigations into the genetic basis of individual variability in drug response started with the discipline of pharmacogenetics [Motulsky, 1957; Kalow, 1962; Kalow, 2002]. These early pharmacogenetic studies focused on candidate SNPs or a limited number of genes. More recent research, however, has clearly demonstrated that the hereditary components of drug effects are often polygenic [Evans and McLeod, 2003; Ozdemir *et al.* 2005]. With the impetus provided by the Human Genome Project (HGP) and the acceleration in the development of high throughput genomic technologies, it became possible to begin exploring complex polygenic factors involved in drug function, variability and disease etiology. An editorial in the September 1997 issue of Nature Biotechnology [Marshall, 1997] introduced, for the first time, the term pharmacogenomics into the research literature [see Hedgecoe, 2003; for a detailed account of the history of evolution of pharmacogenetics/ pharmacogenomics and related biotechnologies]. Although both pharmacogenetics and pharmacogenomics share essentially the same goal of identifying the genetic basis of variability in drug effects, pharmacogenomics takes a broader scope of inquiry, usually on a genome-wide scale. Over the past several years, a number of researchers from the fields of biological psychiatry, human genetics, pharmacology, and bioinformatics have played important roles in the development of the discipline of pharmacogenomics and its applications to clinical medicine. As research on the genetic basis of individual differences in response to atypical antipsychotics and other psychotropic drugs continues to evolve, a number of issues pertinent for the optimal design of study protocols (e.g. the use of haplotypes, genomic controls or strategies for unequivocal description of pharmacological phenotypes) have been described and discussed in detail [Devlin and Roeder, 1999; Bacanu *et al.* 2000; Nebert *et al.* 2003]. Notably, the interpretation of genetic studies of many common complex diseases have been streamlined in 1990s by specific criteria outlined to prevent false positive claims and standardized reporting of linkage results [Lander and Kruglyak, 1995]. Hence, there are lessons that may be drawn from previous experiences dealing with genetics of human diseases [Jorde, 2000]. We herein focus our discussion on how best to harness the promise of pharmacogenomics in therapeutic decisions relating to atypical antipsychotic medications through the integration of molecular genetic data from pharmacokinetic and pharmacodynamic candidate genes.

A typical characteristic of pharmacogenomic studies is the increase in the scope of queried genetic loci. Despite the initial well-deserved enthusiasm for pharmacogenomics in clinical psychiatry, the increased ability of the researchers to characterize multiple genes brings with it a statistical conundrum. In order to allow statistical correction for multiple comparisons in treatment outcomes among various genes or genetic loci, an adequate number of patients - on the order of several thousands - has to be recruited in clinical pharmacogenomic studies. As an alternative, hypothesis testing in small samples of patients, studies with candidate genes chosen by a careful consideration of the disease biology, pharmacokinetics or molecular drug targets have been advocated. On the other hand, candidate gene studies are open to criticism as they may neglect the important contributions of genes located upstream or down-stream the biological pathway where the primary candidate gene of interest is being investigated.

To address the concerns about the scope of molecular genetic analysis and the issue of sample sizes that can be realistically attained in clinical pharmacogenomic studies, a "candidate pathway" approach is being advocated [Fourie and Diasio, 2005]. In this approach, all or most genes positioned on a biological pathway are considered. For example, in the serotonin or dopamine system, it would be necessary to analyze genes encompassing neurotransmitter synthesizing enzymes, neurotransmitter receptors, transporters and the enzymes that contribute to degradation of the neurotransmitter molecules (see Fig. **1**). Evans and McLeod [2003] have recently provided a theoretical illustration of the utility of evaluating genotypic data in tandem, from drug-metabolism and drug-receptor related pathways, yielding therapeutic indexes (efficacy:safety ratios) ranging from 13 to 0.125 (Fig. **2**). Note, for example, that the same drug concentration (e.g. AUC = 200) may lead to markedly different percentage of patients responding to therapy depending on the molecular genetic variation in the target receptor pathway (*middle panel* in Fig. **2**). Conversely, for each genetic subtype of a receptor, different drug concentrations result in varying degrees of therapeutic response and toxicity, illustrating the importance of controlling for genetic or environmental sources of variability in drug metabolism, pharmacokinetics and molecular drug targets in pharmacogenetic association studies.

Using the candidate pathway approach, it should therefore be emphasized that there is much theoretical basis for a joint investigation of genetic variability in pharmacokinetic and/or serotonin-dopamine neurotransmitter pathways that may underlie response to aripiprazole. For example, genetic differences in aripiprazole metabolism mediated by CYP2D6 as well as the primary neurotransmitter receptor targets for aripiprazole (e.g. 5-HT_{1A}, 5-HT_{2A}, and dopamine D2 receptors) can now be investigated in concert with pharmacogenomic studies, as outlined in the subsequent sections.

