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Genetic association between *RGS1* and internalizing disorders

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Abstract

Objective—Quantitative trait loci identified in animal models provide potential candidate susceptibility loci for human disorders. In this study, we investigated whether internalizing disorders (anxiety disorders, major depression, and neuroticism) were associated with a region on human chromosome 1 syntenic with a quantitative trait locus for rodent emotionality.

Methods—We genotyped 31 single-nucleotide polymorphisms in genes located on chromosome 1q31.2 in a two-stage association study of 1128 individuals chosen for a high or a low genetic risk for internalizing disorders from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders.

Results—None of the individual single-nucleotide polymorphisms showed consistent association across stages. A four-marker haplotype in the regulator of G-protein signaling 1 gene (*RGS1*) was significantly associated with decreased internalizing risk in both stages, whereas another showed a nominal association with a higher risk.

Conclusion—Our data suggest that markers in the *RGS1* gene might be in linkage disequilibrium with a protective allele that reduces the risk of anxiety and depressive disorders.

Keywords

anxiety; association study; depression; genetics; personality

Introduction

Internalizing disorders, that is, anxiety and depressive disorders, are quite common in the general population and account for a substantial clinical burden worldwide (Andrews *et al.*, 1998). They are complex genetic syndromes, showing shared familial aggregation (Weissman *et al.*, 1997) and overlapping genetic risk factors (Kendler *et al.*, 2003). The

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Conflicts of interest

There are no conflicts of interest.

personality trait of neuroticism has considerable genetic correlation with internalizing symptoms and disorders (Jardine *et al.*, 1984; Kendler *et al.*, 2006; Hetteema *et al.*, 2006b). Study of these internalizing phenotypes in a coordinated manner may prove effective for identification of the primary susceptibility genes for these conditions.

Gene discovery efforts remain challenging for most polygenic disorders because of small effect sizes; thus, animal models continue to play an important role in the identification of genes involved in human diseases. Rodent ‘emotionality’ has been proposed as a model for human internalizing phenotypes (Flint *et al.*, 1995). A behavioral quantitative trait locus (QTL) for emotionality has been reliably localized to a 4.8Mb region on mouse chromosome 1 (Yalcin *et al.*, 2004). Interestingly, the syntenic region on human chromosome 1 coincides with linkage peaks reported for several human internalizing phenotypes (reviewed in Fullerton, 2006). Further dissection of this region provided support for three separate murine QTLs, including effects attributable to the gene, regulator of the G-protein signaling 1 gene (*RGS2*). The *RGS2* gene has since been reportedly associated with several anxiety-related phenotypes in humans (Leygraf *et al.*, 2006; Smoller *et al.*, 2008; Amstadter *et al.*, 2009; Koenen *et al.*, 2009; Mouri *et al.*, 2010; Otowa *et al.*, 2011). Another study examined the potential relevance, for neuroticism, of human polymorphisms that correspond to functional murine sequence variants in that syntenic region, finding significant evidence for a single-nucleotide polymorphism (SNP) near *RGS18* (Fullerton *et al.*, 2008). However, there are many genes in that region that have not been explored adequately for their potential contribution to these linkage signals. To further examine the relevance of the mouse chromosome 1 emotionality QTLs for human internalizing disorders, in this study, we tested genes in the syntenic human region chr1q31.2 for an association with genetic risk for these phenotypes.

Methods

Participants

The Caucasian participants in this study were from the population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) (Kendler and Prescott, 1999; Kendler and Prescott, 2006). Approval of the local Institutional Review Board was obtained before the study and informed consent was obtained from all participants before data collection.

Diagnostic measures

We obtained lifetime psychiatric diagnoses on the basis of DSM-III-R (American Psychiatric Association, 1987) through a face-to-face or a telephone structured psychiatric interview on the basis of the SCID (Spitzer and Williams, 1985). Neuroticism and extroversion were assessed using the short form of the Eysenck Personality Questionnaire (Eysenck and Eysenck, 1975).

