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ORIGINAL INVESTIGATION

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Serotonin transporter gene promoter polymorphism predicts SSRI response in generalized social anxiety disorder

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Abstract *Objectives:* To determine whether variation in the serotonin transporter gene promoter (5HTTLPR) influences the efficacy of selective serotonin reuptake inhibitors (SSRIs) in generalized social anxiety disorder (GSAD). *Methods:* Consecutive series of *N*=32 patients with DSM-IV GSAD for whom DNA and standardized outcome data from a 12-week SSRI trial were available. After ensuring that neither clinical response [clinical global impression of change scale (CGI-C)] nor 5HTTLPR genotype was confounded by ethnicity or sex, we determined whether the number of copies (0, 1, or 2) of hi-risk alleles using either the diallelic L–S system or the triallelic La–Lg–S system, predicted response and change in Liebowitz social anxiety scale (LSAS) and brief social phobia scale (BSPS) scores during SSRI treatment. *Results:* Twenty-one patients (66%) were responders to SSRI (i.e., CGI-C much or very much improved). A trend was seen for a linear association between 5HTTLPR genotype and likelihood of response to SSRI: diallelic classification L/L 7/8 (88%), L/S 12/18 (67%), S/S 2/6 (33%), p=0.051; triallelic classification L′/L′ 4/5 (80%), L′/S′ 14/19 (74%), S′/S′ 3/8 (38%), p=0.093. Reduction in LSAS (and BSPS) scores during SSRI treatment was significantly (p<0.02) associated with 5HTTLPR genotype using either the diallelic or triallelic classification. *Conclusions:* Variation in a functional polymorphism known to influence serotonin reuptake is associated with SSRI response in patients with GSAD. Independent replication in larger samples is required before the predictive utility of this information can be confirmed and generalized to clinical settings.

Keywords Selective serotonin reuptake inhibitor (SSRI) · Social anxiety disorder (SAD) · Pharmacotherapy · Genetics · Polymorphism · Pharmacogenomics

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Introduction

Social anxiety disorder is a common (lifetime prevalence 12%) (Kessler et al. 2005), frequently disabling disorder (Stein and Kean 2000) whose pathophysiology is poorly understood (Charney 2004). Generalized social anxiety disorder (GSAD) is a more pervasive, chronic, heritable subtype of the disorder with a lifetime prevalence of 4–5% (Schneier 2003; Stein and Gorman 2001). Selective serotonin reuptake inhibitors (SSRIs) are the most widely used, evidence-based treatment for GSAD (Stein et al. 2004) but approximately 40% of patients fail to derive an adequate therapeutic effect (Van Ameringen et al. 2005). Explanations for this heterogeneity of response are lacking but could, theoretically, involve individual differences in brain serotonin metabolism.

There are some evidence that poor SSRI response in major depression, a disorder with which GSAD is frequently comorbid (Kessler et al. 1999), is associated with the short (S) allele of the "long/short (L/S)" polymorphism in the serotonin transporter gene promoter (5HTTLPR) [see (Malhotra et al. 2004) for review]. This functional polymorphism, which conveys reduced transcriptional efficiency (Lesch et al. 1996), has also recently been associated with SSRI response in panic disorder (Perna et al. 2005) and in bulimia nervosa (Monteleone et al. 2005). Both of these are disorders with which GSAD is often comorbid (Kaye et al. 2004; Kessler et al. 1998). To test the hypothesis that the 5HTTLPR is associated with SSRI response in GSAD, we genotyped the L/S polymor-

phism in a consecutive series of patients with GSAD who were participating in controlled trials of SSRIs at our research center. In light of the recent findings that the 5HTTLPR can also be classified by a triallelic system (La/Lg/S) (Hu et al. 2005), where the L allele is subtyped into La and Lg alleles, the latter of which is thought to be similar to the S allele in terms of reuptake efficiency, we also genotyped subjects for this polymorphism.