The search for genetic biomarkers of response to aripiprazole may also carry the risk for excessive compartmentalization of various other biomarkers that may otherwise provide complementary information. As with the need to bridge genetic biomarker data from multiple candidate pathways noted above, it will be necessary to integrate biomarkers of response to atypical antipsychotics along the biological cascade from genes to their expressed products including the encoded proteins. Because the only barrier between the patient and antipsychotic safety or efficacy may rely on the accuracy of a pharmacogenomic test, clinicians need to know both the genetic variants in

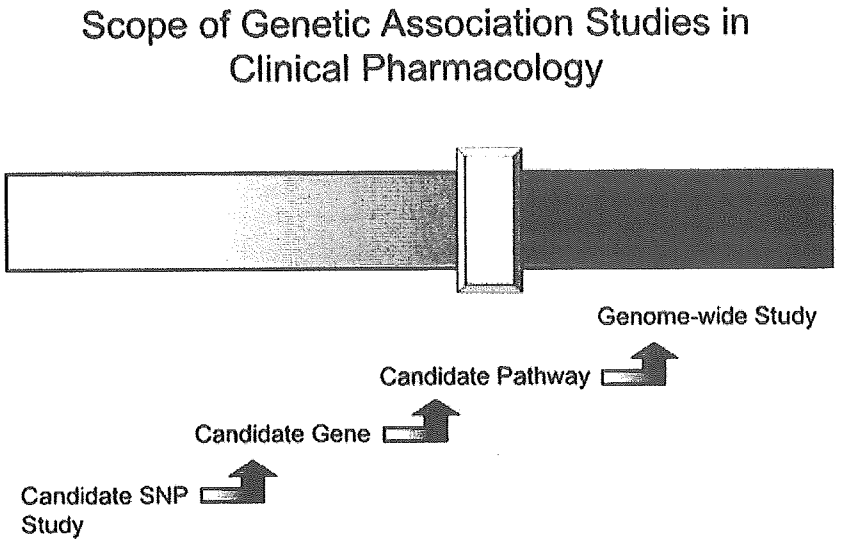

Fig. (1). The scope of molecular genetic analyses in clinical pharmacogenomic association studies ranging from candidate SNP (feasible in limited samples of study subjects) to genome wide investigations (typically in very large samples in the order of hundreds to several thousand patients). A realistic scope of genetic inquiry, in the form of candidate pathway approach, is depicted by the vertical column on this spectrum.

patients' DNA as well as the corresponding protein function. This is essential because (1) proteins are responsible for the eventual functional or clinical significance of genes and, (2) there may be marked differences or fluctuations in protein function (than what is predicted solely by gene structure) due to environmental factors or endogenous physiological rhythms that may influence posttranscriptional/ posttranslational modifications of gene products and proteins. Further, most drug effects are elicited within a matter of minutes, hours or days which may demand a more precise prediction of the present or acute state of the pathophysiological pathway whose function is inferred through a genetic test. Hence, an accurate prediction of antipsychotic treatment outcomes may require a two-step complementary strategy involving both genetic and proteomic tests for the same gene and its protein product, respectively. To this end, there is reason for guarded optimism that theragnostics, a new subspecialty of molecular medicine formed by combination of therapeutics with diagnostics, may allow the synthesis of different types of biomarker data (DNA and protein-based) in the context of antipsychotic therapeutics [Funkhouser, 2002].

3. MOLECULAR TARGETS FOR ARIPIPRAZOLE: MODE OF ACTION IN PSYCHOSIS

3.1. A Move Towards Partial Dopamine Agonists for Treatment of Schizophrenia

The dopamine hypothesis of schizophrenia is predicated on the idea that the positive symptoms of psychosis (e.g. delusions and hallucinations) are in part attributable to an *elevated* dopaminergic activity in the mesolimbic pathway, while *reduced* activity in the mesocortical dopaminergic pathway projecting to the frontal cortex is responsible for the negative symptoms (e.g. avolition and anhedonia) and neurocognitive deficits [Carlsson *et al.* 2004; Lieberman, 2004]. Hence, drugs that can differentially modulate dopaminergic activity in these brain regions would be ideal for alleviating both positive and negative symptoms of schizophrenia.

Strategies in antipsychotic drug development have recently witnessed a shift in emphasis from dopamine-serotonin antagonists, a prime focus of the pharmaceutical industry in 1990s, to dopamine partial agonists with the introduction of aripiprazole in November 2002 by the US Food and Drug Administration [Abilify®, 2002; Carlsson *et al.* 2004]. Because partial agonists by definition have lower intrinsic activity than the endogenous ligands (e.g. dopamine), aripiprazole attenuates dopaminergic neurotransmission in the presence of increased dopaminergic tone while acting as an agonist in synapses with reduced dopaminergic function [Stahl, 2001; Tamminga, 2002]. Moreover, partial agonists may prevent complete blockade of neurotransmission in brain regions with normal dopaminergic activity, thereby reducing the risk for extrapyramidal side effects. Atypical antipsychotics with partial agonist properties at dopamine receptors therefore offer the possibility of being able to modify or 'stabilize' dopaminergic neurotransmission in a graded and nuanced fashion depending on the existing dopaminergic tone in each brain region. By contrast, typical antipsychotics that act as full antagonists at dopamine D2 receptors lead to less desirable "on" or "off" regulation of synaptic function in all brain regions that project or receive dopaminergic innervation (see also Section 3.3 on alternative explanations on the mode of action of aripiprazole, and the "Functional Selectivity

