Published in final edited form as:
Schizophr Res. 2008 April ; 101(1-3): 67–75.

ASSOCIATION OF *RGS2* AND *RGS5* VARIANTS WITH SCHIZOPHRENIA SYMPTOM SEVERITY

Daniel B. Campbell[1], **Leslie A. Lange**[2], **Tara Skelly**[2], **Jeffrey Lieberman**[3], **Pat Levitt**[1,4], and **Patrick F. Sullivan**[2,5]

1*Department of Pharmacology, Vanderbilt University, Nashville, TN 37232 USA*

2*Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA*

3*Department of Psychiatry, Columbia University, New York, NY 10032 USA*

4*Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37203 USA*

5*Department of Medical Epidemiology & Biostatistics, Karolinska Institutet, SE-171 77 Stockholm, Sweden*

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.

Corresponding author: Daniel B. Campbell, Ph.D.; Department of Pharmacology; 8114 MRB3; 465 21st Avenue South; Vanderbilt University; Nashville, TN 37232; email: daniel.campbell@vanderbilt.edu.

Financial Disclosures

Dr. Lieberman reports having received research funding from AstraZeneca Pharmaceuticals, Bristol-Myers Squibb, GlaxoSmithKline, Janssen Pharmaceutica, and Pfizer and consulting and educational fees from AstraZeneca Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly, Forest Pharmaceuticals, GlaxoSmithKline, Janssen Pharmaceutica, Novartis, Pfizer, and Solvay. Dr. Sullivan reports receiving research funding from Eli Lilly. Dr. Campbell, Dr. Lange, Ms. Skelly and Dr. Levitt report no biomedical financial interests or potential conflicts of interest.

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 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 1,460 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 2006).

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). Briefly, patients with schizophrenia were randomly assigned to one of five antipsychotic treatments in Phase 1. The five treatments in Phase 1 consisted of a first-generation antipsychotic, perphenazine, and several second-generation (atypical) antipsychotics, olanzapine, quetiapine risperidone and ziprasidone (Lieberman et al 2005). The analyses reported here are based on the 750 DNA samples available at the close of the CATIE trial.

The primary goal of the CATIE study was to compare the clinical effectiveness of a first-generation antipsychotic, perphenazine, to four newer antipsychotics (Lieberman et al 2005). Therefore, our analyses focused on comparing the response to perphenazine versus each of the second-generation antipsychotics in phase 1 of CATIE. Change in PANSS total score from baseline, measured longitudinally, was used to infer treatment response.

2.3. DNA Samples

High molecular weight DNA samples were obtained from all consenting subjects from cell lines established via EBV transformation at the Rutgers University Cell and DNA Repository (http://www.rucdr.org/quality_control.html). Sample DNA concentrations were quantified and normalized via the use of Picogreen dsDNA Quantitation Kits (Molecular Probes, Eugene, OR) (Sullivan et al 2006).

2.4. Genotyping and quality control

Selection of genotyping markers was performed with TAMAL (http://neoref.ils.unc.edu/tamal) (Hemminger et al 2006) using dbSNP, HapMap, Perlegen and UCSC data available mid-2005. SNPs were selected if they were more likely to lead to

structural variation (i.e., a coding SNP) or had predicted functional significance (e.g., a SNP in a highly conserved region or predicted promoter).

Genotyping was performed with TaqMan 5′ exonuclease assays (Livak 1999) as previously described (Sullivan et al 2006). The overall no-call rate had median 0.67% (range 0.267–2.80%). Genotyping was conducted blind to all clinical data. Automated allele calls were made with SDS Data Collection software with all allele calls reviewed by an experienced operator according to protocol. In order for a SNP to be released for analysis, we required that: (a) 46 duplicated samples per SNP were in agreement, (b) call rates for all plates exceeded 95%, and (c) the clustering patterns were robust per review by an experienced technician. The assay for *RGS8* marker rs1287978 failed to meet these quality control criteria and was thus excluded from the analyses.

