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Influence of *RGS2* on Sertraline Treatment for Social Anxiety Disorder

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Only a minority of patients with social anxiety disorder (SAD) has a robust therapeutic response to evidence-based serotonin reuptake inhibitor (SSRI) treatment. To help improve the personalized medicine approach to psychiatric care, we evaluated several candidate genetic predictors of SSRI response in SAD. At the start of a randomized controlled trial (NCT00282828), 346 patients with SAD at three sites received protocol-driven, open-label treatment with sertraline, up to 200. mg/d over 10 weeks. Efficacy was determined using a continuous measure of outcome (Liebowitz Social Anxiety Scale (LSAS)) and dichotomous indicators of response (LSAS $\leqslant$50) and remission (LSAS $\leqslant$30). Predictors of efficacy were examined in multivariate regression models that included eight polymorphic variants in four candidate genes (four in *RGS2*, two in *HTR2A*, one in *SLC6A2*, and one in *SLC6A4*). Adjusting for genetic ancestral cluster and non-genetic predictors of response, all four single-nucleotide polymorphisms (SNPs) in *RGS2* predicted change in LSAS over time, at study-wise significance ($p = 0.00833$), with the minor allele associated with less improvement over time. After adjusting for genetic ancestral cluster and non-genetic predictors of remission, two of the four *RGS2* SNPs predicted likelihood of remission at or just below study-wise significance ($p = 0.025$): rs4606 (AOR = 0.49 (95% CI = 0.27–0.90), $p = 0.022$) and rs1819741 (AOR = 0.50 (95% CI = 0.28–0.92), $p = 0.027$). Variation in *RGS2*, a gene previously shown to be associated with social anxiety phenotypes and serotonergic neurotransmission, may be a biomarker of the likelihood of substantially benefiting from sertraline among patients with SAD.
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INTRODUCTION

Social anxiety disorder (SAD; also referred to as social phobia) is a common psychiatric condition with a lifetime prevalence of 12% (Ruscio *et al*, 2008) and is associated with significant distress and dysfunction (Stein and Stein, 2008). Despite the availability of evidence-based treatments for SAD, many patients remain symptomatic after initial intervention. Randomized controlled trials from within the serotonin (SSRIs) and serotonin-norepinephrine reuptake inhibitors classes, which include the only FDA-approved medications for SAD, report rates of response in adults (interestingly, response rates is children are much higher (Masi *et al*, 2012; Wagner *et al*, 2004)) in the range of 40–60%; remission rates are even lower, averaging approximately 20% (Stein *et al*, 2004; Stein and Stein, 2008).

Therefore, considering the most widely used class of pharmacological treatments for SAD, SSRIs, only about half of adult patients garner a clinically meaningful benefit (ie, 'respond') and only about half of those again derive substantial benefit (ie, 'remit') after initial intervention. It would be extremely useful if we were able to predict which patients were likely to have a substantial therapeutic response, as this would strongly influence initial choice of treatment. This is a desired aim not only for pharmacotherapy of SAD, but also for all of the mental disorders, where we lack the ability to predict the likelihood of response of a given patient to a given drug (Uher *et al*, 2012). We are aware of only a couple of studies that have attempted to predict response to SSRI in patients with SAD (Stein *et al*, 2006; Van Ameringen *et al*, 2004). Only one of these studies included a genetic predictor, 5HTTLPR, which in that retrospective analysis found an association between number of 's' alleles and lower likelihood of response (Stein *et al*, 2006). To the best of our knowledge, no published studies have attempted to replicate that finding or to seek other genetic predictors of SSRI response in SAD.

We report here the results from analyses attempting to predict likelihood of response and remission to an SSRI coming from the first phase of a three-center trial of pharmacotherapy for SAD. This trial, Social Anxiety Pharmacotherapy Improvement, provides prospective data on a large sample of patients with SAD uniformly treated

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with the same SSRI, sertraline, on an open-label basis according to a standardized protocol.

METHODS

Participants

Participants were recruited between August 2006 and July 2011 through clinical referrals to Massachusetts General Hospital (MGH), McMaster University and University of California at San Diego, and advertisements. Inclusion criteria were: (1) male or female outpatients 18 years of age or older with a current principal diagnosis (including designation by the patient as most important source of distress or impairment) of generalized social anxiety disorder (GSAD) by *Diagnostic and Statistical Manual for Mental Disorders* (4th Edition) criteria, (2) a total score on the Liebowitz Social Anxiety Scale (LSAS; Fresco *et al*, 2001) of 60 or greater, and (3) no clinically significant medical abnormalities based on physical and laboratory examination.