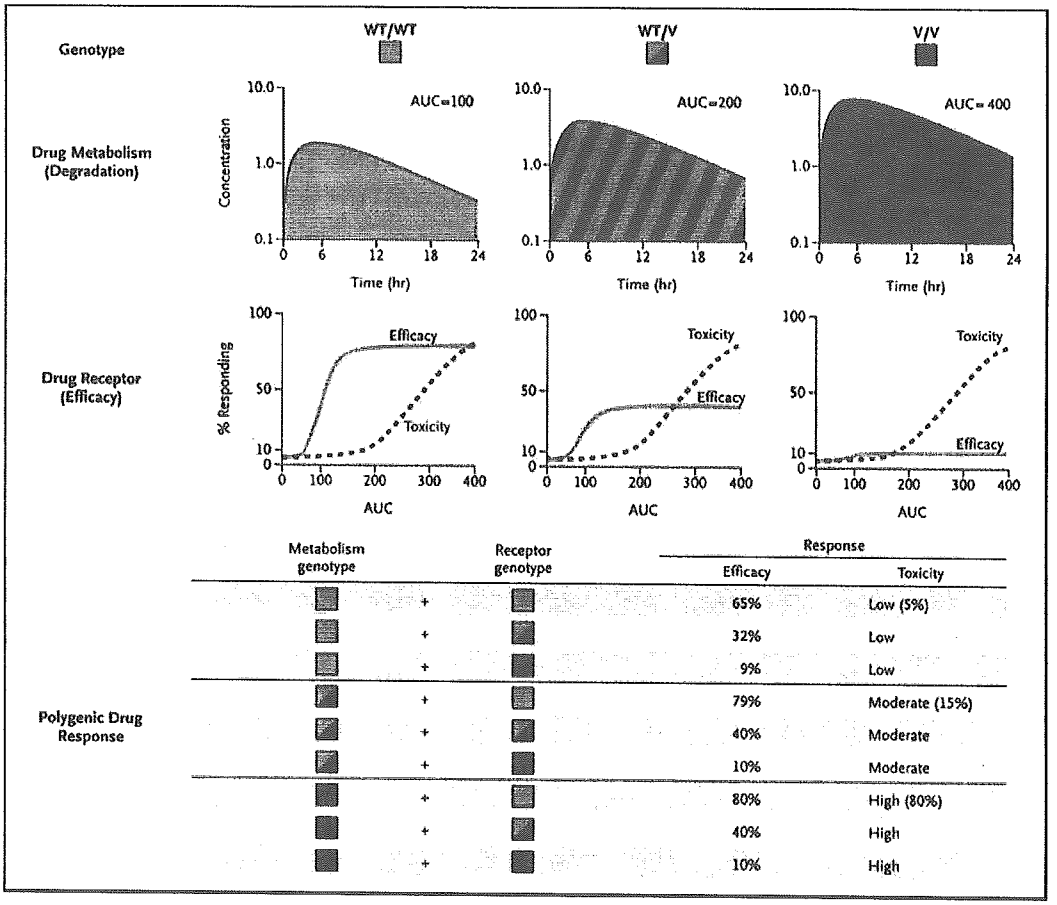

Fig. (2). A conceptual framework for genetic variability in drug metabolism and drug targets, and their integrated influence on response to pharmacotherapy. Two genetic polymorphisms, one in a drug metabolizing enzyme (*top panel*) and the second in a drug receptor (*middle panel*), depict differences in drug clearance (or the area under the plasma concentration–time curve [AUC]) and receptor sensitivity in patients who are homozygous for the wild-type allele (WT/WT), are heterozygous for one wild-type and one variant (V) allele (WT/V), or have two variant alleles (V/V) for the two polymorphisms. The *bottom panel* displays the nine potential combinations of drug-metabolism and drug-receptor genotypes and the corresponding drug-response phenotypes calculated from data at the top. "reprinted with permission from Evans & McLeod, 2003".

Hypothesis" proposed by Lawler *et al.* 1999 and Shapiro *et al.* 2003).

3.2. Aripiprazole Chemistry and Structure-Activity Relationship

Antagonism of postsynaptic dopamine D2 receptors appears to be essential not only for antipsychotic efficacy against positive symptoms of schizophrenia but also contributes to debilitating neurological side effects such as EPS [Lieberman, 2004]. OPC-4392, the predecessor of aripiprazole (OPC-14597), was initially synthesized to modulate dopaminergic neurotransmission indirectly by way of an alternate mechanism through the stimulation of presynaptic dopamine D2 autoreceptors [Yasuda *et al.* 1988]. This therapeutic strategy was based on the idea that dopamine autoreceptors serve as part of an inhibitory feedback mechanism regulating dopamine synthesis and release from the presynaptic nerve terminals. The relatively weak effects of OPC-4392 on the postsynaptic dopamine receptors led to interest in the synthesis and development of compounds such as aripiprazole that have dual actions on both the pre- and postsynaptic dopamine receptors [Kikuchi *et al.* 1995]. As a quinolinone derivative, aripiprazole differs from its structurally related predecessor OPC-4392 by two chloro substituents at positions 2 and 3 of the phenyl-

piperazinyl moiety. The halogen replacement of the phenylpiperazinyl ring is thought to increase the potency of antagonist effects on the postsynaptic dopamine receptors [Kikuchi *et al.* 1995; Oshiro *et al.* 1998; Ozdemir *et al.* 2002]. The affinities of aripiprazole toward the [^{3}H]spiperone-labeled D2 receptors in the rat frontal cortex, limbic forebrain and striatum are about 7- to 20-fold higher than OPC-4392 [Kikuchi *et al.* 1995]. Notably, aripiprazole acts as an antagonist at the postsynaptic D2 receptors at doses that produce agonist effects at the presynaptic dopaminergic nerve terminals [Kikuchi *et al.* 1995; Oshiro *et al.* 1998]. In contrast, the ED_{50} values for the biological effects of OPC-4392 as a presynaptic dopamine autoreceptor agonist and postsynaptic dopamine receptor antagonist differ by two orders of magnitude, thereby constraining the possibility of a simultaneous dual pharmacological action on both dopamine autoreceptors and those located on the postsynaptic membrane [Oshiro *et al.* 1998].