Sample selection

As described previously (Hetteema *et al.*, 2006a), we used a two-stage association design in which candidate loci were screened in stage 1, the positive results of which were tested for replication in stage 2. The parameters for this design were calculated using the LGA program (Robles and Van den Oord, 2004) to achieve 80% power to detect markers that explained 1–2% of the variance of the liability distribution while controlling the false discovery rate at 0.1 (Van den Oord and Sullivan, 2003). If any of the markers genotyped in stage 1 met the estimated threshold *P*-value of 0.1 or less, they were then also tested in the stage 2 sample.

We have incorporated two novel strategies to optimize participant selection. First, we have taken advantage of research suggesting shared genetic susceptibility across internalizing disorders (Scherrer *et al.*, 2000; Middeldorp *et al.*, 2005; Hetteema *et al.*, 2006b). Starting with a total of 9270 twin individuals, we used multivariate structural equation modeling to estimate a latent additive genetic risk factor (A1) for neuroticism that is highly correlated with genetic susceptibility to internalizing disorders (see Hetteema *et al.*, 2006b for details). Second, we used an extreme selection strategy (Van den Oord, 1999; Schork *et al.*, 2000; Van Gestel *et al.*, 2000) to choose one member from each twin pair for whom DNA was available as a ‘case’ (high genetic risk) or ‘control’ (low genetic risk) for genotyping on the basis of scoring above the 80th or below the 20th percentile, respectively, of the genetic factor A1. This led to a total sample size of $N = 1128$ that included 589 cases and 539 controls: 188 cases and controls in stage 1 and 401 cases and 351 controls in stage 2. Overall, the cases had a mean raw neuroticism score of 6.3 (z -score = 1.04) and had the following frequencies of the target internalizing disorders: major depressive disorder (80.1%), generalized anxiety disorder (53.8%), panic disorder (20.5%), agoraphobia (14.1%), and social phobia (17.5%). The controls were free of these five disorders and had a mean raw neuroticism score of 0.55 (z -score = -0.89). These phenotypic distributions did not differ significantly between the stage 1 and stage 2 samples.

Genotyping

DNA was extracted from buccal epithelial cells obtained using cytology brushes. SNPs were genotyped by the 5′ nuclease cleavage assay (TaqMan method) (Livak, 1999). We selected SNP markers with minor allele frequency greater than 0.10 in the human chr1q31.2 region syntenic with the murine emotionality locus (Fig. 1). The region of interest was large, about 1 Mb in length (start position: 190 387 326; end position: 191 399 900 in the March 2006 assembly, NCBI build 36); thus, we focused our analyses on the known genes in the regions. We used the Tagger module of Haploview 4.1 (Barrett *et al.*, 2005) with HapMap (The International HapMap Consortium, 2003) phase II data (release 22) and pairwise tagging to capture the allelic variation (with $r^2 > 0.7$) in and near all of the known genes. We used quality control genotyping thresholds to exclude poorly performing assays: genotyping rate less than 90% and missingness greater than 10%.

Statistical analysis

Pearson’s χ^2 -tests were used to test for allelic or genotypic differences by marker between cases and controls in the two stages. We used Haploview 4.1 to find the regions of high linkage disequilibrium (LD) (i.e. haplotype blocks) using the default confidence interval algorithm (Gabriel *et al.*, 2002). Haplotype association analyses were carried out using the Cocophase module of the UNPHASED program (Dudbridge, 2003).

Results

The genotype and allele frequencies and the results of χ^2 -association tests for the 31 markers genotyped in stage 1 are listed in Table 1. None of the SNPs showed deviations from Hardy–Weinberg equilibrium. Five markers in and around the *RGS1* gene locus (markers 9–13) met the threshold criteria of allelic P -value less than 0.1 in our stage 1 sample (Table 1). However, none of these individual markers were associated in stage 2.