Exclusion criteria included: (1) a history of more than two unsuccessful, adequate pharmacologic treatment trials, operationalized as lack of response to $\geqslant$10 weeks of any of the following: SSRIs at adequate dose (eg, paroxetine 40 mg/d or its equivalent); benzodiazepines (eg, clonazepam $>$2.5 mg/d) plus an antidepressant (at adequate dose as above); MAOIs (eg, phenelzine 60 mg/d or its equivalent); or a single failed trial of $\geqslant$10 weeks of venlafaxine ($\geqslant$150 mg/d); (2) pregnant women, lactating women, and women of childbearing potential not using medically accepted forms of contraception; (3) psychotic disorders, mental retardation, organic medical disorders, bipolar disorder or OCD with YBOCS score $\geqslant$25; recent history of eating disorders or alcohol/substance abuse or dependence. Entry of patients with major depression, panic disorder, generalized anxiety disorder, PTSD, or adult attention-deficit hyperactivity disorder (ADHD) was permitted if the GSAD was judged to be the predominant disorder; (4) concurrent use of other psychotropic medications with discontinuation of regular benzodiazepine or antidepressant therapy required at least 2 weeks (5 weeks for fluoxetine) before baseline: patients with comorbid adult ADHD could remain on stimulant medication if dose had been stable for $\geqslant$1 month; (5) patients with current or recent significant suicidality; (7) any concurrent psychotherapy initiated within 3 months or any ongoing psychotherapy directed specifically toward GSAD (eg, CBT) was excluded.

Study Design and Procedures

Eligible participants were enrolled into the first phase of the study, on which the results presented here are based. Phase 1 comprised 10 weeks of open prospective treatment with sertraline initiated at 25 mg/d, with all symptomatic patients titrated up to their maximally tolerated dose ($\leqslant$200 mg/d) by week 8. Participants unable to reach at least 50 mg/d by the end of Phase 1 were discontinued from the study and referred for clinical treatment. Participants were also randomly assigned to receive instructions encouraging them to interact with others while taking study medication ('exposure instructions') or instructions lacking that encouragement ('neutral instructions'); instruction set had no significant impact on Phase 1 outcome (Pollack *et al*, 2013). Participants were seen weekly for the first 2 weeks of Phase 1 and then bi-weekly thereafter. Efficacy was determined using a continuous measure of outcome (LSAS) and dichotomous indicators of response (LSAS $\leqslant$50) and remission (LSAS $\leqslant$30). At the end of week 10, remitters were discontinued from that phase of the study and offered an additional 12 weeks of continued open-label treatment, and non-remitters were offered randomization to the second phase of the study (the results of which are described elsewhere (Pollack *et al*, 2013)).

A total of 397 patients received at least one dose of sertraline in the study. The present analysis is limited to the 346 patients for whom genetic and phenotypic data were available. Demographic characteristics of the subjects are shown in Table 1.

We chose to investigate a very parsimonious number of genes, and SNPs within those genes, knowing that multiple testing would limit our ability to detect study-wise significance. Candidates (Table 2) were chosen on the basis of published associations with the diagnostic phenotype (SAD) itself (Gelernter *et al*, 2004), with strongly related phenotypes (eg, behavioral inhibition or shyness; Arbelle *et al*, 2003; Smoller *et al*, 2008) or with SSRI response in a prior study of SAD (Stein *et al*, 2006).

Genotyping Methods

SNP genotyping. SNP genotyping was performed at the MGH Center for Human Genetic Research (CHGR) using the Sequenom iPLEX Gold chemistry and the MassARRAY system. The major steps in this process included the following: multiplex assay design using Assay Designer software, DNA amplification by PCR, post-PCR nucleotide deactivation using shrimp alkaline phosphatase to remove phosphate groups from unincorporated dNTPs, single-base extension reaction, salt removal using ion-exchange resin, and mass correlated genotype calling using SpectroCHIP array and MALDI-TOF mass spectrometry.