3.3. Aripiprazole Mode of Therapeutic Action in Psychosis

Aripiprazole displays partial agonist activity on the dopamine D2, D3 and the serotonin 5-HT_{1A} receptors and antagonist effects on the 5-HT_{2A} receptor with K_i values of 3.3, 1.0, 5.6 and 8.7 nM, respectively [Shapiro *et al.* 2003]. *In vitro* receptor binding studies suggest that aripiprazole has high affinity for several other neurotransmitter receptors such as 5-HT_{2B} (K_i = 0.4 nM) and 5-HT_7 (K_i = 10.3 nM) [Shapiro *et al.* 2003].

Aripiprazole dose-dependently inhibits apomorphine-induced stereotypy (an *in vivo* model of dopaminergic hyperactivity) in animals [Kikuchi *et al.* 1995; Semba *et al.* 1995]. In contrast to typical antipsychotics, the latter effect of aripiprazole is observed at doses (ED_{50} = 12 μmol/kg, po) about one order of magnitude lower than that which produces catalepsy (ED_{50} = 150 μmol/kg, po) [Oshiro *et al.* 1998]. Catalepsy has been used as a valid preclinical model for detecting the EPS liability of compounds in humans. For aripiprazole, its weak cataleptogenic effect in animal models appears to correlate well with the lower incidence of EPS in patients treated with aripiprazole [Marder *et al.* 2003].

By virtue of partial dopamine agonist properties, aripiprazole has lesser agonist effects than the endogenous naturally occurring ligand dopamine. It has been suggested that aripiprazole acts as an agonist, or a *functional* antagonist attenuating dopaminergic neurotransmission depending on the endogenous neurotransmitter concentration at the receptor-ligand biophase as well as the receptor reserve on the neuronal membrane [Lieberman, 2004; Tadori *et al.* 2005]. The dopamine autoreceptors are strategically positioned at both the presynaptic nerve terminus and the neuronal soma occurring as somatodendritic receptors. The *agonist* effects of aripiprazole on these dopamine autoreceptors are attributed in part to the high receptor reserve in the presynaptic nerve terminus and the lower (than the synaptic cleft) dopamine concentration in the vicinity of the somatodendritic autoreceptors [Lieberman, 2004]. Consistent with these theoretical considerations, aripiprazole exerts agonistic effects on the inhibitory dopamine autoreceptors as reflected by the blockade of compensatory increase in dopamine synthesis in reserpine treated rats [Kikuchi *et al.* 1995]. Excitability of dopaminergic neurons in the ventral tegmental area as measured by the spontaneous firing of type 1 neurons is inhibited by aripiprazole treatment in rats [Momiyama *et al.* 1996]. Further evidence of this activity is reflected by its reversal of reserpine- and gamma-butyrolactone-induced increase in tyrosine hydroxylase activity in the mouse and rat brain [Kikuchi *et al.* 1995].

There are a number of observations, however, that are at variance with the proposed partial agonist effects of aripiprazole at dopamine D2 receptors. An *in vivo* micro-dialysis study in rats showed a decrease in extracellular dopamine concentration following aripiprazole treatment, but only at doses (10 and 40 mg/kg) markedly higher than those that produce behavioral effects in the animal models described above [Semba *et al.* 1995]. Moreover, aripiprazole did not influence behavioral measures indicative of postsynaptic dopamine receptor stimulation such as hyper-locomotion in mice treated with reserpine, or contralateral rotation in rats with unilateral striatal 6-hydroxydopamine lesions [Kikuchi *et al.* 1995]. Aripiprazole can induce a diverse range of effects at dopamine D2 receptors (agonism, antagonism, partial agonism) in different cell lines, or in the postsynaptic membrane and dopamine autoreceptors, depending on the cellular milieu defined by promiscuous interactions with a host of signaling partners and variability in local G protein complement and concentration [Lawler *et al.* 1999; Shapiro *et al.* 2003]. This ability of aripiprazole to elicit different functional effects at the same molecular isoform of the dopamine receptor expressed in different neuroanatomical or cellular locations has been referred to as the "Functional Selectivity Hypothesis" [Lawler *et al.* 1999; Shapiro *et al.* 2003], as an alternative to explanations based on a dopamine receptor partial agonist mechanism of action [Carlsson *et al.* 2004; Lieberman, 2004; Tamminga, 2002; Stahl, 2001]. To this end, it should be noted that the "Functional Selectivity Hypothesis" raises additional possibilities for future pharmacogenomic research: genetic variations in signaling partners for dopamine receptors may also contribute to individual differences in antipsychotic response to aripiprazole [Roth, 2000].