2.5. Population stratification

Population stratification is a potential limitation of association studies (Sullivan et al 2001) and is of particular concern in CATIE, given its explicit intent to ascertain a diverse, clinically representative sample (Lieberman et al 2005; Stroup et al 2003). There are two preconditions for population stratification to yield false positive results (Gorroochurn et al 2004; Heiman et al 2004). Because population stratification as a confounder exists only if the phenotypic distribution and the marker allele frequency differ across ancestral strata, we can determine the impact of stratification by measuring the marker and disease frequency and predict the extent of stratification. If there is substantial evidence that population substructure exists in the overall CATIE sample for the markers studied here, we default to stratified analysis. CATIE subjects were allowed to select multiple racial categories (White, Black/African-American, American Indian or Alaska native, Asian, Native Hawaiian or Pacific Islander, or Other) and were also asked if they were Hispanic or Latino. Inferred ancestry was defined as “Africa only” if a subject endorsed Black/African-American only, ”Europe only” if a subject endorsed White only, and “Other” if any other racial category was endorsed.

2.6. Statistical analysis

Data management and statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc). For baseline PANSS score analyses, analysis of covariance (ANCOVA) models were used to test for genotypic association, with adjustment for ancestry, sex, and age. In CATIE, the PANSS was administered at baseline and at multiple time points during antipsychotic treatment. Longitudinal analyses were performed using multiple linear regression with generalized estimating equation (GEE) models (Zeger et al 1988), which allow adjustment for the non-independence of PANSS scores on the same individual over multiple time-points, used to test genotype X treatment interaction effects across phase 1. Significant interactions represent altered PANSS score throughout the treatment period when comparing perphenazine response to olanzapine, quetiapine, risperidone and ziprasidone responses. These models included the covariates of baseline PANSS score, treatment, sex, and age. Haplotype association tests for baseline PANSS score were performed using the program HAPLO.STAT (http://www.mayo.edu/statgen), which utilizes a weighting scheme based on EM derived haplotype frequency estimates and weights every haplotype rather than assigning a ‘most likely’ haplotype to an individual. An additive mode of inheritance assumption was made for all genotypic and haplotypic tests of association. HaploView (version 3.2) (Barrett et al 2005) was used to calculate tests of Hardy-Weinberg Equilibrium (HWE), and $|D'|$ and r^2 estimates to assess linkage disequilibrium.

3. Results

3.1. Sample description

The sample for this report is 750 CATIE subjects who provided DNA samples. Details on the sample are provided in Table 1.

3.2. Stratification by ancestry

Supplementary Table 1 contains Hardy-Weinberg Equilibrium (HWE) results for the SNPs genotyped. The allele frequencies for many of the genotyped SNPs were divergent from HWE in the combined sample but absent when analyzed separately by ancestry, suggesting population stratification. We therefore defaulted to analyzing the data by ancestral strata.

3.3. Regional map and linkage disequilibrium (LD)

Figure 1 depicts the 30-Mb region of chromosome 1q23.3-1q31 including *RGS4*, *RGS5*, *RGS16*, *RGS8* and *RGS2* in the 422-sample Europe only strata. A total of 67 markers (including 8 markers genotyped in *RGS4*) (Campbell et al 2008) were genotyped in the CATIE sample. The *RGS4* 5′ markers associated with baseline schizophrenia severity and antipsychotic treatment response (rs951439, rs2661319 and rs2842030) (Campbell et al 2008) were not in LD with the remaining markers genotyped in the R4 family. In contrast, the *RGS4* 3′ markers showed high levels of LD with the nearest twelve markers of the neighboring *RGS5* gene (approximately 32-kb) (Figure 1). Therefore, associations of the *RGS5* gene must be interpreted in the context of its LD with *RGS4*. Thirteen additional markers near the 61-kb *RGS5* gene defined a separate 39-kb LD block (Figure 1). Thirteen markers genotyped near the 6-kb *RGS16* gene defined two LD blocks of approximately 15- and 2-kb (Figure 1). Twelve markers spanning the 28-kb *RGS8* gene defined two LD blocks of approximately 10- and 4-kb (Figure 1). Nine genotyped markers near the 3-kb *RGS2* gene defined two LD blocks of approximately 7-kb each (Figure 1). The LD structures were similar in the African and European ancestry strata (Supplementary Table 2).