Serotonin transporter (5-HTTLPR) genotyping. 5-HTTLPR genotyping was performed at CHGR using Applied Biosystems' instruments and reagents. Genomic DNA was amplified using the following primer sequences: 5-HTTLPR-F 6FAM 5′-GGCGTTGCCGCTCTGAATGC-3′ and 5-HTTLPR-R 5′-GAGGGACTGAGCTGGACAACCAC-3′. Amplified product was then combined with a size standard (GeneScan LIZ-500) before being analyzed. The 'long' allele appears as a product of approximately 528 base pairs, whereas the 'short' allele is approximately 483 base pairs.

Genetic ancestral cluster identification. We used a 40-SNP Ancestry Informative Marker Set to derive genetic ancestral clusters using clustering tools provided in PLINK (Purcell *et al*, 2007). The Ancestry Informative Marker Set SNPs have very different minor allele frequencies across racial groups, and thus are informative for ancestry. They were designed to separate out the Caucasian, African, and Asian population blocks from each other, and they roughly map onto these self-reported racial groups in our sample (which are shown in Table 1).

Table 1 Demographic Characteristics among Genetics Analysis Population

	Patients with genetic data ($n = 346$)	
Site: N (%)		
MGH	123	36%
UCSD	104	30%
McMaster	119	34%
Age at entry: *mean (SD), years*	35	(13.3)
Gender: N (%)		
Male	230	66%
Female	116	34%
Race (self-report): N (%)		
White	268	77%
Asian	27	8%
Black/African American	20	6%
American Indian/Alaska Native/First Nations	2	1%
Other	29	8%
Ethnicity (self-report): N (%)		
Hispanic	32	9%
Non-hispanic	314	91%
Baseline LSAS: N (%)		
<70	44	13%
$\geqslant 70$	302	87%
Phase I instructions received: N (%)		
Exposure	170	49%
Neutral	176	51%
# Comorbidities: N (%)[a]		
0	194	56%
1	98	28%
2	36	10%
3-4	18	5%
Lifetime alcohol abuse/dependence: *N (%)*	62	18%
Age of sad onset: *mean (SD)*	11	(6.8)
MADRS total: *mean (SD)*	11	(7.5)
Avoidant PD # criteria endorsed (7 total): *mean (SD)*	4.4	(1.9)
Sheehan disability total: *mean (SD)*	16	(5.5)
Week 10 sertraline blood levels (ng/ml): *mean (SD)*	68	(46.7)
Completed phase I: *N (%)*	273	79%

[a]Includes MDD, Panic, OCD, and GAD

Statistical Approach

An additive genetic model was used for the primary analysis for each variant, corresponding to a single-predictor variable for the variant in the model, coded as 0, 1, or 2 to reflect the number of minor alleles in a subject's genotype (see Table 2 for tabulations of number of minor alleles for each gene variant). The main effect for each variant is that of adding one additional minor allele to the genotype (comparing 1 minor allele to 0 as well as 2 minor alleles to 1). The use of an additive model is common in genetic association studies because the true underlying model is seldom known and an additive model is considered to be robust to model misspecification. Although genetic ancestral cluster was not associated with LSAS ($p = 0.14$), we nevertheless controlled for potential confounding by race as the genetic allele frequencies for some of the variants differed significantly by self-reported race.

We examined genetic predictors of change in LSAS total score over Phase I (measured at weeks 0, 1, 2, 4, 6, 8, 10) using mixed models with random effects for slope and intercept. Change in LSAS over time was non-linear, and therefore was modeled quadratically with terms for week and week^2. We first univariately tested the predictive value of each genetic variant by using a likelihood ratio test to compare a model with and without the (variant $\times$ week) and (variant $\times$ week^2) interaction terms. To better interpret the results of this non-linear model, we calculated the effect of an increase of one minor allele on LSAS at week 10. In these univariate analyses of change in LSAS, we examined the spectral decomposition of the genetic variants using SNPSpD (Nyholt, 2004) to determine the number of effective independent comparisons being made, in order to control the type I error rate accordingly. The decomposition yielded six independent comparisons, and thus an $\alpha = 0.05/6 = 0.00833$ was applied, based on the Bonferroni correction. Finally, we added number of current comorbidities (the only significant clinical predictor of change in LSAS over time, categorized as 0, 1, 2, 3, 4) and genetic ancestral cluster into the univariate model for each significant genetic variant to determine if the significance was maintained.