Aripiprazole and other atypical antipsychotics uniformly display a high affinity for the 5-HT_{2A} receptor, a property that may contribute to their reduced liability for EPS and tardive dyskinesia. For example, serotonergic neurons projecting from the dorsal raphe nuclei exert a tonic inhibitory control on the nigrostriatal pathway through 5-HT_{2A} receptors located on the dopaminergic neuronal soma in the substantia nigra and the nerve termini in the striatum. In effect, stimulation of the 5-HT_{2A} receptors on the nigrostriatal pathway results in a decrease in dopamine release in the striatum [Lieberman *et al.* 1998]. The blockade of 5-HT_{2A} receptors by antagonists such as aripiprazole counteracts the serotonergic inhibition of dopamine release. Hence, 5-HT_{2A} receptor antagonism can help offset the antipsychotic-induced reduction in dopaminergic function in the striatum and the basal ganglia where excessive blockade of dopamine function can lead to EPS.

Aripiprazole displays antipsychotic efficacy both for positive and negative symptoms of schizophrenia [Marder *et al.* 2003; DeLeon *et al.* 2004]. Despite these advantages, the extent and the time course of antipsychotic response to aripiprazole may vary considerably among patients. For example, in a 52-week trial assessing long-term efficacy, as defined by a 30% or more reduction in PANSS scores, about half of the patients could be classified as nonresponders [Kasper *et al.* 2003; DeLeon *et al.* 2004]. Advances in our understanding of pharmacogenomic factors that influence patient-to-patient variability in response to aripiprazole may thus contribute to rational prescription of partial dopamine receptor agonists in patients with schizophrenia. In addition, genetic biomarkers of anti-psychotic response may help to further differentiate aripiprazole from other atypical antipsychotics.

For antipsychotic safety related endpoints, the available clinical data thus far suggest that aripiprazole is not associated with a marked increase in prolactin levels and EPS associated with typical antipsychotics, nor does it appear to pose a significant risk for metabolic disturbances observed with other atypical antipsychotics [Swainston-Harrison and Perry, 2004]. We suggest, therefore, that the study of inter-individual variability in aripiprazole efficacy toward various clinical dimensions of schizophrenia may warrant priority over those phenotypes related to safety endpoints in future pharmacogenomic investigations.

Endophenotypes of psychotic disorders or intermediary biochemical and neuroimaging endpoints are receiving increasing attention in pharmacogenomics [Heinz *et al.* 2003; Noble, 2003; Reist *et al.* 2004]. For aripiprazole and partial receptor agonists, clinical interpretations of neuro-imaging findings may require additional considerations. For example, Yokoi *et al.* [2002] found that administration of aripiprazole in humans for 14 days led to a dose-dependent (0.5 to 30 mg/day) increase in dopamine D2 and D3 receptor occupancy of between 40% and 95% as measured by positron emission tomography (PET). In patients treated with typical antipsychotics, the risk of EPS increases at D2 receptor occupancies above 80% [Nyberg *et al.* 1998]. Interestingly, EPS was not observed with aripiprazole even at striatal D2 receptor occupancy values above 90%, likely attesting to its low intrinsic activity; further, this suggests that the endophenotypes dealing with *in vivo* receptor occupancy need to be complemented with other measures of treatment outcome in search for genetic biomarkers of aripiprazole response [Grunder *et al.* 2003].

Taken together, and from a clinical pharmacogenomic standpoint, individual variations in dopamine D2 or serotonin 5-HT_{1A} or 5-HT_{2A} receptor genes (*DRD2*, *HTR1A* and *HTR2A*, respectively) emerge as prime candidates for developing genetic biomarkers of therapeutic response (or failure) to aripiprazole treatment. It should be mentioned that other receptors such as 5-HT_{2B} and 5-HT_7 for which aripiprazole displays a high binding affinity may deserve additional attention as putative molecular targets. However, genetic variation in these receptors and their pathophysiological significance remain less well understood. Because genetic variability in dopamine D2 and D3 receptors has been reviewed in detail previously [Noble, 2003; Staddon *et al.* 2005], we focus our attention in this synopsis on recent advances in our understanding of genetic differences in *HTR2A* and *HTR1A* genes that are likely to impact the attendant receptor function and treatment response to aripiprazole. Pharmacogenomic variations in other elements of the serotonergic pathway are beyond the scope of the present review and can be found elsewhere [Veenstra-VanderWeele *et al.* 2000; Glatt *et al.* 2004].