3.4 Genotype association with baseline PANSS total score

RGS5 intron 1 marker rs10799902 demonstrated significant genotypic association with baseline PANSS total score in both the African and European ancestry strata (Table 2). The association did not survive Bonferroni correction in either strata (Table 2). In both ancestral strata, individuals of rs10799902 genotype A/A displayed higher baseline PANSS total scores than individuals with genotype G/G (Table 2). Of the 25 markers genotyped near the RGS5 gene, six showed association in the Africa only strata and two displayed association in the Europe only strata; however, none of these associations survived Bonferroni correction for multiple comparisons (Table 2; Supplementary Table 3). Of the seven *RGS5* markers with significant uncorrected differences in baseline PANSS total score, only rs2456899 has a high degree of LD with markers in *RGS4* (r^2 with *RGS4* marker rs2063142 = 0.458); however rs2456899 is not in LD (r^2<0.001) with the *RGS4* markers that showed association with baseline PANSS total score in our previous report (rs2661319 and rs2842030) (Campbell et al, 2008) (Supplementary Table 1). Haplotype-based analysis of a 6-marker LD block in the Africa only strata (rs4657248-rs4657251-rs10799902-rs7513108-rs2999967-rs2999965) including 3 markers with uncorrected association (rs10799902, rs2999967 and rs2999965) failed to show significant global association (P=0.33).

None of the 25 markers genotyped near the *RGS16* and *RGS8* genes demonstrated significant uncorrected genotypic differences in baseline PANSS total score (Supplementary Table 3).

Of the 9 markers genotyped near *RGS2*, uncorrected significant association was observed for five markers in the Europe only strata and one marker in the Africa only strata (Table 2).

However, none of these differences survived correction for multiple comparisons. In a 5-marker LD block spanning the 3′ end of the *RGS2* gene (rs2746073-rs4606-rs3767488-rs1819741-rs10801156; r^2>0.61), four markers revealed uncorrected differences in baseline PANSS total score in the Europe only sample: rs2746073 (P=0.0331), rs4606 (P=0.0261), rs3767488 (P=0.0325) and rs10801156 (P=0.0355) (Table 2). Haplotype-based analyses of this 5-marker LD block revealed a significant global association (P=0.0254) in the Europe only strata. There were only two haplotypes with frequency >1%. Haplotype A-G-G-C-C (frequency=0.257) was associated with significantly lower PANSS total score (P=0.0093); haplotype T-C-A-T-A (frequency=0.732) was associated with significantly higher baseline PANSS total score (P=0.0196) (Table 3). Permutation analyses confirmed the significant global haplotype association (P=0.030), the A-G-G-C-C association with lower PANSS total score (P=0.015) and the T-C-A-T-A association with higher PANSS total score (P=0.024).

3.5. Genotype association with antipsychotic treatment response

Using GEE models to include all available longitudinal data, SNP by treatment analyses for PANSS total indicated that, after correction for multiple comparisons, none of the genotype markers exhibited significant association (Supplementary Table 4). Of the 59 genotyped markers, we observed uncorrected significant differences for 3 markers in the Africa only sample and 5 markers in the Europe only sample (Supplementary Table 4). Notably, four consecutive markers in *RGS8*, all in LD (r^2>0.5), demonstrated uncorrected evidence for SNP by treatment interactions in the Europe only strata: rs3845459 (P=0.0291), rs10489965 (P=0.0358), rs1287978 (P=0.0517) and rs4652741 (P=0.0317). None of these markers show a main effect of genotype on treatment response in a longitudinal setting (Supplementary Table 4).

Tests of the main effect of genotype on treatment response revealed uncorrected significant differences for one marker in the Africa only and two markers in the Europe only strata (Supplementary Table 4). Notably, the two markers with uncorrected evidence for a main effect of genotype in the Europe only strata were consecutive markers in *RGS2*: rs2179652 (P=0.0446) and rs1890397 (P=0.0486) (Supplementary Table 4). None of these differences survive Bonferroni correction for multiple comparisons.

4. Discussion

The present exploratory study revealed an association of genetic variants in the *RGS5* gene and a haplotype block in the *RGS2* gene with baseline schizophrenia symptom severity. Thus, along with another R4 family member *RGS4*, *RGS2* and *RGS5* may contribute to schizophrenia symptom severity. In contrast to *RGS4*, none of the other R4 family members tested here contributed to differential antipsychotic treatment response in the CATIE Phase 1.

The results of this study should be interpreted cautiously as none of the uncorrected single marker associations survive correction for multiple comparisons. A limitation of this study was the necessity to stratify by ethnicity to account for population stratification, and thus decrease the sample size significantly.