As we used tagging SNPs that are unlikely to be the functional variants themselves, we constructed haplotype blocks using the default confidence interval procedure in Haploview 3.2 to better understand the LD structure around these markers. Markers 10, 11, and 12 occurred on a single haplotype block in our sample, although the LD between these and marker 9 was high, consistent with CEU HapMap data, suggesting one large block structure

across the SNPs in this region. We therefore tested the association of the four-marker haplotypes created from combinations of markers 9–12. In Table 2, we present the results, by stage, for these four-marker haplotypes as calculated using UNPHASED (results for three-marker and five-marker sliding window haplotypes produced similar patterns). As indicated, the most consistent result across stages is for the common ‘protective’ C-T-G-G haplotype, showing higher frequencies in controls than cases ($P = 0.0036$ in the combined sample). The less common T-T-A-A haplotype showed a nominal association with an increased risk ($P = 0.029$). As we are testing five markers in both stages (or five haplotypes derived from four of these markers), we applied a corrected P -value of 0.01 to indicate significance, which is fulfilled by the C-T-G-G haplotype. Permutation testing estimates the empirical 5% of the best P -value equals 0.00787, indicating that this haplotype (with $P = 0.0036$) occurs less than 5% of the time by chance.

Discussion

In this study, we examined whether human genes syntenic to the murine chromosome 1 emotionality region were associated with genetic susceptibility to human internalizing phenotypes, including anxiety disorders, major depression, and neuroticism. This susceptibility was indexed by a latent genetic factor common to these phenotypes derived from multivariate twin modeling. We entered the resulting sample of 589 high genetic risk and 539 low genetic risk individuals into a two-stage association study in which markers from the candidate loci were screened in stage 1, the positive results of which were tested for replication in stage 2. Individual markers and relevant haplotypes were analyzed.

Out of the 31 markers tested in this region, five in and around the *RGS1* gene fulfilled the threshold screening criterion in stage 1 of P -value less than 0.1, but these associations were not replicated in stage 2. The C-T-G-G haplotype of four of these SNPs in high LD showed an association across the two stages with a lower internalizing risk. However, as individual SNPs did not show a consistent association, either (a) these haplotype results occurred by chance or (b) this haplotype is in LD with another functional variant that we did not test. *RGS1* is a small gene (4.3 kb) that codes for one of the many members of the class of proteins known as regulators of G-protein signaling. These proteins attenuate the signaling activity of G-proteins by binding to activated, GTP-bound Ga subunits and increase the rate of conversion of GTP into GDP. As summarized on the UCSC Genome Browser website (www.genome.ucsc.edu), *RGS1* has little known brain expression, limited to the hypothalamus and the corpus callosum. Extant research supports its role in B-cell inflammatory responses (Moratz *et al.*, 2000), with possible involvement in conditions such as celiac disease (Hunt *et al.*, 2008), melanoma progression (Rangel *et al.*, 2008), and multiple sclerosis (International Multiple Sclerosis Genetics Consortium, 2010). To our knowledge, this is the first report of an association with a psychiatric phenotype.

In a previous study, Fullerton *et al.* (2008) identified human SNPs corresponding to functional and conserved regions in the murine emotionality locus and tested them for association in a large, extreme-selected sample for neuroticism. They reported a significant association for SNP rs6428058, about 600 kb upstream of *RGS18* (outside of our selected region). They did not genotype any markers overlapping the *RGS1* block implicated in the current study. We note that neither that study nor the current one found evidence supporting a role for the *RGS2* gene in internalizing disorder susceptibility.

The results of this study should be interpreted in the context of several potential limitations. First, this sample, although chosen to maximize power to directly test an association with a common genetic risk for internalizing phenotypes and selected from the informative tails of a much larger sample, may nonetheless lack sufficient power to detect an association with

common genetic polymorphisms that have modest effect sizes. In particular, although we attempted to balance type I and type II errors and maintain an available sample for replication using a two-stage study design with less stringent stage 1 screening *P*-values, the stage 1 sample size is nonetheless quite modest. Second, because of the large number of SNPs in the region and cost limitations of the study, we chose to genotype 31 tagging SNPs with less stringent criteria in the regions of the known genes only, and thus, could not capture all of the major allelic variation in the region.

