Genome-Wide Pharmacogenomic Study of Neurocognition As an Indicator of Antipsychotic Treatment Response in Schizophrenia

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Neurocognitive deficits are a core feature of schizophrenia and, therefore, represent potentially critical outcome variables for assessing antipsychotic treatment response. We performed genome-wide association studies (GWAS) with 492K single nucleotide polymorphisms (SNPs) in a sample of 738 patients with schizophrenia from the Clinical Antipsychotic Trials of Intervention Effectiveness study. Outcome variables consisted of a neurocognitive battery administered at multiple time points over an 18-month period, measuring processing speed, verbal memory, vigilance, reasoning, and working memory domains. Genetic mediation of improvements in each of these five domains plus a composite neurocognitive measure was assessed for each of five antipsychotics (olanzapine, perphenazine, quetiapine, risperidone, and ziprasidone). Six SNPs achieved genome-wide significance using a pre-specified threshold that ensures, on average, only 1 in 10 findings is a false discovery. These six SNPs were located within, or in close proximity to, genes *EHF*, *SLC26A9*, *DRD2*, *GPR137B*, *CHST8*, and *IL1A*. The more robust findings, that is those significant across multiple neurocognitive domains and having adjacent SNPs showing evidence for association, were rs286913 at the *EHF* gene (p -value 6.99×10^{-8} , q -value 0.034, mediating the effects of ziprasidone on vigilance), rs11240594 at *SLC26A9* (p -value 1.4×10^{-7} , q -value 0.068, mediating the effects of olanzapine on processing speed), and rs11677416 at *IL1A* (p -value 6.67×10^{-7} , q -value 0.081, mediating the effects of olanzapine on working memory). This study has generated several novel candidate genes for antipsychotic response. However, our findings will require replication and functional validation. To facilitate replication efforts, we provide all GWAS p -values for download.

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INTRODUCTION

Schizophrenia is an often devastating neuropsychiatric illness. Biological factors have been strongly implicated and considerable efforts are currently being devoted to finding genes that increase susceptibility to the disease. One

eventual goal of these efforts is the identification of novel drug targets to develop better therapeutic compounds (Sanger, 2004; Williams, 2003). However, the development of new drugs is extremely expensive and takes many years (Roses, 2000). Arguably, a more immediate role for genetic markers in the treatment and management of schizophrenia is to improve our ability to tailor the prescription of existing drugs to individual patients (Malhotra *et al*, 2004). Typically, only a proportion of patients respond to any given antipsychotic drug (Kane, 1999). The several weeks it may take clinicians to declare a treatment ineffective leaves the patient vulnerable to continuing dysfunction and suicide (Meltzer and Okayli, 1995). The clinical benefits of

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a genetic test to identify the most effective antipsychotic treatment for each patient at time of first presentation would be considerable (Basile *et al*, 2002).

Despite some encouraging signs, efforts to predict antipsychotic treatment response have met with limited success (Malhotra *et al*, 2004). One of the complications is that neuropsychiatric clinical phenotypes are complex and heterogeneous and, therefore, may not be optimal measures in pharmacogenomic studies. An alternative is to focus on the amelioration of specific deficits related to the disorder. Patients with schizophrenia show deficits across a large number of neurocognitive domains and these deficits are regarded as the single strongest correlate of real world functioning (Green, 1996). Although there is variability among patients (Kremen *et al*, 2004), neurocognitive deficits are present by the first episode and persist throughout the course of the illness (Mesholam-Gately *et al*, 2009). Substantial evidence points to neurocognitive deficits in the relatives of schizophrenic probands (Hill *et al*, 2008) and a large consortium study found moderate, but significant, heritabilities for all neurocognitive measures investigated, with estimates ranging from 24 to 55% (Greenwood *et al*, 2007). The motivational and cognitive impairments of schizophrenia are strongly associated with the long-term disability typically produced by the disease, and this is why cognitive deficits are increasingly being seen as targets for treatment (Hyman and Fenton, 2003). Crucially, for our purposes, previous studies have shown improvement in neurocognitive function following treatment with several different antipsychotic drugs (Keefe *et al*, 2007a,b). Taken together, these observations suggest that neurocognitive deficits represent potentially critical outcome variables for the pharmacogenomic study of antipsychotic drug response.

To date, pharmacogenomics studies have typically focused on candidate genes, selected for encoding of drug targets (pharmacodynamic candidates) or for involvement in the metabolism of the drug itself (pharmacokinetic candidates). Pharmacodynamic candidates in schizophrenia include dopamine or serotonin receptors and several studies have shown positive associations between, for example, dopamine receptors D2 (*DRD2*) and D3 (*DRD3*) and antipsychotic response (see Arranz and de Leon, 2007, for a review). Pharmacokinetic candidates, particularly the cytochrome P450 genes, are well known to harbor genetic variation affecting the metabolism of antipsychotics (Kirchheiner *et al*, 2004). Nevertheless, a limitation of prior studies in this area is that the selection of candidate genes is restricted to current knowledge about mechanisms of drug action. More exploratory methods that systematically screen the whole genome for association with drug response likely represent a superior strategy for discovering relevant genetic variation. Such genome-wide association studies (GWAS) have become feasible in recent years and have already shown considerable promise to identify novel associations between genetic variants and complex traits (Altshuler *et al*, 2008). Indeed, several large-scale GWAS for schizophrenia have been recently published. Associations have been found with, for example, the MHC region (Shi *et al*, 2009) and novel loci such as *ZNF804A* (O'Donovan *et al*, 2008). To date, though, the number of GWAS on antipsychotic response is considerably more modest (Zandi and Judy, 2010).