3.4. Response to Aripiprazole and Genetic Variation in 5-HT_{2A} and 5-HT_{1A} Receptors

Aripiprazole is a high affinity (K_i = 8.7 nM) antagonist at the 5-HT_{2A}, a G protein-linked receptor that activates phosphoinositide hydrolysis [Shapiro *et al.* 2003]. Antagonism of the 5-HT_{2A} receptor is a shared pharmacological attribute of clozapine and other atypical antipsychotics [Meltzer *et al.* 2003]. Conversely, stimulation of the 5-HT_{2A} receptor by agonists such as lysergic acid diethylamide (LSD) can mimic psychosis, for example, by induction of hallucinations [Aghajanian and Marek, 1999]. The 5-HT_{2A} receptor gene, *HTR2A*, maps to chromosome 13q14.1-14.2. Pharmacogenomic studies of *HTR2A* have thus far concentrated on three single nucleotide polymorphisms (SNPs), one in the promoter (A-1438G) and two in the coding region (T102C, synonymous; His452Tyr, nonsynonymous) [Veenstra-VanderWeele *et al.* 2000]. Among these, the frequency of the commonly occurring 102C-allele of the T102C SNP in healthy controls was reportedly 58.3% in Caucasians of British origin and 45.9% in an Israeli sample [Spurlock *et al.* 1998; Segman *et al.* 2001]. Notably, T102C genetic variation in *HTR2A* was previously associated with antipsychotic response to clozapine, serotonin induced platelet aggregation, prolactin response to fenfluramine and for predisposition to tardive dyskinesia, a movement disorder associated primarily with typical antipsychotic drugs [Arranz *et al.* 1995; Segman *et al.* 2001; Reist *et al.* 2004; Ozdener *et al.* 2005]. In addition, some, but not all, genetic studies of schizophrenia suggest a possible association with *HTR2A* [Veenstra-VanderWeele *et al.* 2000]. Postmortem allele specific gene expression studies in the temporal cortex of normal individuals found a lower expression of the 102C-allele than the T102 variant in *HTR2A* [Polesskaya and Sokolov, 2002], although another postmortem study could not replicate this observation [Bray *et al.* 2004].

The T102C SNP is in complete linkage disequilibrium with the A-1438G SNP in the *HTR2A* promoter region [Spurlock *et al.* 1998; Segman *et al.* 2001]. A study of A-1438G and T102C polymorphisms found an association with 5-HT_{2A} receptor binding in postmortem brains [Turecki *et al.* 1999], but this finding could not be confirmed in another study [Kouzmenko *et al.* 1999]. The A-1438G polymorphism does not affect basal or protein kinase C-induced gene transcription in HeLa cells [Spurlock *et al.* 1998]. However, the A-1438G SNP is positioned upstream of two alternative promoters for the *HTR2A*. Using two reporter gene assays and cell lines that express endogenous 5-HT_{2A}, a recent study found that the promoter activity was higher in the presence of the A allele compared to the G allele [Parsons *et al.* 2004]. Due to the significance of the 5-HT_{2A} receptor in serotonergic neurotransmission and the high

frequency of the T102C and A-1438G SNPs in human populations, further studies are necessary to delineate the mechanisms by which these genetic polymorphisms may lead to differences in 5-HT_{2A} receptor function.

A less common His452Tyr SNP in the C-terminal region of the 5-HT_{2A} receptor (9% frequency for the 452Tyr-allele in Caucasians) was previously associated with 5-HT-induced intracellular calcium mobilization [Ozaki *et al.* 1997]. Thus, the His452Tyr genetic variation may also explain individual differences in pharmacological effects of aripiprazole on the 5-HT_{2A} receptor.

HTR1A is an intronless gene encoding the 5-HT_{1A}, a G protein-linked receptor expressed both on pre- and postsynaptic membranes, acting primarily by inhibition of adenylate cyclase activity. *HTR1A* maps to human chromosome 5q12.3. Interest in the 5-HT_{1A} receptor, and by extension in *HTR1A*, stems from its role in the pathophysiology of anxiety and affective disorders [Strobel *et al.* 2003; Lesch and Gutknecht, 2004]. For example, *HTR1A* knockout mice display increased anxiety [Parks *et al.* 1998]. *In vitro*, several clinically efficacious atypical antipsychotics (such as ziprasidone) have high affinity for the 5-HT_{1A} [Richelson and Souder, 2000], while clozapine displays partial agonist activity at this receptor [Newman-Tancredi *et al.* 1996; Richelson and Souder, 2000]. Moreover, the documented anxiolytic and antidepressant properties of the 5-HT_{1A} receptor agonists (e.g. buspirone) [Blier and Ward, 2003] suggest that *HTR1A* may serve as an ancillary molecular target for the development of antipsychotic drugs directed both at psychosis and mood disorders that can occasionally co-exist, for example, in schizoaffective disorder or psychotic depression.

Allelic variation in *HTR1A* coding sequence has been extensively studied in African-American and Caucasian populations [Glatt *et al.* 2004]. Although a number of rare or low frequency nonsynonymous SNPs were identified within the *HTR1A* coding region, their low abundance (<3% allele frequency) in these populations would require large patient samples to discern clinical significance for individualization of antipsychotic therapy with aripiprazole. In an earlier functional study of a low frequency Gly22Ser variant (0.2% in Caucasians), Rotondo *et al.* [1997] found that the rare 22Ser allele did not influence receptor binding profile, although this variant was insensitive to receptor down-regulation [Nakhai *et al.* 1995]. 5-HT_{1A} receptor concentration-response curves were not influenced by the Ile28Val SNP, another rare nonsynonymous variant (0.55% in Caucasians) [Bruss *et al.* 1995; Nakhai *et al.* 1995].