The region of chromosome 1q harboring the R4 gene family may include multiple genes that contribute to schizophrenia vulnerability. Several schizophrenia genome-wide linkage scans have identified peaks in the chromosome 1q21-42 region (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). However, the location of the peak varies across linkage studies: linkage evidence has been reported for chromosome 1q21-24.2 (near the *RGS4* and *RGS5* genes) (Brzustowicz et al 2000; Gurling et al 2001; Hwu et al 2003; Shaw et al 1998), chromosome 1q31.1-32.2 (near the *RGS2* gene) (Ekelund et al 2001; Hovatta et al

1999; Jang et al 2007; Shaw et al 1998) and chromosome 1q42 (near the *DISC1* gene) (Ekelund et al 2004; Ekelund et al 2000; Hwu et al 2003). *RGS4* and *DISC1* are among the most promising schizophrenia candidate genes with multiple replications of the original positive association; however, multiple reports failing to replicate association with schizophrenia have been reported for both genes (Levitt et al 2006; Norton et al 2006; Talkowski et al 2006). At chromosome 1q21-23, recent positional candidate approaches have identified variants associated with schizophrenia within both the *CAPON* (Brzustowicz et al 2004) and *UHMK1* genes (Puri et al 2007). Association of *CAPON* was replicated in one sample (Zheng et al 2005), but failed to replicate in a different sample (Puri et al 2006). Near the chromosome 1q31-32 linkage peak, a single whole genome association study identified convincing evidence for association of markers in the *PLXNA2* gene in the discovery sample and four replication samples (Mah et al 2006); however, an independent attempt to replicate these findings failed to confirm the association (Fujii et al 2007). Perhaps as can be expected for a clinically heterogeneous disorder, although several promising schizophrenia candidate genes have been identified in the chromosome 1q21-42 region, conflicting results for each gene have been reported. The associations of *RGS2* and *RGS5* variants with baseline PANSS total scores, along with previous associations of *RGS4* variants with schizophrenia, suggest that multiple RGS proteins may modulate the function of circuits implicated in schizophrenia.

In conclusion, the data presented here suggest that variants in two genes, *RGS5* and *RGS2*, may influence the severity of schizophrenia symptoms. It is also conceivable that the baseline differences in the schizophrenia symptoms may reflect differential antipsychotic treatment responses to medications that patients had received prior to entry into CATIE Phase 1. In this context, it could be speculated that the RGS proteins are associated with an aspect of illness severity that influences patients' capacity to respond to antipsychotic drug treatment. Further experiments with a much larger sample collection will be required to test these hypotheses. As with all genetic association studies, precise replication is required in order to determine whether these findings are sample-specific or whether they apply more generally to clinical samples of individuals with schizophrenia (Sullivan 2007).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The CATIE project was supported by NIMH contract NO1 MH90001. The project reported here was funded by the Vanderbilt Kennedy Center P30 HD15052 (PL), and NIMH Conte Center MH45156 (D. Lewis, PI), Project 4 (PL, PI). PFS was supported by R01 MH-074027. Funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

References

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]

Bowden NA, Scott RJ, Tooney PA. Altered expression of regulator of G-protein signalling 4 (RGS4) mRNA in the superior temporal gyrus in schizophrenia. Schizophr Res 2007;89:165–168. [PubMed: 17071056]

Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. Science 2000;288:678–682. [PubMed: 10784452]

Brzustowicz LM, Simone J, Mohseni P, et al. Linkage disequilibrium mapping of schizophrenia susceptibility to the CAPON region of chromosome 1q22. Am J Hum Genet 2004;74:1057–1063. [PubMed: 15065015]

Buckholtz JW, Meyer-Lindenberg A, Honea RA, et al. Allelic variation in RGS4 impacts functional and structural connectivity in the human brain. J Neurosci 2007;27:1584–1593. [PubMed: 17301167]

Campbell DB, Ebert PJ, Skelly T, et al. Ethnic stratification of the association of RGS4 variants with antipsychotic treatment response in schizophrenia. Biol Psychiatry 2008;63:32–41. [PubMed: 17588543]

Chen X, Dunham C, Kendler S, et al. Regulator of G-protein signaling 4 (RGS4) gene is associated with schizophrenia in Irish high density families. Am J Med Genet B Neuropsychiatr Genet 2004;129:23–26. [PubMed: 15274033]