RESULTS

Table 3 shows the results for each of the genetic variants tested. Based on the likelihood ratio test simultaneously testing the (variant $\times$ week) and (variant $\times$ week^2) interaction terms, we found that all four RGS2 SNPs showed study-wise significance ($\alpha = 0.00833$) when tested univariately. These four RGS2 SNPs are all in high linkage disequilibrium with each other (all pairwise $R^2 > 0.8$ in the study sample), and thus were not entered together into a multivariate model. Two haplotypes of these four SNPs are common: (1) TGGC—the all minor alleles haplotype of rs10801152-rs6428136-rs4606-rs1819741, with a frequency of $\sim$25% in the study sample, and (2) ATCT—the all major alleles haplotype, with a frequency of $\sim$72%. These two haplotypes were also tested for association with change in LSAS over Phase I, and both were significantly associated with change in LSAS, with the TGGC haplotype even more so than the best individual RGS2 SNP, rs1819741. After controlling for number of current comorbidities and genetic ancestral cluster, significance of all four RGS2 SNPs as well as both haplotypes was maintained.

Figure 1 shows of the direction of effects of all four RGS2 SNPs (Figure 1a–d), as well as the TGGC (Figure 1e) and ATCT (Figure 1f) haplotypes. For the RGS2 SNPs and the TGGC haplotype, the minor allele was associated with smaller changes in LSAS total score over time, whereas for

Table 2 Frequency of Number of Minor Alleles by Gene Variant

SNP	Minor allele	Gene symbol	Gene name	0 Copies (%)	1 Copy (%)	2 Copies (%)
rs10801152	T	*RGS2*	Regulator of G protein signaling 2	51	41	8
rs6428136	G	*RGS2*	Regulator of G protein signaling 2	55	38	7
rs4606	G	*RGS2*	Regulator of G protein signaling 2	52	39	9
rs1819741	C	*RGS2*	Regulator of G protein signaling 2	54	38	8
rs3742278	C	*HTR2A*	Serotonin 2A receptor	71	26	3
rs6313	T	*HTR2A*	Serotonin 2A receptor	32	52	16
rs1532701	G	*SLC6A2*	Norepinephrine transporter	33	47	20
5-HTTLPR	S	*SLC6A4*	Serotonin transporter	24	51	25
TGGC haplotype	—	—	All minor alleles haplotype	57	36	7
ATCT haplotype	—	—	All major alleles haplotype	8	41	50

Table 3 Genetic Variants as Predictors of Phase I (10-week) Change in LSAS[a]

SNP[b]	Minor allele	Gene symbol	Gene name	Week 10 estimate (SE)[c]	LRT *p*-value[d]
rs10801152	T	*RGS2*	Regulator of G protein signaling 2	2.68 (2.36)	0.0045*
rs6428136	G	*RGS2*	Regulator of G protein signaling 2	2.89 (2.42)	0.0043*
rs4606	G	*RGS2*	Regulator of G protein signaling 2	3.08 (2.36)	0.0051*
rs1819741	C	*RGS2*	Regulator of G protein signaling 2	3.54 (2.37)	0.0025*
rs3742278	C	*HTR2A*	Serotonin 2A receptor	1.31 (2.87)	0.029
rs6313	T	*HTR2A*	Serotonin 2A receptor	3.66 (2.28)	0.20
rs1532701	G	*SLC6A2*	Norepinephrine transporter	1.43 (2.09)	0.30
5-HTTLPR	S	*SLC6A4*	Serotonin transporter	0.44 (2.25)	0.39
TGGC haplotype	—	—	All minor alleles haplotype	3.56 (2.42)	0.0025*
ATCT haplotype	—	—	All major alleles haplotype	−2.46 (2.38)	0.0068*

*p-value $< 0.05/6 = 0.00833$ after principal components analysis and Bonferroni correction.
[a]All models controlled for number of co-morbidities, the only significant clinical predictors of change in LSAS over time (modeled categorically as 0, 1, 2, 3-4)—as well as genetic ancestral cluster (which roughly mapped onto White, Black, Asian, all other).
[b]Each variant was modeled linearly as 0, 1, 2 minor alleles (ie, additive model was used).
[c]The estimate is based on the longitudinal model and represents the difference in week 10 LSAS total score when the number of minor alleles is increased by 1.
[d]The *p*-value is based on a likelihood ratio test comparing a model with and without the (variant × week) and (variant × week^2) interaction terms, and represents the effect of each variant on the change in LSAS over time.

the ATCT haplotype, the minor allele was associated with larger gains over time.