In this study, we use the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study (Lieberman *et al*, 2005; Stroup *et al*, 2003) to detect genetic variation underlying changes in neurocognitive functioning following treatment with five different antipsychotics. The sample consisted of 738 CATIE subjects with DSM-IV schizophrenia diagnoses who were genotyped using the Affymetrix 500K platform plus a custom 164K chip to improve genome-wide coverage (Sullivan *et al*, 2008). Treatment outcome was measured using a comprehensive neurocognitive assessment battery in five domains: processing speed, verbal memory, vigilance, reasoning, and working memory, in addition to a composite measure that condensed information from these five sub-domains (Keefe *et al*, 2003, 2006).

MATERIALS AND METHODS

Study Sample

A total of 765 subjects from the CATIE study gave informed consent to participate in genetic studies. The patients were diagnosed with schizophrenia using the Structured Clinical Interview for DSM-IV (First *et al*, 1994). This sample has been carefully described elsewhere (Lieberman *et al*, 2005; Stroup *et al*, 2003). In short, CATIE is a multiphase randomized controlled trial of antipsychotic medications, including the second-generation drugs, olanzapine, quetiapine, risperidone, and ziprasidone, compared with a mid-potency first-generation drug, perphenazine. Patients were followed for up to 18 months and, to maximize representativeness, the participants were recruited from 57 clinical settings around the United States. The mean age for the participants was 40.9 years and on average they first received antipsychotic medication 14.3 years previously. All participants or their legal guardians gave written informed consent and the institutional review board at each site approved the study.

Neurocognitive Assessment Battery

For CATIE, a neurocognitive advisory group was convened and arrived at consensus regarding the contents, standardization, and methodology of the neurocognitive assessment battery (Keefe *et al*, 2003, 2006). Eleven tests were administered, resulting in 24 individual scores. Factor analysis showed that a model comprised of five domain scores provided the best fit (Keefe *et al*, 2006). The five neurocognitive domains were processing speed that is based on the standardized mean of grooved pegboard (Lafayette Instrument Company, 1989), WAIS-R digit symbol test (Wechsler, 1981), and the mean of the two verbal fluency measures (Benton, 1978); verbal memory was assessed with the Hopkins verbal learning test (average of three trials) (Brandt, 1991); vigilance was based on the continuous performance test d-prime scores (average of 2-digit, 3-digit, and 4-digit) (Cornblatt *et al*, 1988); reasoning summary score was the mean of Wisconsin card sorting test (Heaton *et al*, 1993) and WISC-R mazes (Wechsler, 1991); working memory summary score was the average of a computerized test of visuospatial working memory (sign reversed) (Lyons-Warren *et al*, 2004) and letter number sequencing

Table 1 Number of Subjects/Assessments Analyzed for Each Antipsychotic and Neurocognitive Outcome

	Olanzapine	Perphenazine	Quetiapine	Risperidone	Ziprasidone
Verbal memory	223/459	109/223	198/325	213/413	138/254
Vigilance	215/430	102/203	190/305	202/381	133/234
Processing speed	223/459	109/223	198/325	213/413	138/254
Reasoning	223/459	109/223	198/325	213/413	138/254
Working memory	223/459	108/222	198/325	213/413	138/254
Neurocognitive composite	223/459	109/223	198/325	213/413	138/254

The slightly smaller numbers in vigilance are attributable to invalid scores from computerized testing (see Keefe *et al*, 2006).

(Gold *et al*, 1997). The correlations among the five domain scores were medium to high and we, therefore, also analyze the standardized composite score of these five domains (Keefe *et al*, 2006).