Chowdari KV, Mirnics K, Semwal P, et al. Association and linkage analyses of RGS4 polymorphisms in schizophrenia. Hum Mol Genet 2002;11:1373–1380. [PubMed: 12023979]

Ekelund J, Hennah W, Hiekkalinna T, et al. Replication of 1q42 linkage in Finnish schizophrenia pedigrees. Mol Psychiatry 2004;9:1037–1041. [PubMed: 15197400]

Ekelund J, Hovatta I, Parker A, et al. Chromosome 1 loci in Finnish schizophrenia families. Hum Mol Genet 2001;10:1611–1617. [PubMed: 11468279]

Ekelund J, Lichtermann D, Hovatta I, et al. Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. Hum Mol Genet 2000;9:1049–1057. [PubMed: 10767329]

Erdely HA, Tamminga CA, Roberts RC, Vogel MW. Regional alterations in RGS4 protein in schizophrenia. Synapse 2006;59:472–479. [PubMed: 16565965]

Fallin MD, Lasseter VK, Avramopoulos D, et al. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am J Hum Genet 2005;77:918–936. [PubMed: 16380905]

Fatemi SH, Reutiman TJ, Folsom TD, et al. Chronic olanzapine treatment causes differential expression of genes in frontal cortex of rats as revealed by DNA microarray technique. Neuropsychopharmacology 2006;31:1888–1899. [PubMed: 16407901]

Fujii T, Iijima Y, Kondo H, et al. Failure to confirm an association between the PLXNA2 gene and schizophrenia in a Japanese population. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:873–877. [PubMed: 17346868]

Gorroochurn P, Hodge SE, Heiman G, Greenberg DA. Effect of population stratification on case-control association studies. II. False-positive rates and their limiting behavior as number of subpopulations increases. Hum Hered 2004;58:40–48. [PubMed: 15604563]

Gurling HM, Kalsi G, Brynjolfson J, et al. Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. Am J Hum Genet 2001;68:661–673. [PubMed: 11179014]

Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 2005;10:40–68. [PubMed: 15263907]image 5

Heiman GA, Hodge SE, Gorroochurn P, Zhang J, Greenberg DA. Effect of population stratification on case-control association studies. I. Elevation in false positive rates and comparison to confounding risk ratios (a simulation study). Hum Hered 2004;58:30–39. [PubMed: 15604562]

Hemminger BM, Saelim B, Sullivan PF. TAMAL: an integrated approach to choosing SNPs for genetic studies of human complex traits. Bioinformatics 2006;22:626–627. [PubMed: 16418238]

Hovatta I, Varilo T, Suvisaari J, et al. A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. Am J Hum Genet 1999;65:1114–1124. [PubMed: 10486331]

Hwu HG, Liu CM, Fann CS, Ou-Yang WC, Lee SF. Linkage of schizophrenia with chromosome 1q loci in Taiwanese families. Mol Psychiatry 2003;8:445–452. [PubMed: 12740602]

Jang YL, Kim JW, Lee YS, et al. Linkage of schizophrenia with chromosome 1q32 in Korean multiplex families. Am J Med Genet B Neuropsychiatr Genet 2007;144:279–284. [PubMed: 17171664]

Kampman O, Illi A, Hanninen K, et al. RGS4 genotype is not associated with antipsychotic medication response in schizophrenia. J Neural Transm. 2006

Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261–276. [PubMed: 3616518]

Levitt P, Ebert P, Mirnics K, Nimgaonkar VL, Lewis DA. Making the case for a candidate vulnerability gene in schizophrenia: Convergent evidence for regulator of G-protein signaling 4 (RGS4). Biol Psychiatry 2006;60:534–537. [PubMed: 16860780]

Lieberman JA, Stroup TS, McEvoy JP, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 2005;353:1209–1223. [PubMed: 16172203]

Lipska BK, Mitkus S, Caruso M, et al. RGS4 mRNA expression in postmortem human cortex is associated with COMT Val158Met genotype and COMT enzyme activity. Hum Mol Genet 2006;15:2804–2812. [PubMed: 16905560]

Liu YL, Shen-Jang Fann C, Liu CM, et al. Evaluation of RGS4 as a candidate gene for schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2006;141:418–420. [PubMed: 16526029]

Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999;14:143–149. [PubMed: 10084106]