Given the findings with change in LSAS, we decided to examine whether variants of the *RGS2* gene and the TGGC and ATCT haplotypes predicted Phase I response and remission in the ITT population (ie response and remission were based on the last Phase I LSAS score for patients who terminated early) using logistic regression. Spectral decomposition of the genetic variants (to control the type I error rate; Nyholt, 2004) yielded two independent comparisons, and thus we used an α of 0.025 for these analyses, based on the Bonferroni correction. We also examined the non-genetic covariates shown in Table 1 as predictors of response using univariate logistic regression (at the $\alpha = 0.05$ type I error rate), and subsequently controlled for any significant clinical predictors in our models examining genetic predictors of response and remission.

As seen in Table 4, none of the genetic variants predicted Phase I response after controlling for genetic ancestral cluster and significant clinical predictors of response, which included (1) Avoidant PD Score (number of questions, out of 7, scored as a 3 = threshold/true); (2) lifetime alcohol abuse or dependence (Yes/No); and (3) impairment severity (sum of Sheehan Disability (Leon *et al*, 1997) scores for work, social life, and family life/home responsibilities). However, the SNP rs4606 was a significant predictor of Phase I remission ($p < 0.025$), rs1819741 was borderline significant ($p = 0.027$), and the TGGC haplotype was nominally significant ($p < 0.05$).

DISCUSSION

SSRIs are the most widely prescribed evidence-based form of pharmacotherapy for SAD (Stein and Stein, 2008). Despite their widespread use, only approximately 50% of adult patients with SAD derive significant therapeutic benefit. In the present controlled study—the largest ever to provide standardized SSRI treatment to patients with SAD—the response rate was low (33%) and the remission