Estimating Treatment Effects

Multiple assessments were available per patient (Table 1). There was a mean of 3.4 assessments per subject. Conventional approaches to calculating treatment effects (eg, post-treatment—pre-treatment difference scores) define treatment using only two observations. Alternatively, using all available assessments can substantially improve statistical power to detect true associations. For this reason, we have developed a systematic method (van den Oord *et al*, 2009) using mixed effects modeling (Goldstein, 1995; Searle *et al*, 1992) to estimate treatment effects. We first determine the functional form of the drug response trajectory, then screen many possible covariates to select those that improve the precision of the treatment effect estimates, and finally generate individual drug effect estimates based on the best fitting model using best linear unbiased predictors (Pinheiro and Bates, 2000) (see Supplementary Material for details). Intuitively, these treatment effects quantify how much each subject's phenotype changes in response to a given drug, relative to the average effect for all subjects who took the drug. Each treatment effect measure was screened for outliers using the multivariate outlier method developed by Hadi (Hadi and Simonoff, 1993). This procedure quantifies the (Mahalanobis) distance of each observation from the centroid of all observations. We excluded observations that were outlying at $p < 0.05$. The majority of distributions showed 0 outliers and none exhibited > 2 (7 total outliers out of 30 outcome distributions). After excluding outliers, outcomes were all virtually perfectly normally distributed: mean skewness = -0.025 or 0.209 when taking the mean of the absolute values, while mean kurtosis = 0.534 (signed) or 0.585 (absolute).

Genotyping and Genotype Quality Control

DNA sampling, GWAS genotyping, and genotype quality control (QC) have been described previously for this sample (Sullivan *et al*, 2008). Briefly, a total of 665 439 single nucleotide polymorphisms (SNPs) were genotyped for each subject using both the Affymetrix 500K 'A' chipset (Santa Clara, CA, USA) and a custom 164K SNP-chip created by Perlegen (Mountain View, CA, USA). Highly stringent QC protocols were implemented. Of the 500 568 SNPs on the

Affymetrix arrays, 79.8% passed initial QC, while 66.1% of the 164 871 SNPs on the custom Perlegen chip passed initial QC. Additional SNPs with allele frequencies < 0.01 and those with 2 disagreements among 36 pairs of replicate samples were removed. Subjects with sex discrepancies, cryptic duplicates, and those with excessive missing genotypes were removed (see Supplementary Materials). After QC, a total of 492 900 SNP genotypes from 738 individuals remained for analysis.

Ancestral Background

Approximately 57% of the CATIE subjects self-identify themselves as white/European American (EA) and 29% as black/African American (AA). The remaining 14% of the patients consider themselves to have 'other' ancestral origins or to belong to multiple ancestral categories. To avoid false positives due to population stratification, Sullivan *et al* (2008) previously performed an extensive evaluation of multiple methods to control for ancestral heterogeneity in CATIE. They concluded that the principal component and multi-dimensional scaling (MDS) approach worked best. We, therefore, proceeded with the MDS approach as implemented in PLINK (Purcell *et al*, 2007). For our analyses, we used five MDS dimensions that appeared to capture the majority of ancestry-related effects in CATIE (see Supplementary Materials for details).

Association Testing and False Discovery Rate Control

All association testing was conducted in PLINK (Purcell *et al*, 2007) using a linear regression model with the five population stratification MDS dimensions as covariates. We used a false discovery rate (FDR) (Benjamini and Hochberg, 1995)-based approach to declare significance. In comparison with controlling a family-wise error rate (eg, Bonferroni's correction), the FDR (a) provides a better balance between finding true effects *vs* controlling false discoveries, (b) results in comparable standards for declaring significance across studies because it does not directly depend on the number of tests, and (c) is relatively robust against having correlated tests (Brown and Russell, 1997). The FDR is commonly used in many high-dimensional applications and has also successfully been applied in the context of GWAS (Beecham *et al*, 2009; Lei *et al*, 2009; Liu *et al*, 2009). As motivated previously, we chose an FDR threshold of 0.1 for declaring genome-wide significance (van den Oord and Sullivan, 2003). This means that on average 10% of the SNPs declared significant are expected to be false discoveries.

Operationally (Black, 2004), the FDR was controlled using q -values that are FDRs calculated using the p -value of the markers as thresholds for declaring significance (Storey, 2003; Storey and Tibshirani, 2003). It is important to note that performing many GWAS analyses does not present a problem for the FDR because it controls the expected ratio of false to all discoveries. Thus, when many GWAS are performed, the number of false positives will increase and so will the number of true positives. The expected ratio of false to all discoveries will, therefore, remain 0.1 with our threshold for declaring genome-wide significance regardless of how many GWAS are performed.

To avoid an all-or-nothing conclusion about whether an SNP is significant and improve the interpretation of our GWAS results, we also estimated for each SNP the local FDR (lFDR) (Efron *et al*, 2001) using a precise method specifically designed for GWAS studies (Bukaszar *et al*, 2009). This estimated lFDR is the posterior probability that the SNP has no effect. In addition to reporting p - and q -values, we also report the effect size for our top findings, that is increase in explained variance (r^2) for each of the genome-wide significant SNPs when the SNP in question is added to the regression model (without the MDS dimensions).