Mah S, Nelson MR, Delisi LE, et al. Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia. Mol Psychiatry 2006;11:471–478. [PubMed: 16402134]

Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P. Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. Mol Psychiatry 2001;6:293–301. [PubMed: 11326297]

Morris DW, Rodgers A, McGhee KA, et al. Confirming RGS4 as a susceptibility gene for schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004;125:50–53. [PubMed: 14755443]

Norton N, Williams HJ, Owen MJ. An update on the genetics of schizophrenia. Curr Opin Psychiatry 2006;19:158–164. [PubMed: 16612196]

Prasad KM, Chowdari KV, Nimgaonkar VL, Talkowski ME, Lewis DA, Keshavan MS. Genetic polymorphisms of the RGS4 and dorsolateral prefrontal cortex morphometry among first episode schizophrenia patients. Mol Psychiatry 2005;10:213–219. [PubMed: 15381923]

Puri V, McQuillin A, Choudhury K, et al. Fine mapping by genetic association implicates the chromosome 1q23.3 gene UHMK1, encoding a serine/threonine protein kinase, as a novel schizophrenia susceptibility gene. Biol Psychiatry 2007;61:873–879. [PubMed: 16978587]

Puri V, McQuillin A, Thirumalai S, et al. Failure to confirm allelic association between markers at the CAPON gene locus and schizophrenia in a British sample. Biol Psychiatry 2006;59:195–197. [PubMed: 16202394]

Rizig MA, McQuillin A, Puri V, et al. Failure to confirm genetic association between schizophrenia and markers on chromosome 1q23.3 in the region of the gene encoding the regulator of G-protein signaling 4 protein (RGS4). Am J Med Genet B Neuropsychiatr Genet 2006;141:296–300. [PubMed: 16508931]

Shaw SH, Kelly M, Smith AB, et al. A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 1998;81:364–376. [PubMed: 9754621]

Sierra DA, Gilbert DJ, Householder D, et al. Evolution of the regulators of G-protein signaling multigene family in mouse and human. Genomics 2002;79:177–185. [PubMed: 11829488]

Sobell JL, Richard C, Wirshing DA, Heston LL. Failure to confirm association between RGS4 haplotypes and schizophrenia in Caucasians. Am J Med Genet B Neuropsychiatr Genet 2005;139:23–27. [PubMed: 16082709]

Stanwood GD, Parlaman JP, Levitt P. Genetic or pharmacological inactivation of the dopamine D1 receptor differentially alters the expression of regulator of G-protein signalling (Rgs) transcripts. Eur J Neurosci 2006;24:806–818. [PubMed: 16930410]

Stroup TS, McEvoy JP, Swartz MS, et al. The National Institute of Mental Health Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project: schizophrenia trial design and protocol development. Schizophr Bull 2003;29:15–31. [PubMed: 12908658]

Sullivan PF. Spurious genetic associations. Biol Psychiatry 2007;61:1121–1126. [PubMed: 17346679]

Sullivan PF, Eaves LJ, Kendler KS, Neale MC. Genetic case-control association studies in neuropsychiatry. Arch Gen Psychiatry 2001;58:1015–1024. [PubMed: 11695947]

Sullivan PF, Keefe RS, Lange LA, et al. NCAM1 and Neurocognition in Schizophrenia. Biol Psychiatry. 2006

Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 2003;60:1187–1192. [PubMed: 14662550]

Talkowski ME, Seltman H, Bassett AS, et al. Evaluation of a Susceptibility Gene for Schizophrenia: Genotype Based Meta-Analysis of RGS4 Polymorphisms from Thirteen Independent Samples. Biol Psychiatry. 2006

Williams NM, Preece A, Spurlock G, et al. Support for RGS4 as a susceptibility gene for schizophrenia. Biol Psychiatry 2004;55:192–195. [PubMed: 14732600]

Xie GX, Palmer PP. How regulators of G protein signaling achieve selective regulation. J Mol Biol 2007;366:349–365. [PubMed: 17173929]

Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988;44:1049–1060. [PubMed: 3233245]

Zhang F, St Clair D, Liu X, et al. Association analysis of the RGS4 gene in Han Chinese and Scottish populations with schizophrenia. Genes Brain Behav 2005;4:444–448. [PubMed: 16176390]