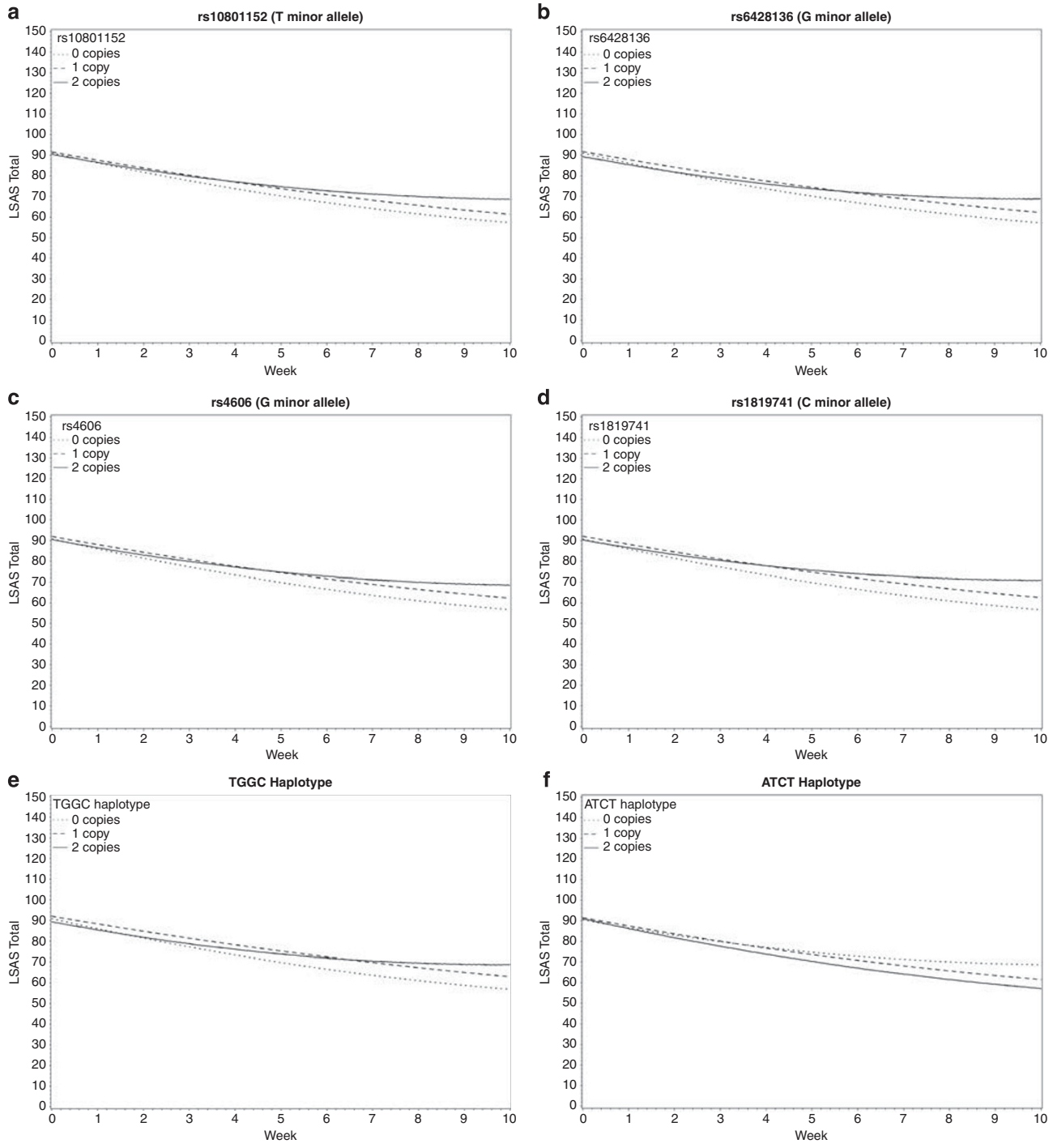

Figure 1 Change in Liebowitz Social Anxiety Disorder (LSAS) total score over Phase I by minor allele copies for select SNPs. Shown are predicted trajectories of symptom (LSAS) change over time in patients with social anxiety disorder receiving selective serotonin reuptake inhibitor treatment, stratified by number of copies of minor allele in each single-nucleotide polymorphism as noted. Predicted values were based on a quadratic model (ie, $\text{LSAS} \sim \beta_0 + \beta_1 \text{week} + \beta_2 \text{week}^2$).

rate even lower (13%). These data speak to the need for predictive biomarkers of SSRI response in SAD with the aim of identifying *a priori* who is more or less likely to respond before embarking on a therapeutic trial.

In the present study, looking at a very limited, pre-specified panel of potential genetic predictors, we found that several markers in *RGS2* predicted less reduction in symptoms and lower likelihood of remission with SSRI treatment. We chose to look at *RGS2* variants because of their association with the phenotype SAD-related traits such as introversion and behavioral inhibition, as well as functional MRI markers such as enhanced amygdala and

Table 4 Select Genetic Variants as Predictors of Phase I Response and Remission

SNP[a]	Minor allele	Phase I response[b]			Phase I Remission[b]		
		Odds ratio	(95% CI)	*P*-value	Odds ratio	(95% CI)	*p*-value
rs10801152	T	0.60	(0.58–1.27)	0.49	0.60	(0.34–1.05)	0.079
rs6428136	G	0.79	(0.54–1.17)	0.24	0.62	(0.36–1.12)	0.10
rs4606	G	0.84	(0.58–1.23)	0.37	0.49	(0.27–0.90)	0.022*
rs1819741	C	0.79	(0.54–1.16)	0.23	0.50	(0.28–0.92)	0.027
TGGC haplotype	—	0.76	(0.52–1.13)	0.18	0.55	(0.30–0.99)	0.049
ATCT haplotype	—	1.13	(0.78–1.65)	0.52	1.73	(0.99–3.05)	0.063

*p-value $< 0.05/2 = 0.025$ after principal components analysis and Bonferroni correction.
[a]Each variant was modeled linearly as 0, 1, 2 minor alleles (ie, additive model was used).
[b]All models controlled for genetic ancestral cluster (which roughly mapped onto White, Black, Asian, all other) as well as significant clinical predictors of response, ie, Avoidant PD Score (number of questions, out of 7, scored as a 3 = threshold/true); lifetime alcohol abuse or dependence (yes/no); and impairment severity (sum of Sheehan Disability scores for work, social life, and family life/home responsibilities).