Recently, Lemonde *et al.* [2003] proposed a transcriptional model for a new functional C(-1019)G SNP, located in a 26-bp palindrome, that binds transcription factors such as NUDR (nuclear deformed epidermal autoregulatory factor (DEAF-1)) in the transcriptional control region of the *HTR1A*. Interestingly, the (-1019)G variant of this SNP abolished the repression of 5-HT_{1A} autoreceptor expression, thereby leading to reduction in serotonergic neurotransmission. The regulatory C(-1019)G SNP of the *HTR1A* occurs in high frequency in the population. In Ontario, Canada, for example, the (-1019)G allele frequency was 37.3% in healthy controls of predominantly Caucasian descent [Lemonde *et al.* 2003]. A spectrum of psychopathologies ranging from schizophrenia to substance abuse [Huang *et al.* 2004] as well as therapeutic response to tricyclic and selective serotonin reuptake inhibitor antidepressants [Lemonde *et al.* 2004; Serretti *et al.* 2004], appear to be associated with the *HTR1A* C(-1019)G allelic variation. We suggest, therefore, that *HTR1A* genetic variation deserves further study in future clinical pharmacogenomic studies, particularly in relation to the clinical effects of aripiprazole on affective dimensions of psychopathology co-morbid with schizophrenia.

4. PHARMACOKINETICS OF ARIPIPRAZOLE AND PHARMACOGENETIC VARIATION

There are limited published data on mechanisms of inter-individual variability in aripiprazole pharmacokinetics [Mallikaarjun *et al.* 2004; Raggi *et al.* 2004]. The absolute oral bioavailability of aripiprazole is reportedly 87% [Abilify®, 2002]. After a single oral dose of [^{14}C]-labeled aripiprazole, less than 1% of the dose is excreted as unchanged parent drug in the urine while about 18% is recovered unchanged in the feces. In healthy male volunteers, aripiprazole displays linear pharmacokinetics at doses ranging from 5 mg to 30 mg daily. In healthy volunteers, the coefficient of variation (CV) for area under the plasma concentration-time curve (AUC_{0-24}) and the elimination half-life ($t_{1/2}$) at a dose of 20 mg/day was 51% and 34%, respectively [Mallikaarjun *et al.* 2004]. It should be noted that the extent of variability in aripiprazole disposition in patients with schizophrenia or other populations under real life clinical settings deserves further investigations. *In vitro*, it appears that aripiprazole is not subject to metabolism by CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2E1 enzymes and does not undergo direct glucuronidation [Abilify®, 2002]. Conversely, the effects of aripiprazole (inhibition or induction) on drug metabolizing enzymes are not presently known.

The primary routes of aripiprazole metabolism are reportedly dehydrogenation, hydroxylation and N-dealkylation [Abilify®, 2002], but virtually no information is available in the public domain on the relative quantitative or clinical significance of these metabolic pathways. Both CYP2D6 and CYP3A4 enzymes appear to contribute to formation of the dehydrogenated metabolite which, according to the drug label by the manufacturer, exhibits activity at the dopamine D2 receptor similar to the parent compound [Abilify®, 2002]. The AUC for the active dehydrogenated metabolite is about 40% of that for aripiprazole. To this end, the pharmacological activity profile of the dehydrogenated aripiprazole metabolite toward other neurotransmitter receptors is unknown.

CYP2D6 is one of the most extensively studied genetically polymorphic drug metabolizing enzymes with, for example, 7% of Caucasians classified as poor metabolizers (PMs) while the rest are extensive metabolizers (EMs) [Aklillu *et al.* 2002; Bertilsson *et al.* 2002; Ingelman-Sundberg, 2005]. Moreover, there are marked inter-ethnic variations in CYP2D6 catalytic function. In Asian populations (Chinese,

Japanese and Koreans), the prevalence of PM phenotype is only 1% but the distribution of enzyme activity is significantly shifted toward lower values in EMs compared to Caucasian EMs [Kalow, 1991]. In PMs, aripiprazole exposure is increased by 80% and accompanied by a 30% decrease in exposure to the dehydrogenated putative active metabolite, leading to a net increase in the total active moieties from a given dose of aripiprazole, compared to EMs [Abilify®, 2002]. Similarly, co-administration of aripiprazole with quinidine, a potent inhibitor of CYP2D6 enzyme, results in a more than two-fold (112%) increase in aripiprazole exposure in EMs. Hence, it is conceivable that an increase in aripiprazole concentration can be anticipated with other potent CYP2D6 inhibitors (e.g. paroxetine) that may be co-prescribed with aripiprazole.

Pharmacokinetic bridging-studies are usually conducted when regulatory drug approval is sought in various countries. For aripiprazole, pharmacogenetic-guided pharmacokinetic bridging-studies focusing on CYP2D6 appear to be warranted among Asian, Caucasian and other populations who display genetically determined inter-ethnic differences in CYP2D6 activity. These data may provide guidance for rational use of aripiprazole and facilitate its registration in different populations or countries as well.

The CYP3A4 enzyme also contributes to metabolism of aripiprazole *via* dehydrogenation and is subject to genetic regulation. It is estimated that 60% to 90% of interindividual variation in catalytic function is determined by hereditary factors [Ozdemir *et al.* 2000]. However, the identity of the precise genetic loci regulating CYP3A4 function remains elusive. More than 30 SNPs have been discovered within *CYP3A4*, but the majority either occur at low frequency (<5%) in human populations or have a minimal impact on enzyme function [Lambda *et al.* 2002a; Lambda *et al.* 2002b]. An unequivocal prediction of CYP3A4 catalytic function solely with a genotypic test is not yet feasible. A further complicating factor is the extensive overlap in substrate selectivity between CYP3A4 and CYP3A5, suggesting that a genetic deficiency in CYP3A4 activity can be partially compensated by the CYP3A5 enzyme. Taken together, these data suggest that variability in CYP2D6 function due to genetic factors, or drug-drug interactions, influences the pharmacokinetics, clinical efficacy and, presumably, concentration-dependent side effects of aripiprazole [Kubo *et al.* 2005].