In summary, this study finds a suggestive association between variants in the *RGS1* gene and internalizing disorders. As with any novel genetic association finding, these results should be considered as tentative until adequate replication is shown.

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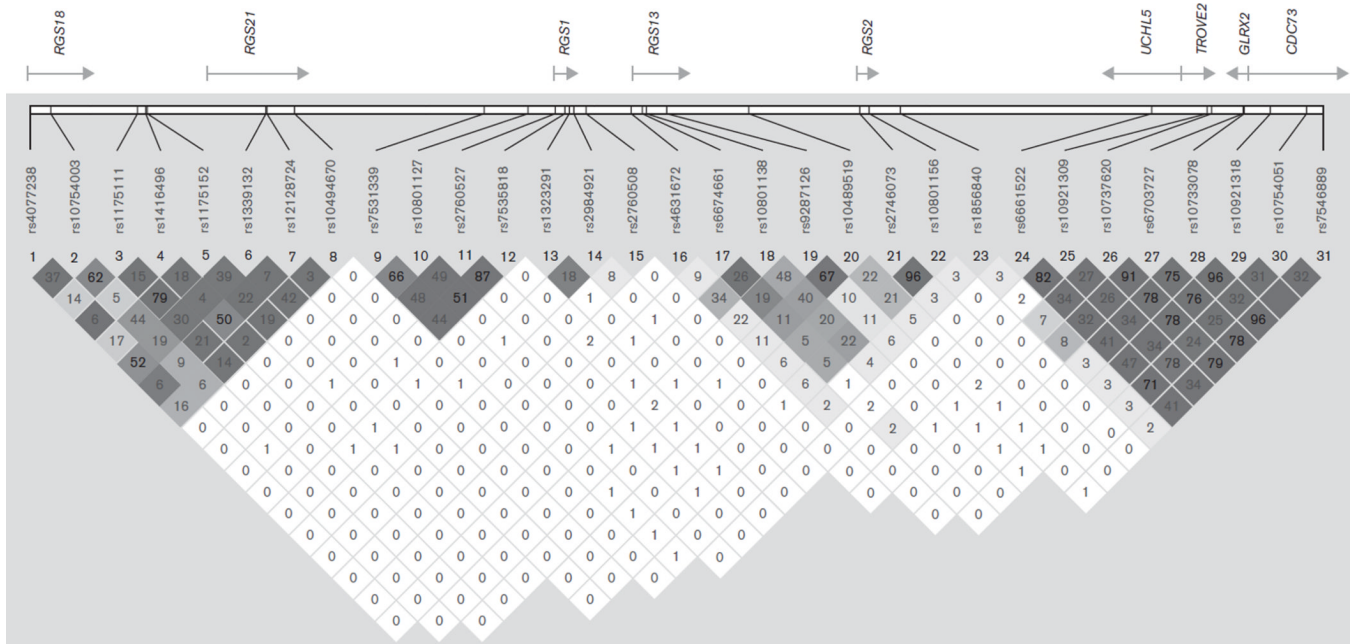


Fig. 1. Relative gene locations and linkage disequilibrium (r^2) for all single-nucleotide polymorphisms genotyped in the stage 1 sample.