Candidate Gene Analysis

To select candidate genes for drug effects, we selected all genes that had previously shown positive associations with response to any antipsychotic drug using any outcome measure, as reviewed by Arranz and de Leon (2007), plus additional genes with reported positive associations since publication of that article (Anttila *et al*, 2007; Campbell *et al*, 2008; Hamdani *et al*, 2008; Hwang *et al*, 2007; Mancama *et al*, 2007; Meary *et al*, 2008). We also included all genes involved in the major and secondary metabolic pathways of antipsychotic drugs (Arranz and de Leon, 2007). Finally, we also included two genes (*RIMS1* and *GRM8*) that showed promising associations in a recent candidate gene study of antipsychotic response in CATIE (Need *et al*, 2009), using a different analytical approach to that presented here. This gave a total of 41 candidate genes. SNPs were selected in these genes using a liberal definition of the gene boundary ($\pm \sim 50$ kb), to avoid excluding SNPs in the region that may be in linkage disequilibrium (LD) with others within the gene itself, or that may be in proximal regions of regulatory importance. In total, we identified 1595 SNPs corresponding to the 41 candidate genes— ~ 39 SNPs per gene (see Supplementary Table S4; Supplementary Material).

RESULTS

When generating the outcome measures, two were found to show limited individual differences in treatment response, that is variance was close to zero. These were perphenazine with vigilance and perphenazine with working memory. GWAS were not performed for these outcomes because any findings would not be associated with individual differences in treatment, but simply sampling error. Quantile–Quantile (QQ) plots and Manhattan plots for each of the remaining successful GWAS are available in the online Supplementary

Table 2 Number of GWAS q -values Below Various Thresholds

	q-value threshold				
	0.1	0.25	0.5	0.75	0.95
<i>By neurocognitive scale</i>					
Neurocognitive composite	0	0	3	78	160
Working memory	4	12	43	95	162
Reasoning	0	10	27	49	171
Processing speed	1	1	8	61	97
Verbal memory	0	0	2	29	126
Vigilance	1	2	4	8	97
Total	6	25	87	320	813
<i>By drug</i>					
Olanzapine	5	10	23	32	141
Quetiapine	0	11	48	206	286
Risperidone	0	2	9	31	184
Ziprasidone	1	2	5	29	141
Perphenazine	0	0	2	22	61
Total	6	25	87	320	813

Material. All p -values for each GWAS are available for download at <http://www.people.vcu.edu/~jlmccclay>. Table 2 shows the number of significant GWAS results at various FDR thresholds. For concision, Table 2 presents significant results grouped by outcome or by drug. Six SNPs exceeded our pre-specified genome-wide significance threshold, which controls the FDR at the 0.1 level (van den Oord and Sullivan, 2003). When grouped by medication, the largest numbers of genome-wide significant results were found for olanzapine ($m = 5$); when grouped by neurocognitive outcome, the largest number of findings were found for working memory ($m = 4$). Figure 1 shows the regional plots of the genome-wide significant findings, while Figure 2 shows the outcome scores by genotype.

Table 3 provides details on those SNPs that were either genome-wide significant (q -value < 0.1) or 'potentially interesting' at a q -value threshold < 0.25 . The most significant SNP overall, rs286913, reached genome-wide significance (p -value $= 6.99 \times 10^{-8}$, q -value $= 0.034$, $r^2 = 0.213$) and appeared to mediate the effects of ziprasidone on vigilance. This SNP lies within an intron of the *EHF* (ETS homologous factor) gene that is located on chromosome 11p12. An additional SNP in *EHF*, rs286928, also mediated the effects of ziprasidone on vigilance and was located some 16 kb from rs286913. LD between these two markers was high, with $r^2 = 0.87$ in EAs and 0.46 in AAs. A regional plot of p -values for all CATIE SNPs at this locus (Figure 1) shows their position relative to the gene. Haplotype analysis of these two SNPs plus an additional six flanking markers yielded several highly significant findings in the EA subsample (the largest single ethnic group in CATIE). However, the best p -value among the haplotypes tested ($p = 1.18 \times 10^{-5}$) was only marginally more significant than the single marker p -value for rs286913 in EA ($p = 1.8 \times 10^{-5}$), suggesting that this marker alone is

Figure 1 (a and b) Regional plots of p -values for SNPs flanking the six genome-wide significant findings (solid black dots). The y axis is the $-\log_{10}$ of the p -values for each SNP, while the x axis denotes genomic position according to genome build 36 (hg18). All genotyped SNPs within $\sim \pm 100$ kb of the primary findings are plotted.

Figure 2 Box and whisker plots of treatment response, by genotype, for the six genome-wide significant SNP associations. The y axis units are SD. Vigil_zipras = vigilance with ziprasidone; Speed_olanza = processing speed with olanzapine; Memory_olanza = working memory with olanzapine. For rs4805924 and rs11214606, only two genotype groups are plotted. Although rare allele homozygotes were present for both SNPs in CATIE, none of these had at least two observations in the relevant neurocognitive outcome/drug combination (working memory and olanzapine) to allow a treatment effect to be calculated.

probably sufficient to capture the association. In addition to mediating the effects of ziprasidone on vigilance, rs286913 showed association ($p < 0.05$) for additional drug and outcome combinations. These included ziprasidone with the neurocognitive composite measure ($p = 0.0082$),

risperidone and reasoning ($p = 0.0015$), and risperidone with working memory ($p = 0.035$). The p -values for all six genome-wide significant SNPs across all 28 drug/outcome combinations are shown in Supplementary Tables S4A and B in the Supplementary Material.