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Aripiprazole is thought to stabilize dopamine and serotonin neurotransmitter systems in various brain regions in a graded and selective manner depending on the existing endogenous dopaminergic or serotonergic tone. The underlying mechanism of action of aripiprazole in psychotic disorders is likely more complex than what would have been anticipated solely by simple partial agonist effects at the dopamine D2 receptor. In particular, differences in local cellular environment and variability in the type or concentration of the signaling partners for neurotransmitter receptors may also influence clinical response to aripiprazole [Lawler *et al.* 1999; Roth, 2000; Shapiro *et al.* 2003]. Available data from clinical trials in carefully selected patient populations suggest that aripiprazole is largely devoid of metabolic side effects frequently observed with atypical antipsychotics. Still, patients do not uniformly respond to aripiprazole while other patients fail to benefit from drug therapy. Insomnia, anxiety, akathisia, and worsening of psychosis have been noted during aripiprazole treatment in a minority of patients [Swainston-Harrison and Perry, 2004; Ramaswamy *et al.*, 2004; Reeves and Mack, 2004]. Pharmacogenomic biomarkers of therapeutic response to aripiprazole would be desirable to identify the patient subpopulations in whom aripiprazole is more likely to display its antipsychotic effects while minimizing the risk for adverse effects. This would further support its position as a first line agent in the treatment of psychosis and provide unequivocal therapeutic differentiation from other frequently prescribed antipsychotics such as olanzapine.

From the point of therapeutic sciences and psychotropic drug development, the implications of genetic testing for antipsychotic drug targets can be dramatic; it may mean that the choice of drugs – not only the dosage - will be guided by the genetic make up of individuals. Hence, pharmacogenomic tests may decrease the segment of the population for which and to whom drugs can be marketed [Williams-Jones and Corrigan, 2003]. Conversely, prior knowledge of genetic determinants of efficacy and safety may allow targeting of discrete subpopulations in clinical trials and demonstration of "proof of concept" in a smaller number of patients. In theory, this should significantly reduce research and development costs and expedite the regulatory approval of newer atypical antipsychotic candidates. This would also be an aid for rational prescription of aripiprazole as well as its therapeutic differentiation from other atypical antipsychoti c compounds. It is plausible that pharmaco-genomic biomarkers for aripiprazole may inform and guide the development of other partial dopamine-serotonin agonists such as bifeprunox that share similar drug targets [Newman-Tancredi *et al.* 2005].

It should be emphasized that some of the atypical antipsychotics will soon be eligible for regulatory approval as a generic formulation (e.g. risperidone and olanzapine within the next 5 years) [Grady *et al.* 2003]. It is conceivable that a combination of factors ranging from unmet patient needs to market forces, amendments in regulatory policies and competition by generic formulations may provide further motivation on the part of the pharmaceutical industry to develop genetic biomarkers of response to aripiprazole [Melzer *et al.* 2003; Williams-Jones and Burgess, 2004; Williams-Jones, 2005]. The discipline of science and technology studies (STS) and the attendant research literature focus on precisely such complex issues dealing with implementation of pharmacogenomics and other emerging biotechnologies in the clinic [Williams-Jones and Graham, 2003; Hedgecoe and Martin, 2003; Hedgecoe, 2003]. Unfortunately, the expertise already available in the STS research community does not always find its way to the mainstream medical research literature [Hedgecoe, 2004; Webster *et al.* 2004]. Therefore, there is an acute need for more extensive collaborations and consultations among pharmacogeneticists, bioethicists and experts dealing with

STS for an expeditious and equitable development of genetic biomarkers of treatment outcomes with newer atypical antipsychotic agents [Corrigan and Williams-Jones, 2005; Melzer *et al.* 2005; Ozdemir *et al.* 2005; Williams-Jones and Ozdemir, 2005; Smart *et al.* 2004; Webster *et al.* 2004].

As clinical utility of aripiprazole is extended to more diverse groups of patients, well-beyond those who meet the stringent and narrowly-defined inclusion and exclusion criteria used in clinical trials, psychiatrists should continue to use their clinical judgment and pharmacovigilance against previously unrecognized safety or efficacy issues. When pharmacogenomic testing is made available for use as part of routine patient care in psychiatry, it will be necessary to take into account that the human genome displays a high level of plasticity in regulation of gene expression, not to mention our incomplete understanding of the mechanisms responsible for posttranscriptional and posttranslational modifications on products of gene expression [Nebert *et al.* 2003; Collier, 2003]. On the path from the discovery of a genetic biomarker of psychotropic drug response in the laboratory, to a commercially available test applied at the point of patient care, the attendant ethical and therapeutic policy implications of pharmacogenomic tests and their integration with other types of (e.g. proteomics-based) biomarkers will also need to be considered.

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ABBREVIATIONS

EPS	=	Extrapyramidal symptoms
HGP	=	Human genome project
HTR1A	=	Serotonin-1A receptor gene
HTR2A	=	Serotonin-2A receptor gene
NUDR	=	Nuclear deformed epidermal autoregulatory factor
OPC-14597	=	Aripiprazole
SNP	=	Single nucleotide polymorphism
STS	=	Science and technology studies

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