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Genotypic variation in the *SV2C* gene impacts response to atypical antipsychotics the CATIE Study☆

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ABSTRACT

Pharmacogenetic (PGx) predictors of response would improve outcomes in antipsychotic treatment. Based on both biological rationale and prior evidence of an impact on Parkinson's disease, we conducted an association study for 106 SNPs in the synaptic vesicle protein 2C (SV2C) gene using genetic and treatment response data from the Clinical Trial of Antipsychotic Intervention Effectiveness (CATIE). We examined response to the atypical antipsychotics for Caucasian subjects in the blinded phases, Phases 1A, 1B, and 2, of CATIE with sample sizes as follows: olanzapine (N = 134), quetiapine (N = 124), risperidone (N = 134), and ziprasidone (N = 74). Response was defined as change in the Positive and Negative Syndrome Scale (PANSS) score using a mixed model repeat measures approach. Subjects homozygous for the T allele of rs11960832 displayed significantly worse response to olanzapine treatment, the only finding with study-wide significance ($p = 2.94 \times 10^{-5}$; false discovery rate $= 2.18 \times 10^{-2}$). These subjects also displayed worse response to quetiapine with nominal significance ($p = 4.56 \times 10^{-2}$). While no other SNP achieved study-wide significance, one SNP (rs10214163) influencing Parkinson's disease displayed nominally significant association with olanzapine and quetiapine response, while the second such SNP (rs30196) showed a statistical trend toward correlating with olanzapine and quetiapine response. Furthermore, both coding SNPs examined (rs31244 and rs2270927) displayed nominally significant correlations with treatment response: one for olanzapine (rs227092), and one for quetiapine (rs31244). The fact that multiple SNPs in SV2C may impact response to atypical antipsychotics suggests that further evaluation of SNPs in this gene as PGx predictors of antipsychotic response is warranted.

1. Introduction

Variation in response to antipsychotic treatment complicates the treatment of patients with schizophrenia, and pharmacogenetic (PGx) predictors of efficacy for antipsychotics have proven challenging to find. Several studies have been published on PGx response to antipsychotics, most of which have focused on Genome Wide Association Study (GWAS) approaches using an additive genetic model (Volpi et al., 2009; Adkins et al., 2011; McClay et al., 2011; Aberg et al., 2012; Fijal et al., 2012; Liu et al., 2012). Though often overlooked, genetic variants might just as well impact treatment response via other modes of inheritance (as tested by dominant and recessive genetic models) (Lavedan et al., 2009; Need et al., 2009; Fijal et al., 2012).

Recently, the synaptic vesicle protein 2C (*SV2C*) gene was identified as a potential pharmacogenetic (PGx) modulator of nicotine's protective effect on Parkinson's disease incidence (Hill-Burns et al., 2012). Following a genome-wide scan for interaction with smoking and Parkinson's disease, the authors identified eighteen single nucleotide polymorphisms (SNPs) in the promoter region and early 5′ portion of the gene that moderated the impact of smoking on Parkinson's susceptibility. Interestingly, rather than experiencing a protective effect from nicotine consumption, subjects homozygous for the minor alleles of rs10214163 and rs30196 experienced an increased incidence of Parkinson's disease.

The SV2C protein has a number of characteristics that may have relevance to the pathophysiology and pharmacological treatment of affective disorders and psychosis. SV2C is found on the surface of synaptic vesicles, and it has been hypothesized that SV2C plays a role in synaptic function (Dardou et al., 2013). In mice, SV2C has restricted distribution in the brain, being found in specific regions of the striatum, substantia nigra, neostriatum, and pallidum (Dardou et al., 2011). Expression of SV2C is inversely linked to tyrosine hydroxylase expression, with SV2C knockout mice exhibiting large increases in production of tyrosine hydroxylase mRNA (Dardou et al., 2013). As tyrosine hydroxylase is the rate-limiting enzymatic step in the production of dopamine and norepinephrine, SV2C may play a critical role in catecholamine-mediated activities of the basal ganglia (Dardou et al., 2013). Consistently, decreased SV2C expression

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produces a deficit in the cocaine conditioned place preference procedure, abolishing the preference for cocaine-associated locations (Dardou et al., 2013). Additionally, SV2C appears to preferentially co-localize with Gamma Amino Butyric Acid (GABA) vesicles but not glutamate vesicles (Gronborg et al., 2010). Outside the brain, SV2C is highly expressed in the pancreas, especially in islet cells. It has been linked to glucose-mediated but not Ca^{++}-mediated insulin release (Iezzi et al., 2005).

SV2C has many attributes that make it an interesting candidate PGx gene for antipsychotic response. While the exact mechanism of action for SV2C with regard to vesicle transport of hormones and neurotransmitters remains unclear, its involvement with both GABA and insulin transporters may have implications in the etiology of schizophrenia and its treatment. It is expressed in dopaminergic neurons located in brain areas associated with the pathology of psychosis (Dardou et al., 2013). The entire group of synaptic vesicle family 2 proteins (SV2A, SV2B, and SV2C) provides key components of neurotransmitter vesicles (Gronborg et al., 2010). These proteins interact with many components of the vesicle structure. SV2C appears to be associated preferentially with GABA vesicles and not associated with glutamate vesicles, which instead show enrichment of SV2B protein levels (Gronborg et al., 2010). Several laboratories have shown that down-regulation of mRNAs encoding glutamic acid decarboxylase 67 and reelin decreases the cognate proteins coexpressed in prefrontal cortex GABAergic neurons of post-mortem schizophrenic and bipolar brains (Guidotti et al., 2005). GABAergic neurons release reelin that can interact with integrin receptors on cortical pyramidal neurons and regulate mRNA translation. The hypoplasticity of the prefrontal cortex in schizophrenia and bipolar disorder may be associated with such downregulation and may play a role in negative symptomatology.