Table 3 Summary of top findings with q -values below 0.25.

Outcome		Locus					Test				
Drug	Measure	SNP ID	Gene	Chr	Posn (bp)	MAF	N	p-val	q-val	IFDR	Eff
Ziprasidone	Vigilance	rs286913	EHF	11	34 611 939	0.051	127	6.99E-08	0.034	0.241	+
Ziprasidone	Vigilance	rs286928	EHF	11	34 596 076	0.044	126	6.06E-07	0.147	0.689	+
Olanzapine	Processing speed	rs11240594	SLC26A9	1	204 162 858	0.191	213	1.40E-07	0.068	0.390	+
Olanzapine	Working memory	rs11214606	DRD2	11	112 815 079	0.034	203	4.84E-07	0.081	0.442	—
Olanzapine	Working memory	rs7520258	GPR137B	1	234 379 750	0.066	203	5.10E-07	0.081	0.453	—
Olanzapine	Working memory	rs4805924	CHST8	19	38 860 113	0.024	202	6.17E-07	0.081	0.493	—
Olanzapine	Working memory	rs11677416	IL1A	2	113 245 711	0.253	200	6.67E-07	0.081	0.509	+
Olanzapine	Working memory	rs17561	IL1A	2	113 253 694	0.253	203	1.05E-06	0.102	0.604	+
Quetiapine	Working memory	rs11110077	ANKS1B	12	98 790 244	0.109	176	3.70E-07	0.129	0.448	—
Quetiapine	Working memory	rs12726652		1	107 234 099	0.264	177	5.31E-07	0.129	0.522	—
Quetiapine	Working memory	rs6856328	LPHN3	4	62 119 913	0.074	178	8.26E-07	0.133	0.612	—
Quetiapine	Working memory	rs7770731		6	14 706 799	0.292	182	1.23E-06	0.149	0.690	+
Quetiapine	Reasoning	rs4611189		11	40 794 289	0.034	186	6.16E-07	0.161	0.584	—
Quetiapine	Reasoning	rs17126180	ANKRD33	12	50 550 223	0.059	189	9.13E-07	0.161	0.658	+
Quetiapine	Reasoning	rs17108533	MAP3K9	14	70 319 555	0.102	188	9.96E-07	0.161	0.674	+
Quetiapine	Working memory	rs16865258	CLDN1	3	191 480 090	0.015	182	1.74E-06	0.169	0.750	—
Olanzapine	Reasoning	rs2833556	HUNK	21	32 207 171	0.314	212	4.59E-07	0.188	0.633	+
Olanzapine	Reasoning	rs2286720	LYZL4	3	42 423 475	0.073	215	1.00E-06	0.188	0.760	—
Olanzapine	Reasoning	rs17706989	WVVOX	16	77 127 458	0.031	214	1.16E-06	0.188	0.781	—
Quetiapine	Reasoning	rs1264202	ZNF706	8	102 297 099	0.313	189	1.72E-06	0.209	0.764	—
Quetiapine	Working memory	rs4293296	CDKN3	14	53 927 036	0.291	181	2.94E-06	0.237	0.825	—
Olanzapine	Working memory	rs6707600	ITSN2	2	24 375 270	0.019	203	2.97E-06	0.240	0.792	—
Risperidone	Reasoning	rs3753242	PRKCZ	1	1 548 310	0.057	199	9.71E-07	0.243	0.794	+
Risperidone	Reasoning	rs17689437	ZFP90	16	67 157 852	0.131	204	1.00E-06	0.243	0.797	+
Quetiapine	Reasoning	rs6436839	PID1	2	229 678 042	0.289	183	2.58E-06	0.249	0.820	—

Summary of top findings with q -values below 0.25. Where more than one marker at a locus has findings $q < 0.25$, these have been grouped and shaded in grayscale. Genomic position is indicated according to genome build 36 (hg18). MAF is the minor allele frequency and IFDR is the local false discovery rate, which provides a marker-specific estimate of the posterior probability that the finding is false positive. 'Eff' is the direction of effect of the minor allele, eg, '+' indicates a greater improvement in neurocognitive function for the minor allele compared with the major allele.

The second most significant locus was rs11240594 on chromosome 1q32 that appeared to mediate the effects of olanzapine on processing speed (p -value = 1.4×10^{-7} , q -value = 0.068, $r^2 = 0.125$). The SNP is located in an intron of *SLC26A9* (solute carrier family 26 (sulfate transporter), member 9). Multiple neighboring SNPs in LD with rs11240594 also appeared to tag the same signal (see Figure 1). These included rs12727528 (p -value = 5.65×10^{-5} in EA) and several other markers with p -values $\sim 10^{-3}$. In addition to mediating the effects of olanzapine on processing speed, rs11240594 showed association ($p < 0.05$) with olanzapine and verbal memory ($p = 0.0377$), vigilance ($p = 0.0434$), and the neurocognitive composite ($p = 0.0022$), in addition to ziprasidone with processing speed ($p = 0.0012$).

