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Association of *RGS2* and *RGS5* variants with schizophrenia symptom severity

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Abstract

Background: Several lines of evidence indicate that Regulator of G Protein Signaling 4 (*RGS4*) contributes to schizophrenia vulnerability. RGS4 is one of a family of molecules that modulate signaling via G-protein coupled receptors. Five genes encoding members of this family (*RGS2*, *RGS4*, *RGS5*, *RGS8* and *RGS16*) map to chromosome 1q23.3–1q31. Due to overlapping cellular functions and chromosomal proximity, we hypothesized that multiple RGS genes may contribute to schizophrenia severity and treatment responsiveness.
Methods: Subjects were 750 individuals with schizophrenia who participated in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). Inferred ancestries were: 221 (30%) 'Africa only', 422 (56%) 'Europe only' and 107 (14%) 'Other'. Fifty-nine single nucleotide polymorphisms (SNPs) in or near the *RGS5*, *RGS16*, *RGS8* and *RGS2* genes were genotyped. Multiple linear regression was used to analyze association of markers with Positive and Negative Symptoms Scale (PANSS) total scores at baseline and throughout antipsychotic treatment.
Results: RGS5 marker rs10799902 was associated with altered baseline PANSS total score in both the Africa only ($P=0.0440$) and Europe only ($P=0.0143$) strata, although neither association survived multiple comparisons correction. A common five-marker haplotype of the *RGS2* gene was associated with more severe baseline PANSS total score in the Europe only strata (global $P=0.0254$; haplotype-specific $P=0.0196$). In contrast to *RGS4*, none of the markers showed association with antipsychotic treatment response.
Conclusions: RGS2 and *RGS5* genotypes predicted severity of baseline symptoms in schizophrenia. Although these analyses are exploratory and replication is required, these data suggest a possible role for multiple RGS proteins in schizophrenia.

Keywords: Genetics; Candidate gene; Pharmacogenetics; G protein coupled receptors; Regulator of G-protein signaling; Cerebral cortex

1. Introduction

Schizophrenia is a neurodevelopmental disorder with a substantial genetic component contributing to risk (Sullivan et al., 2003). Independent functional and

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genetic studies indicate that the regulator of G-protein signaling 4 (*RGS4*) gene is among a small group of promising schizophrenia vulnerability genes (Harrison and Weinberger, 2005; Norton et al., 2006). The initial focus on *RGS4* arose from gene microarray and *in situ* hybridization studies showing decreased levels of *RGS4* mRNA across cortical regions (Mirnics et al., 2001). Subsequent analyses in postmortem cerebral cortex of patients with schizophrenia have confirmed decreased levels of *RGS4* transcript in superior temporal gyrus (Bowden et al., 2007) and decreased RGS4 protein in frontal cortex (Erdely et al., 2006). A genetic association between *RGS4* and schizophrenia was detected initially in three different populations by Chowdari et al. (Chowdari et al., 2002), and confirmed in five subsequent replication studies (Chen et al., 2004; Fallin et al., 2005; Morris et al., 2004; Williams et al., 2004; Zhang et al., 2005). There have been four reports of failures to replicate an association of *RGS4* and schizophrenia (Kampman et al., 2006; Liu et al., 2006; Rizig et al., 2006; Sobell et al., 2005). However, there also are recent reports of association of certain intermediate phenotypes with *RGS4* variants (Buckholtz et al., 2007; Lipska et al., 2006; Prasad et al., 2005). Thus, *RGS4* remains a strong schizophrenia candidate gene, but its contributions must be considered in the context of heterogeneity (Levitt et al., 2006; Talkowski et al., 2006). We recently described association of *RGS4* variants with both baseline schizophrenia symptom severity and antipsychotic treatment response in the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) sample (Campbell et al., 2008).

RGS4 is one of more than 20 genes that encode RGS proteins, important regulatory components of G-protein coupled receptor (GPCR) complexes. Antipsychotic medications act to modulate GPCRs stimulated by dopamine, acetylcholine and serotonin. RGS proteins shorten the duration of signaling via GPCRs by acting as GTPase activating proteins (GAPs), thereby accelerating the deactivation of the heterotrimeric G protein following receptor activation. Therefore, alterations in RGS availability or function could alter the effectiveness of antipsychotic medications.

Based on homologous domain structure, the RGS proteins are categorized into families (Sierra et al., 2002; Xie and Palmer, 2007). Genes encoding five of the ten "R4" family proteins lie within a 30-Mb region of human chromosome 1q23.3–31, within a schizophrenia linkage region identified in several genome-wide scans (Brzustowicz et al., 2000; Ekelund et al., 2004; Ekelund et al., 2001; Ekelund et al., 2000; Gurling et al., 2001; Hovatta et al., 1999; Hwu et al., 2003; Jang et al., 2007; Shaw et al., 1998). Transcripts for each of these five chromosome 1q R4 family proteins – RGS2, RGS4, RGS5, RGS8 and RGS16 – are abundantly expressed in the cerebral cortex. Additionally, *Rgs2* was found to be decreased in the prefrontal cortex of rats following chronic treatment with the antipsychotic olanzapine (Fatemi et al., 2006) and expression levels of the *Rgs2* and *Rgs5* transcripts were found to be altered in the *Drd1* receptor knockout mice (Stanwood et al., 2006). The confluence of these functional data with the chromosomal locations of the five R4 family genes led us to hypothesize that other members of the R4 family, in addition to *RGS4*, may contribute to schizophrenia pathogenesis and differential antipsychotic treatment response.

To test the hypotheses that *RGS2*, *RGS5*, *RGS8* and *RGS16* contribute to schizophrenia symptom severity and antipsychotic treatment response, we performed an exploratory study to determine association of genetic markers in or near these four genes with clinical traits involved in the diagnosis of schizophrenia using the data generated by the CATIE trial (Lieberman et al., 2005; Stroup et al., 2003). Our analyses show that baseline Positive and Negative Symptoms Scale (PANSS) scores differed among *RGS2* and *RGS5* marker genotypes.

2. Experimental/materials and methods

2.1. Subjects

The parent study has been described at length elsewhere (Lieberman et al., 2005; Stroup et al., 2003). Briefly, all subjects participated in CATIE (January 2001 to December 2004), a multi-phase randomized controlled trial of antipsychotic medications involving 1460 persons with schizophrenia followed for up to 18 months. All subjects provided written informed consent (including an additional consent for genetic studies), and the full study protocol was reviewed by IRBs at the University of North Carolina and at participating study sites. Establishment of schizophrenia diagnosis, inclusion criteria and exclusion criteria have been described elsewhere (Sullivan et al., 2007).

2.2. Phenotypes

Analyses were restricted to the total score of the Positive and Negative Symptoms Scale (PANSS), a broadly accepted measure for reliably ascertaining severity of schizophrenia symptoms (Kay et al., 1987).

The CATIE treatment protocol is described elsewhere (Lieberman et al., 2005; Stroup et al., 2003).