insula response to emotion faces (Smoller *et al*, 2008). The current findings are intriguing in light of the biological actions of the RGS2 protein. Among other functions, RGS2 accelerates the deactivation of G proteins to reduce neuronal G protein-coupled receptor response to neurotransmitters including serotonin (Kimple *et al*, 2011). Deletion of *rgs2* in mice produces increased anxiety-related behavior (Lifschytz *et al*, 2012; Oliveira-Dos-Santos *et al*, 2000; Yalcin *et al*, 2004) and avoidance behavior in novel social situations (Lifschytz *et al*, 2012). Mice lacking *rgs2* have also been reported to show reduced expression of $5HT_{1a}$ G protein-coupled presynaptic autoreceptors (thought to be involved in the therapeutic activity of SSRIs) in the raphe (Lifschytz *et al*, 2012). Of note, the G allele of rs4606, which we found to predict reduced sertraline response, has been associated with reduced expression of RGS2 (Semplicini *et al*, 2006). Along with our previous observation that this allele (and those in LD with it) are associated with temperamental indices of social anxiety (Smoller *et al*, 2008), these findings suggest that individuals carrying hypofunctional alleles of *RGS2* may be relatively resistant to sertraline due to dysregulation of serotonergic neurotransmission and increased anxiety proneness. Of note, however, mutations of the G-protein subunit Gαi2 that result in RGS insensitivity have been reported to enhance frontal cortex and hippocampal postsynaptic 5HT1a signaling and potentiate the antidepressant effect of the SSRI fluvoxamine in mice (Talbot *et al*, 2010). Thus, the effects of RGS2 on SSRI response may vary depending on anatomic and synaptic location. Further research is needed to clarify the mechanism through which *RGS2* variants mediate SSRI response.

Strengths of the study are the fairly large sample size and the standardized protocol-driven treatment with a single SSRI (sertraline) and the systematic outcomes assessment. A limitation is the narrow focus on only a few candidate SNPs as opposed to a much broader (or even genomewide) approach such as has been applied to pharmacogenetic studies of major depressive disorder (Laje *et al*, 2009; Perlis, 2010). Given our findings with even this small set of markers, further attempts at the identification of genetic predictors of treatment response in SAD should be undertaken. In addition, although we observed statistically significant association of treatment outcomes with *RGS2* genotypes, the predictive utility of these markers in clinical practice remains to be established. Another feature of the study that limits its clinical interpretation is the prediction of response to only one type of treatment, an SSRI. Whereas it is useful knowing that likelihood of remission to sertraline, as in the present study, is half as likely in some patients than others, it would be even more useful to know what those individuals would respond to. That, too, is a topic for future research. We consider the current findings to be an important step along the way to the identification of genomic predictors of treatment response in SAD, but are fully cognizant of the many steps that need to follow in the delineation of such biomarkers (Perlis, 2011), not the least of which is replication.

With regard to replicating, the present study failed to replicate the only other published finding of genetic prediction of SSRI response in SAD, where the short variant of 5HTTLPR had been associated with poorer response (Stein *et al*, 2006). Similar findings have occasionally, although by no means widely, seen in studies of SSRI response in major depression (Huezo-Diaz *et al*, 2009). We are unable to explain why the previous finding in SAD would not have been replicated in the present study, particularly given that the original finding was seen in a much smaller sample size. One possibility is that the prior study looked at response to a variety of SSRIs, whereas the present study looked solely at response to sertraline. Regardless, these observations speak to the need for replication before any conclusions about clinical utility can be drawn.

In summary, we found evidence that variants in *RGS2* moderate the response to SSRIs in patients with SAD. Additional research is needed to replicate this finding, to place it in context of other yet-to-be-identified predictors of treatment response, and to determine the mechanism(s) by which these effects are seen. This line of research has the potential to lead to better personalization of treatments for patients with SAD and other mental disorders.

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REFERENCES

Arbelle S, Benjamin J, Golin M, Kremer I, Belmaker RH, Ebstein RP (2003). Relation of shyness in grade school children to the genotype for the long form of the serotonin transporter promoter region polymorphism. *Am J Psychiatry* **160**: 671–676.

Fresco DM, Coles ME, Heimberg RG, Liebowitz MR, Hami S, Stein MB *et al* (2001). The Liebowitz Social Anxiety Scale: a comparison of the psychometric properties of self-report and clinician-administered formats. *Psychol Med* **31**: 1025–1035.

Gelernter J, Page GP, Stein MB, Woods SW (2004). Genome-wide linkage scan for loci predisposing to social phobia: evidence for a chromosome 16 risk locus. *Am J Psychiatry* **161**: 59–66.

Huezo-Diaz P, Uher R, Smith R, Rietschel M, Henigsberg N, Marusic A *et al* (2009). Moderation of antidepressant response by the serotonin transporter gene. *Br J Psychiatry* **195**: 30–38.