Zheng Y, Li H, Qin W, et al. Association of the carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase gene with schizophrenia in the Chinese Han population. Biochem Biophys Res Commun 2005;328:809–815. [PubMed: 15707951]

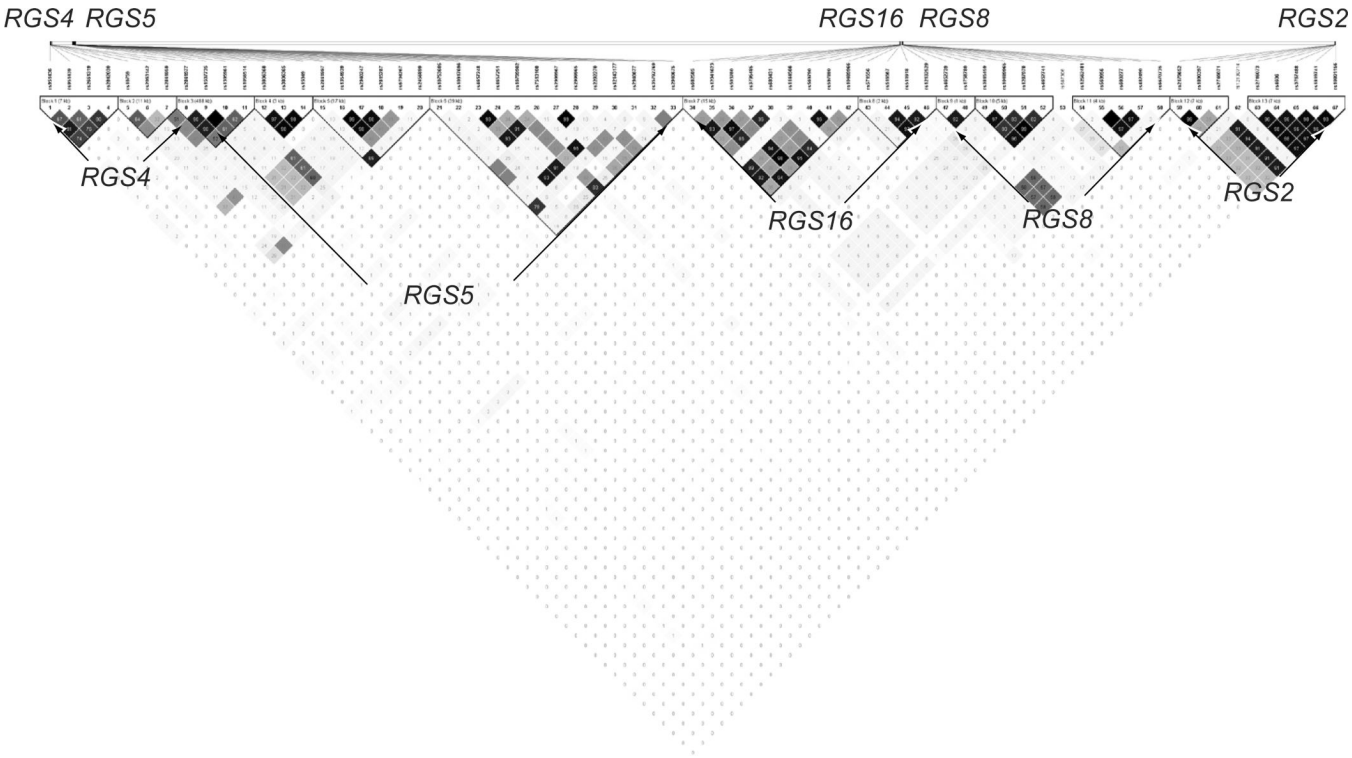

Figure 1.

Genomic region of chromosome 1q23.3-31 including *RGS4*, *RGS5*, *RGS16*, *RGS8* and *RGS2*. All five genes are included in a 30-Mb region; the chromosome is drawn to scale. LD blocks (Haploview, version 3.1.2) for the 422-sample Europe only sample are designated. Arrows indicate boundaries of markers near indicated genes. Pairwise r^2 values (X 100) are indicated for the CATIE Europe only sample. D′ and r^2 values for the Africa only and Europe only ethnic strata are listed in Supplementary Table 2.

Table 1

Description of genotyped CATIE sample.