Table 1

Individual marker association results for stage 1 ($N=188$ cases, 188 controls)

Marker #	Marker ID	Locus	MAF	P-value	
				Genotypic	Allelic
1	rs4077238	<i>RGS18</i>	0.286	0.63	0.79
2	rs10754003	<i>RGS18</i>	0.497	0.71	0.46
3	rs1175111	<i>RGS18</i>	0.490	0.40	0.50
4	rs1416496	Intergenic	0.143	0.59	0.45
5	rs1175152	Intergenic	0.440	0.12	0.74
6	rs1339132	<i>RGS21</i>	0.254	0.91	0.70
7	rs12128724	<i>RGS21</i>	0.171	0.92	0.71
8	rs10494670	<i>RGS21</i>	0.130	1.00	0.98
9	rs7531339	Intergenic	0.273	0.05	0.067
10	rs10801127	<i>RGS1</i>	0.276	0.05	0.041
11	rs2760527	<i>RGS1</i>	0.170	0.06	0.019
12	rs7535818	<i>RGS1</i>	0.163	0.06	0.020
13	rs1323291	<i>RGS1</i>	0.084	0.06	0.029
14	rs2984921	<i>RGS1</i>	0.270	0.72	0.45
15	rs2760508	<i>RGS1</i>	0.208	0.62	0.35
16	rs4631672	<i>RGS13</i>	0.482	0.83	0.72
17	rs6674661	<i>RGS13</i>	0.244	0.97	0.70
18	rs10801138	<i>RGS13</i>	0.455	0.77	0.94
19	rs9287126	<i>RGS13</i>	0.464	0.29	0.24
20	rs10489519	Intergenic	0.395	0.32	0.15
21	rs2746073	<i>RGS2</i>	0.257	0.97	0.86
22	rs10801156	<i>RGS2</i>	0.260	0.85	0.56
23	rs1856840	<i>RGS2</i>	0.360	0.86	0.67
24	rs6661522	<i>UCHL5</i>	0.490	0.99	0.82
25	rs10921309	<i>TROVE2</i>	0.438	0.95	0.99
26	rs10737620	<i>TROVE2</i>	0.278	0.25	0.13
27	rs6703727	<i>GLRX2; CDC73</i>	0.258	0.33	0.40

Marker #	Marker ID	Locus	MAF	<i>P</i> -value	
				Genotypic	Allelic
28	rs10733078	<i>GLRX2; CDC73</i>	0.305	0.73	0.52
29	rs10921318	<i>CDC73</i>	0.309	0.49	0.35
30	rs10754051	<i>CDC73</i>	0.427	0.86	0.66
31	rs7546869	<i>CDC73</i>	0.301	0.25	0.10

P-values are calculated using χ^2 -tests.

Boldface indicates $P < 0.1$ (stage 1 threshold).

Locus, genes within 50 kb of marker; MAF, minor allele frequency.

Table 2

Haplotype analysis results for RGS1 block single-nucleotide polymorphisms in stage 1, stage 2, and the combined samples^a

Stage	Markers	Global P-value	Haplotype	Frequency cases	Frequency controls	Haplotype P-value
1 (N= 376)	9, 10, 11, 12	0.062	T-G-A-A	0.72	0.66	0.076
			T-T-A-A	0.024	0.023	0.96
			C-G-A-A	0.042	0.032	0.48
			C-T-A-A	0.068	0.079	0.64
			C-T-G-G	0.12	0.18	0.027
2 (N= 752)	9, 10, 11, 12	0.00029	T-G-A-A	0.64	0.68	0.087
			T-T-A-A	0.046	0.021	0.0092
			C-G-A-A	0.046	0.029	0.14
			C-T-A-A	0.087	0.073	0.37
			C-T-G-G	0.14	0.18	0.043
Combined (N= 1128)	9, 10, 11, 12	0.0024	T-G-A-A	0.67	0.68	0.71
			T-T-A-A	0.038	0.022	0.029
			C-G-A-A	0.045	0.029	0.091
			C-T-A-A	0.079	0.075	0.69
			C-T-G-G	0.13	0.18	0.0036

Boldface indicates $P < 0.05$ for specific haplotypes.

Haplotype: '1' represents the major allele and '2' represents the minor allele, respectively, for each SNP marker in a haplotype.

^aResults shown only for common haplotypes (frequency >0.01).