Among the six genome-wide significant results was rs11214606 at *DRD2* that mediated the effect of olanzapine on working memory (p -value 4.84×10^{-7} , q -value 0.081, $r^2 = 0.120$). As *DRD2* is a well-known candidate for antipsychotic response and a drug target of all antipsychotic medications, this was an encouraging finding. Nevertheless, as can be seen in Figure 1, rs11214606 is an orphan finding,

with no other SNPs in the region showing substantial evidence for association. Consistent with HapMap data, however, LD between rs11214606 and neighboring markers is very low, that is r^2 does not exceed 0.05 in the CATIE sample. Therefore, we cannot expect neighboring markers to tag the same signal. SNP rs11214606 did not show association at the $p < 0.05$ level with any other drug or outcome combination, although there were trends approaching significance with other outcomes related to olanzapine (see Supplementary Material).

All of the remaining SNPs achieving genome-wide significance were also with olanzapine and working memory. These included rs7520258 (p -value 5.1×10^{-7} , q -value 0.081, $r^2 = 0.120$), located in an intron of the *GPR137B* (G protein-coupled receptor 137B) gene on chromosome 1q42–43 and rs4805924 (p -value 6.1×10^{-7} , q -value 0.08, $r^2 = 0.119$), which is situated ~ 7 kb from the start of transcription at *CHST8* (carbohydrate (*N*-acetyl-galactosamine 4-0) sulfotransferase 8) on chromosome 19q13. (However, the more recent genome build 37.1 provisionally locates rs4805924 in an intron of *CHST8*). As can be seen

from the regional plots in Figure 1, both of these findings are isolated with no neighboring SNPs showing robust evidence for association. In particular, it is worth remarking that rs7520258 at *GPR137B* is almost invariant in EAs (MAF < 0.01 in CATIE), suggesting that the signal, if true, is almost entirely coming from the non-EA ethnic groups. For the finding at *CHST8*, HapMap shows rs4805924 to be in an LD block of 22 kb. However, within this block only two HapMap SNPs were in high LD with rs4805924 (rs16968363 and rs10500266, both with $r^2 = 0.79$) and these were not genotyped in CATIE. Of the remaining SNPs in the block that were genotyped in our sample, none showed LD with $r^2 > 0.15$ and, therefore, could not be expected to tag the same signal. Nevertheless, despite a lack of suitable SNP proxies, both of these genome-wide significant SNPs showed some evidence for association ($p < 0.05$) across different neurocognitive domains and drugs. In addition to the primary finding with olanzapine and working memory, rs7520258 appeared to mediate the effects of ziprasidone on processing speed ($p = 0.0133$), while rs4805924 showed association with olanzapine and processing speed ($p = 0.0045$), in addition to olanzapine and the neurocognitive composite ($p = 0.0029$).

The final genome-wide significant finding, again with olanzapine and working memory, is rs11677416 (p -value 6.67×10^{-7} , q -value 0.081, $r^2 = 0.119$), located ~ 2 kb from the boundary of *IL1A* (interleukin-1 α) on chromosome 2q14. This SNP showed evidence for association across other neurocognitive domains, including olanzapine with reasoning ($p = 0.0045$), vigilance ($p = 0.0084$), and the neurocognitive composite ($p = 0.0003$), in addition to perphenazine and reasoning ($p = 0.0065$). Another SNP, rs17561, ~ 8 kb from rs11677416, was almost genome-wide significant with a q -value of 0.102. This latter SNP is located in the coding sequence of *IL1A* and gives rise to a non-synonymous amino-acid substitution of alanine to serine at protein position 114. Consistent with HapMap data, the two SNPs were in almost perfect LD in our sample ($r^2 = 0.97$ in AA and 0.99 in EA), suggesting that both markers are tagging the same signal.

Candidate Gene Analysis

In addition to the GWAS results, we also report our findings for markers located in, or in the vicinity of, specific candidate genes. Examination of 1595 SNPs at 41 candidate genes, that is ~ 39 SNPs per gene (see Supplementary Table S5; Supplementary Material, p15) yielded only three findings with q -values below 0.5. The remaining tests all had q -values very close to 1. This was somewhat surprising, because far fewer tests are performed relative to GWAS and FDR-based q -values are known to be too liberal in these scenarios (Zaykin *et al*, 2000). However, the lack of complete coverage for many important candidate genes using these arrays, as we have discussed before (McClay *et al*, 2009), means that we cannot reliably exclude association for many of these loci. The top finding in this analysis was rs11214606 at *DRD2*, mediating the effects of olanzapine on working memory (p -value $= 4.84 \times 10^{-7}$, q -value = 0.022). This is the same SNP at *DRD2* that was genome-wide significant in the main analysis. The remaining findings with 'potentially interesting' q -values

were rs7766029 at cannabinoid receptor 1 (*CNR1*), mediating the effects of perphenazine on verbal memory (p -value $= 1.76 \times 10^{-5}$, q -value = 0.391) and rs936465 at *DRD4* that mediated the effects of ziprasidone on the neurocognitive composite measure (p -value $= 3.13 \times 10^{-5}$, q -value = 0.463).