Variable	n	Mean	St Dev	Min	Max
Subject's Age	750	40.9	11.0	18	67
Number of Years of Patient's Education	746	12.1	2.2	3	21
Years Since First Prescribed an Antipsychotic Med	721	14.3	10.8	0	56
Derived PANSS Total Score	749	74.0	17.4	33	133
Derived PANSS Positive Scale Score	749	17.9	5.5	7	38
Derived PANSS Negative Scale Score	749	19.9	6.4	7	41
PANSS General Psychopathology Scale	749	36.2	9.0	16	69
Ancestry	221	Africa only			
	422	Europe only			
	107	Other ancestry			
Sex	200	Female			
	550	Male			

Table 2

RGS5 and *RGS2* variants with uncorrected significant differences among baseline PANSS total scores in the Africa only and Europe only strata. The complete data set is presented in Supplementary Table 3.

RGS Gene	SNP	Strata	Unadjusted P-value	Bonferonni-adjusted P-value	PANSS Total Score LSMean1 (95% CI)	PANSS Total Score LSMean2 (95% CI)	PANSS Total Score LSMean3 (95% CI)
5	rs2456899	Europe only	0.0426	0.9232	C/C 84.993 (74.050, 95.936)	C/T 71.223 (67.725, 74.721)	T/T 70.853 (68.632, 73.073)
5	rs10753605	Africa only	0.0143	0.5724	C/C 57.546 (44.345, 70.748)	C/T 72.450 (68.051, 76.848)	T/T 75.917 (73.232, 78.603)
5	**rs10799902**	**Africa only**	**0.0440**	**0.9296**	**A/A 75.609 (72.179, 79.039)**	**A/G 75.785 (72.392, 79.178)**	**G/G 67.949 (62.243, 73.655)**
5	**rs10799902**	**Europe only**	**0.0143**	**0.5713**	**A/A 75.912 (70.406, 81.418)**	**A/G 72.352 (69.805, 74.899)**	**G/G 68.221 (65.298, 71.144)**
5	rs2999967	Africa only	0.0143	0.5716	A/A 75.092 (71.758, 78.426)	A/G 76.495 (73.071, 79.918)	G/G 66.574 (60.626, 72.522)
5	rs2999965	Africa only	0.0138	0.5606	A/A 66.834 (61.002, 72.665)	A/G 76.811 (73.258, 80.364)	G/G 74.889 (71.623, 78.154)
5	rs2940677	Africa only	0.0331	0.8629	C/C 75.449 (72.873, 78.026)	C/T 72.709 (67.716, 77.702)	T/T 58.330 (45.049, 71.611)
5	rs2940675	Africa only	0.0249	0.7743	C/C 77.912 (74.059, 81.764)	C/T 74.463 (71.255, 77.671)	T/T 68.856 (63.401, 74.311)
2	rs1890397	Africa only	0.0277	0.8096	A/A 75.523 (70.327, 80.720)	A/G 77.089 (73.939, 80.239)	G/G 70.396 (66.433, 74.359)
2	rs2746071	Europe only	0.0352	0.8790	A/A 73.326 (70.781, 75.871)	A/G 68.959 (66.105, 71.813)	G/G 68.335 (61.727, 74.943)
2	rs2746073	Europe only	0.0331	0.8632	A/A 67.161 (60.454, 73.868)	A/T 69.062 (66.151, 71.973)	T/T 73.195 (70.707, 75.684)
2	rs4606	Europe only	0.0261	0.7899	C/C 73.317 (70.807, 75.827)	C/G 68.878 (65.963, 71.792)	G/G 67.553 (60.836, 74.270)
2	rs3767488	Europe only	0.0325	0.8574	A/A 73.219 (70.740, 75.699)	A/G 69.014 (66.119, 71.910)	G/G 67.423 (60.723, 74.124)
2	rs10801156	Europe only	0.0355	0.8818	A/A 73.004 (70.512, 75.497)	A/C 68.510 (65.611, 71.410)	C/C 68.808 (61.895, 75.721)

Table 3

RGS2 haplotype association with baseline PANSS total scores in Europe only strata.

Global: Score=7.3462; df=2; P=0.0254								
Haplotype	**rs2746073**	**rs4606**	**rs3767488**	**rs1819741**	**rs10801156**	**Frequency**	**Z Score**	**P**
1	T	C	A	T	A	0.7315	2.3348	0.0196
2	A	G	G	C	C	0.2575	−2.5996	0.0093