In the current study, we explored the impact of SNPs in the *SV2C* gene on response to atypical antipsychotics in Caucasian subjects in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study of antipsychotic response in schizophrenia. This analysis evaluated the three common models of genetic variation (additive, dominant, and recessive) using the mixed model approach developed previously as a measure of pharmacological response (van den Oord et al., 2009; Liu et al., 2012). We report the top results for these analyses and discuss the implications for further evaluation of genetic variation in the *SV2C* gene as potential PGx markers for antipsychotic response.

2. Materials and methods

2.1. Patients

The patient population and the CATIE data used are described in detail elsewhere (Stroup et al., 2003; Lieberman et al., 2005; Sullivan et al., 2008; Liu et al., 2012). Briefly, this study was limited to self-described Caucasian subjects, diagnosed with schizophrenia and participating in the CATIE trial. All subjects in this study provided informed consent for genetic testing and participated in at least one of the randomized phases of the study, Phases 1A, 1B, and 2 (Lieberman et al., 2005). For each drug, Table 1 presents total number of patients treated, mean and standard deviation for baseline PANSS score and age, and distribution between the sexes. The Center for Collaborative Genomic Studies on Mental Disorders (CCGSMD) provided the genotypes and phenotypes, including drug response data.

2.2. Measures of response and drugs used

As described previously, we used data from all time points available from Phases 1, 1A, and 2 in CATIE from Caucasian subjects treated with atypical antipsychotics to develop a treatment response variable. The sample sizes were as follows: olanzapine, 134; perphenazine, 75; quetiapine, 124; risperidone, 134; and ziprasidone, 74. We implemented the mixed model repeated measures (MMRM) approach developed by Van den Oord et al. (van den Oord et al., 2009; McClay et al., 2011) as a method of modeling antipsychotic response. This model predicted an overall response as defined as change in Total Score on Positive and Negative Syndrome Scale (PANSS-T). PANSS-T provided a continuous variable for use in genetic analysis.

Table 1
Sample characteristics.

Treatment	Sample Size	Mean Baseline(SD)[a]	Mean age (SD)[b]	# of Females[b]	# of Males[b]
Olanzapine	134	73.5 (18.8)	40.5 (11.6)	34	100
Quetiapine	124	73.5 (18.0)	40.5 (11.2)	29	95
Risperidone	134	77.5 (17.2)	42.2 (11.4)	29	105
Ziprasidone	74	71.3 (16.2)	40.1 (11.1)	21	53

[a] Baseline PANSS was a significant contributor to drug response and therefore was incorporated into the mixed model that provided the response variable used for the genetic analysis.
[b] Both sex and age were considered for inclusion in the response model; however as they did not significantly contribute to response they were not included in the final mixed model.

2.3. Statistical methods

For each atypical antipsychotic arm, we conducted genetic association analysis in SVS version 7.3.1 software package (Golden Helix Inc. Bozeman, MT) using the additive, dominant, and recessive models with change in PANSS-T as the dependent variable. The genetic association analysis tested the null hypothesis that subjects with varying combinations of minor alleles did not display a differences in mean PANSS-T for a given drug treatment.

When choosing a p-value threshold to report, one must strike a balance between concerns for false positive and disregarding true signals due to sample size issues. Because this study was a candidate gene study with several lines of evidence suggesting that the *SV2C* gene might have a PGx impact on antipsychotic drug response, we have reported all nominally significant results. We evaluated the impact of tobacco use and alcohol consumption status for all nominally significant findings (using CCGSMD-provided status at study enrollment) by analysis of variance (ANOVA).

To mitigate false positives resulting from the small sample sizes found for some rarer groups comprising homozygous individuals, only those SNPs with homozygosity for the minor allele at a frequency ≥ 0.03 in a given drug arm were used. For SNPs for which there were no cases of homozygosity for the minor alleles, we tested only the dominant model. We analyzed a total of 106 SNPs beginning with rs11960832 in the promoter region and concluding with rs2913257, the 3′ most SNP lying within the transcribed region that was included in the CCGSMD-provided data. Excluding the rare and missing genotype groups resulted in a total of 742 individual tests needed to evaluate the four atypical antipsychotics using the three genetic models. The Benjamini method for False Discovery Rate (FDR) was used to correct for multiple comparisons (Benjamini et al., 2001).

To evaluate if multiple findings for a given drug were due to correlation between the SNPs, we determined the pairwise r^2 values for all nominally significant findings. These linkage disequilibrium (LD) values were calculated along with a graphical representation of the results using Haploview software (version 4.2) (Barrett et al., 2005).

3. Results

3.1. SV2C SNP association results for response to atypical antipsychotics

Table 2 lists results ($p < 0.05$) from the SNP association analysis for PGx impact on the atypical antipsychotics in the CATIE trial. Of