DISCUSSION

Understanding the genetic variation affecting response to antipsychotic drugs is important to develop novel diagnostic tests to match individual patients with schizophrenia to the most effective and safe medication. In this study, we performed GWAS of antipsychotic response, using neurocognitive function as the outcome variable(s). In addition to being a core deficit in schizophrenia, neurocognitive functions can be viewed as endophenotypes for the disorder and as such provide outcome measures that are potentially more genetically simple and tractable. In total, six markers achieved genome-wide significance in our study. In comparison with a previous study that we carried out in CATIE that used schizophrenia symptoms on the positive and negative syndrome scale (PANSS) as the outcome variables, this study with neurocognitive phenotypes would appear to be the more successful. Our study using the PANSS found only a single SNP to be significant at a genome-wide level and only three SNPs in genes with q -values < 0.5 (McClay *et al*, 2009).

An SNP in *DRD2* reached genome-wide significance, mediating the effect of olanzapine on working memory. *DRD2* is a well-known candidate for antipsychotic response and is a drug target of olanzapine (Kapur *et al*, 1999). However, despite the strong theoretical rationale for this gene being associated with antipsychotic drug response, our association evidence for *DRD2* is perhaps less robust than is the case with some of the other genome-wide significant findings. First, no neighboring SNPs in our sample showed evidence for association, although LD between neighboring markers was low. Furthermore, the SNP was not significantly associated with other outcome variables in our study, although there were trends toward significance with some other olanzapine-related variables that were in the same direction as the primary finding (eg, reasoning, neurocognitive composite—see Supplementary Table S4A in the Supplementary Material). If the effect is real, it is likely quite small and, therefore, difficult to detect consistently in samples of the size used here. Even in view of *DRD2*'s status as a likely antipsychotic pharmacogene, our finding at this locus should still be viewed as preliminary and requiring replication.

Our top finding overall was an SNP in the *EHF* (ETS homologous factor) gene on chromosome 11. This gene is a member of the ETS family of transcription factors, which are involved in many aspects of gene regulation and development. *EHF* is expressed at high levels in epithelial tissues, primarily in glandular epithelium such as prostate, pancreas, salivary gland, and trachea (Kas *et al*, 2000). Out of 438 *EHF* tissue-specific human cDNA clones in AceView (Thierry-Mieg and Thierry-Mieg, 2006) (<http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html>), only a single clone of *EHF* was found in brain. Thus, while expression in brain cannot be completely ruled out, it is likely to be at

very low levels. Current information on this gene and its function is relatively sparse. One of the few specific known actions of *EHF* is as a transcriptional repressor downstream of mitogen-activated protein kinase (MAPK) signaling cascades (Tugores *et al*, 2001). This is possibly of note because mitogen-activated protein kinase kinase 9 (*MAP3K9*), an epithelial-specific MAPK (Dorow *et al*, 1993), also emerged as one of our most significant findings (Table 3).

The second best genome-wide significant finding was with *SLC26A9* (solute carrier family 26, sulfate transporter, member 9), which is a member of the SLC26 sulfate/anion transporter gene family. These are anionic transporters involved in chloride and bicarbonate ion absorption or secretion in epithelia (Loriol *et al*, 2008). The product of *SLC26A9* is a highly selective chloride ion channel that is regulated by WNK kinases (Dorwart *et al*, 2007). It is expressed at high levels in airway and gastric surface epithelia, but is also expressed in the brain (based on the AceView (Thierry-Mieg and Thierry-Mieg, 2006) and UniGene (Wheeler *et al*, 2003) databases). It is a relatively uncharacterized gene, but its regulation by WNK kinases, specifically WNK4, may be significant. WNK4 has been shown to interact with intersectin (He *et al*, 2007) and we found *ITSN2* (intersectin 2) among our top findings (q -value = 0.24). WNK4 is also known to regulate cellular chloride ion permeability and is localized to tight junctions (Kahle *et al*, 2004), that is networks of sealing strands that join epithelial cell membranes, thereby creating a virtually impermeable barrier to fluid. One of the two major classes of tight junction proteins is the claudins, the other being the occludins. We found *CLDN1* (claudin 1) to be associated with quetiapine and working memory with a q -value of 0.169. Claudins are well recognized to have a major function in cellular permeability (Tsukita and Furuse, 2000).