Materials and methods

Our sample consisted of a consecutive series of 32 patients [23 (71.9%) men; 25 (78.1%) Caucasian] ages 19–58 years (mean 37.6, SD 9.9) with GSAD who took an SSRI as part of their participation in randomized controlled trials (RCTs) of SSRIs and gave consent for DNA analysis under the auspices of the University of California San Diego (UCSD) Anxiety Disorders Research Program. Subjects met DSM-IV criteria for a clinically predominant diagnosis of GSAD on the basis of a structured clinical interview (SCID-IV or MINI) by an experienced rater; six subjects (18.8%) had comorbid DSM-IV major depressive disorders. The same rater also completed the Liebowitz social anxiety disorder scale (LSAS) (Heimberg et al. 1999) and, in a subset 25 patients, the Brief social phobia scale (BSPS) (Davidson et al. 1997) before and at the end of 12 weeks of SSRI treatment, and the clinical global impression of change scale (CGI-C) (Guy 1976). Mean LSAS score before SSRI treatment was 85.4 (SD 23.0). Mean BSPS score before SSRI treatment was 42.3 (SD 12.5).

Treatment was with paroxetine (N=27) or fluvoxamine (N=5), at maximally tolerated dose (modal dose 30 mg/d paroxetine or 150 mg/d fluvoxamine). Raters were blind to 5HTTLPR status, which was not determined until after the clinical trials were ended. All subjects gave informed written consent to participate.

Genomic DNA was extracted from whole blood and 5HTTLPR was analyzed by polymerase chain reaction (PCR) amplification, as described elsewhere for the diallelic L–S classification (Gelernter et al. 1997). To genotype the additional SNP that occurs in the VNTR repeat region of the SLC6A4 promoter (Hu et al. 2005), we digested the same PCR product that can be size-fractionated to separate "L" and "S" alleles (without digestion) in MspI (New England Biolabs, Beverly, MA). Undigested, these primers amplify a 410-basepair (bp) segment for the L allele, and a 366 bp segment for the S allele. The A→G substitution (i.e., La→Lg) creates an additional MspI site; there are also two constant MspI sites. After restriction digestion, the digest mixture was size-fractionated on agarose gels. Band sizes were: 184, 131, 62, and 33 bp for the Lg allele; 315, 62, and 33 bp for the La allele; and 271, 62, and 33 bp for the S allele. Thus, a single PCR reaction and digest can provide triallelic classification.

Statistical analysis

PowerMarker (Liu and Muse 2005) was used to compute exact Hardy Weinberg equilibrium (HWE) statistics. The triallelic genotypes were reclassified into a biallelic model by their level of expression as follows: Lg/S, Lg/Lg, and S/S were reclassified as S′S′; La/S and La/Lg were reclassified as L′S′; and La/La was reclassified as L′L′ (Parsey et al. 2006).

After ensuring that neither clinical response (CGI-C) nor 5HTTLPR genotype (using either the diallelic or triallelic classification) was confounded by sex, ethnicity, or presence of comorbid major depression (Chi-square or Fisher's exact tests, all p values>0.10), we determined whether the number of copies (coded as 0, 1, or 2) of the S allele using the diallelic classification (or the S′ allele, using the triallelic classification) predicted dichotomous response status and change in LSAS (and BSPS) scores during SSRI treatment, using logistic and linear regression analyses, respectively.

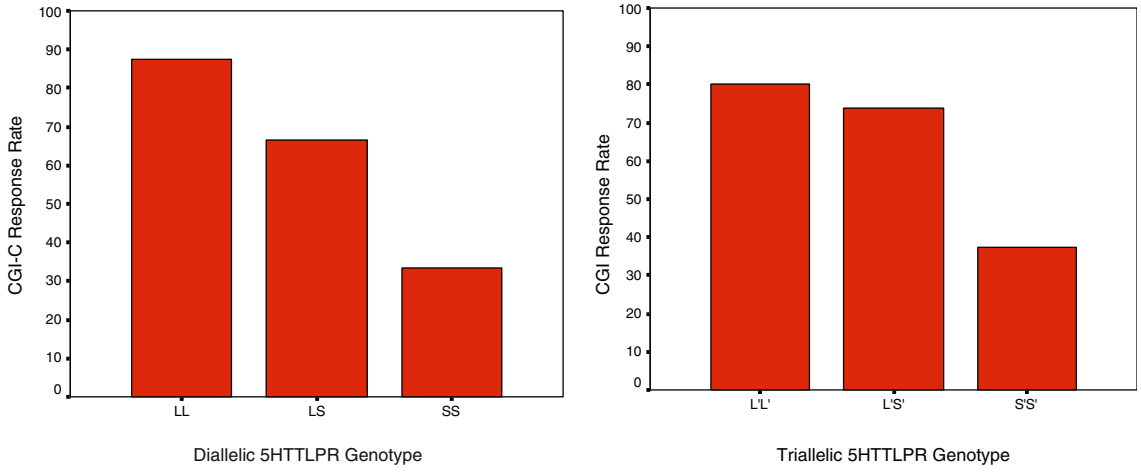

Fig. 1 Response rate to 12 weeks of SSRI treatment by 5HTTLPR genotype (diallelic classification on the *left*; triallelic classification on the *right*)