Kimple AJ, Bosch DE, Giguere PM, Siderovski DP (2011). Regulators of G-protein signaling and their Galpha substrates: promises and challenges in their use as drug discovery targets. *Pharmacol Rev* **63**: 728–749.

Laje G, Perlis RH, Rush AJ, McMahon FJ (2009). Pharmacogenetics studies in STAR*D: strengths, limitations, and results. *Psychiatr Serv* **60**: 1446–1457.

Leon AC, Olfson M, Portera L, Farber L, Sheehan DV (1997). Assessing psychiatric impairment in primary care with the Sheehan Disability Scale. *Int J Psychiatry Med* **27**: 93–105.

Lifschytz T, Broner EC, Zozulinsky P, Slonimsky A, Eitan R, Greenbaum L *et al* (2012). Relationship between Rgs2 gene expression level and anxiety and depression-like behaviour in a mutant mouse model: serotonergic involvement. *Int J Neuropsychopharmacol* **15**: 1307–1318.

Masi G, Pfanner C, Mucci M, Berloffa S, Magazu A, Parolin G *et al* (2012). Pediatric social anxiety disorder: predictors of response to pharmacological treatment. *J Child Adolesc Psychopharmacol* **22**: 410–414.

Nyholt DR (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* **74**: 765–769.

Oliveira-Dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ *et al* (2000). Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci USA* **97**: 12272–12277.

Perlis RH (2010). Can single genes matter in a polygenic world? *Biol Psychiatry* **68**: 783–784.

Perlis RH (2011). Translating biomarkers to clinical practice. *Mol Psychiatry* **16**: 1076–1087.

Pollack MH, Van Ameringen M, Simon NM, Worthington JW, Hoge EA, Keshaviah A *et al* (2013). A double-blind randomized controlled trial of augmentation and switch strategies for refractory social anxiety disorder. *Am J Psychiatry* (in press).

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al* (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**: 559–575.

Ruscio AM, Brown TA, Chiu WT, Sareen J, Stein MB, Kessler RC (2008). Social fears and social phobia in the USA: results from the National Comorbidity Survey Replication. *Psychol Med* **38**: 15–28.

Semplicini A, Lenzini L, Sartori M, Papparella I, Calo LA, Pagnin E *et al* (2006). Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II. *J Hypertens* **24**: 1115–1124.

Smoller JW, Paulus MP, Fagerness JA, Purcell S, Yamaki LH, Hirshfeld-Becker D *et al* (2008). Influence of RGS2 on anxiety-related temperament, personality, and brain function. *Arch Gen Psychiatry* **65**: 298–308.

Stein DJ, Ipser JC, Balkom AJ (2004). Pharmacotherapy for social phobia. *Cochrane Database Syst Rev* CD001206.

Stein MB, Seedat S, Gelernter J (2006). Serotonin transporter gene promoter polymorphism predicts SSRI response in generalized social anxiety disorder. *Psychopharmacology (Berl)* **187**: 68–72.

Stein MB, Stein DJ (2008). Social anxiety disorder. *Lancet* **371**: 1115–1125.

Talbot JN, Jutkiewicz EM, Graves SM, Clemans CF, Nicol MR, Mortensen RM *et al* (2010). RGS inhibition at G(alpha)i2 selectively potentiates 5-HT1A-mediated antidepressant effects. *Proc Natl Acad Sci USA* **107**: 11086–11091.

Uher R, Tansey KE, Malki K, Perlis RH (2012). Biomarkers predicting treatment outcome in depression: what is clinically significant? *Pharmacogenomics* **13**: 233–240.

Van Ameringen M, Oakman J, Mancini C, Pipe B, Chung H (2004). Predictors of response in generalized social phobia: effect of age of onset. *J Clin Psychopharmacol* **24**: 42–48.

Wagner KD, Berard R, Stein MB, Wetherhold E, Carpenter DJ, Perera P *et al* (2004). A multicenter, randomized, double-blind, placebo-controlled trial of paroxetine in children and adolescents with social anxiety disorder. *Arch Gen Psychiatry* **61**: 1153–1162.

Yalcin B, Willis-Owen SA, Fullerton J, Meesaq A, Deacon RM, Rawlins JN *et al* (2004). Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. *Nat Genet* **36**: 1197–1202.