These associations with *EHF* and other epithelial-related genes would not appear to have such an obvious connection to antipsychotics as compared, for example, with *DRD2*. However, with the caveat that these findings still require replication, a plausible hypothesis is that these genes are involved in epithelial barrier permeability. The role of epithelial barriers in pharmacokinetics has long been established. As such, our GWAS may have simply uncovered specific loci harboring variation that contribute to individual differences in barrier permeability, and by extension, antipsychotic availability and efficacy. As most antipsychotics are administered orally, absorption across gut epithelia is a plausible mechanism, as is elimination of drug across hepatic or renal epithelia. Also, it is conceivable that the closely related endothelial tissues of the blood-brain barrier may be involved.

Two SNPs among our top hits were at interleukin-1- α (*IL1A*), which also appeared to mediate the effect of olanzapine on working memory. It has been shown that intravenous administration of human interleukin-1- α protein induces memory impairments in mice (Banks *et al*, 2001). However, there does not appear to be any existing evidence in the literature linking *IL1A* to antipsychotics or schizophrenia. Interleukin-1- β (*IL1B*), which is located ~45 kb from *IL1A*, has been previously associated with altered brain structure in patients with schizophrenia (Meisenzahl *et al*, 2001). However, the

association in this study is clearly localized to *IL1A* (Figure 1) and as mentioned in Results, one of the two top SNPs in *IL1A* (rs17561) is non-synonymous, leading to an amino-acid change at protein position 114. This SNP, therefore, makes an appealing target for future functional study.

We previously reported *ANKK1B* as one of our top findings in a CATIE pharmacogenomics GWAS, mediating the effects of olanzapine on negative symptoms (McClay *et al*, 2009), as measured by the PANSS. In this study, we again find *ANKK1B* among our top findings, this time mediating the effect of quetiapine on working memory. As these findings are both in the same sample, and negative symptoms are known to be correlated with neurocognitive functioning, the fact that we see the same gene appearing in both analyses may not appear too surprising. However, in CATIE, the subjects being treated with one drug are for an important part different from those treated with a second drug. For example, only 49 CATIE subjects received both quetiapine and olanzapine. Furthermore, correlations between drug effects on negative symptoms and cognitive function (working memory in this specific instance) are present, but not particularly large (typically in the 0.1–0.2 range). Thus, due to largely non-overlapping samples and modest correlation between drug effects on negative symptoms and cognitive function, the *ANKK1B* findings in CATIE involve to a large extent independent observations. Furthermore, it is of note that *ANKK1B* also appeared among the top findings in a GWAS of citalopram response carried out by another group in the sequenced treatment alternatives to relieve depression (STAR*D) sample (Garriock *et al*, 2010). While this gene remains to be fully characterized, a recent study identified the *ANKK1B* protein to be part of a large post-synaptic complex, containing many neurotransmitter receptors and other proteins of relevance to schizophrenia (Fernandez *et al*, 2009). Clearly, the next step with *ANKK1B*, in addition to replication, is to identify possible causative variants. In our GWAS, we observed another ankyrin-related gene, *ANKRD33* (ankyrin repeat domain 33), among the top findings. While there is currently little to no literature on this gene, its association in our GWAS may suggest a more general role for ankyrin proteins in antipsychotic response.

CHST8 (carbohydrate *N*-acetylgalactosamine 4-0 sulfo-transferase 8) was also significant at the genome-wide level. The function of its gene product is to carry out the sulfation of carbohydrates. Little published material is available on this gene, but it is considered a plausible pharmacokinetics candidate gene. For example, markers at this locus are included on the Affymetrix DMET plus array, which is designed to detect variation relevant to drug metabolizing enzymes and transporters (Burmester *et al*, 2010). The remaining genes among our top findings (ie, q -value < 0.25) are either uncharacterized or have biological functions that do not immediately suggest obvious links to antipsychotic efficacy. For example, *GPR137B* (G protein-coupled receptor 137B), which achieved genome-wide significance for mediating the effects of olanzapine on working memory, is a member of the G protein-coupled receptor superfamily and is known to be expressed in brain (UniGene). However, its associated ligand has not yet been discovered and no other information on the function of this receptor is available currently.

We present our results with some caveats and cautions. First, it has been shown previously that practice/placebo effects can be apparent with the types of neurocognitive outcome described here (Goldberg *et al*, 2007; Keefe *et al*, 2008). It is possible that the significant findings reported here reflect a genetically mediated ability to benefit from practice or general placebo effects such as expectation bias. Second, the reported effect sizes for the associated SNPs are most certainly overestimates. That is, because of sampling error, effect sizes in an initial study are often greater than those reported in subsequent replications (Goring *et al*, 2001; Ioannidis *et al*, 2001). Finally, chance findings are a possibility, as with any association study. Even with FDR control at the 0.1 level, 1 in 10 findings, on average, will be a false positive. This issue can only be addressed through replication in independent samples. To facilitate this, we provide all *p*-values (<http://www.people.vcu.edu/~jlmccclay>) for download as a resource for investigators with the requisite samples to carry out replication. However, to some extent, the GWAS would appear to have served its purpose, in that it immediately suggests novel follow-up experiments, in addition to simple replication.

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