Results

Distribution of 5HTTLPR alleles according to the diallelic classification was L 53% and S 47%; there was no significant deviation from HWE (chi-square=0.54, *df*=1, *p*=0.46). According to the triallelic system, the distribution of alleles was La 45%, Lg 8%, and S 47% with no significant deviation from HWE (exact test, *p*=0.71).

Twenty-one patients (65.6%) were deemed responders to SSRI (i.e., CGI-C much or very much improved). Response rate was predicted by 5HTTLPR genotype at the level of a statistical trend: logistic regression for number of copies of the S allele in the diallelic system, Beta=−1.33 (SE 0.68), *df*=1, *p*=0.051 [L/L 7/8 (88%), L/S 12/18 (67%), S/S 2/6 (33%)]; logistic regression for number of copies of the S′ allele in the triallelic classification, Beta=−1.14 (SE 0.68), *df*=1, *p*=0.093 [L′/L′ 4/5 (80%), L′/S′ 14/19 (74%), S′/S′ 3/8 (38%)] Fig. 1.

Reduction in LSAS score during treatment was also significantly predicted by 5HTTLPR genotype: linear regression for number of copies of the S alleles in the diallelic system, adjusted R^2=0.176, standardized Beta=0.451, *p*=0.011; linear regression for number of copies of S′ alleles in the triallelic system, adjusted R^2=0.195, standardized Beta=0.471, *p*=0.008). As can be seen in Fig. 2, the S allele in the diallelic system or the S′ allele (i.e., Lg or S) in the triallelic system is associated with poorer response to SSRI treatment (i.e., a smaller reduction in LSAS scores from pre- to posttreatment).

Similarly, in the subjects (*N*=25) for whom BSPS data were available, change in BSPS score during treatment was also significantly predicted by 5HTTLPR genotype: linear regression for number of copies of S alleles in the diallelic system, adjusted R^2=0.206, standardized Beta=0.489, *p*=0.013; linear regression for number of copies of S′ alleles in the triallelic system, adjusted R^2=0.180, standardized Beta=0.463, *p*=0.02) (data not shown).

Discussion

Earlier age at onset and longer duration of treatment have been reported as modest predictors of SSRI response in GSAD (Stein et al. 2002a; Van Ameringen et al. 2004), but neither of these factors is informative with regard to mechanism of action. We found that allelic variation in 5HTTLPR predicts response to SSRIs in patients with GSAD, thereby reinforcing the notion that synaptic (extracellular) serotonin availability is requisite for response. Considering the increased amygdala response to fearful and angry faces seen in GSAD (Stein et al. 2002b), the mediation of this response by 5HTTLPR genotype (Furmark et al. 2004; Hariri et al. 2005), and the preclinical evidence that serotonergic neurons influence amygdala activation (Bauman and Amaral 2005), the role for serotonin in the disorder and its treatment is strongly suspected.

It is unclear whether SSRI effects in GSAD are mediated through similar pathways as in major depression or other SSRI-responsive anxiety disorders though, as in major depression, reversal of clinical response is observed with acute tryptophan depletion (Argyropoulos et al. 2004). Serotonin reuptake may, in fact, be necessary for response to antidepressants in GSAD, whereas—in contrast to major depression or panic disorder—norepinephrine reuptake may not contribute to therapeutic effects (Stein et al. 2005). In light of the recent finding from an in vivo human PET study that 5HTTLPR genotype (according to either the diallelic or triallelic system) is not associated with the degree of serotonin transporter binding potential (Parsey et al. 2006), the precise mechanism by which variation in 5HTTLPR would lead to differences in SSRI treatment outcome remains to be determined.

A number of factors should be considered in interpreting our findings. First and foremost, the small sample size mandates that our results be replicated. Using a dichotomous measure of outcome (i.e., response vs nonresponse),

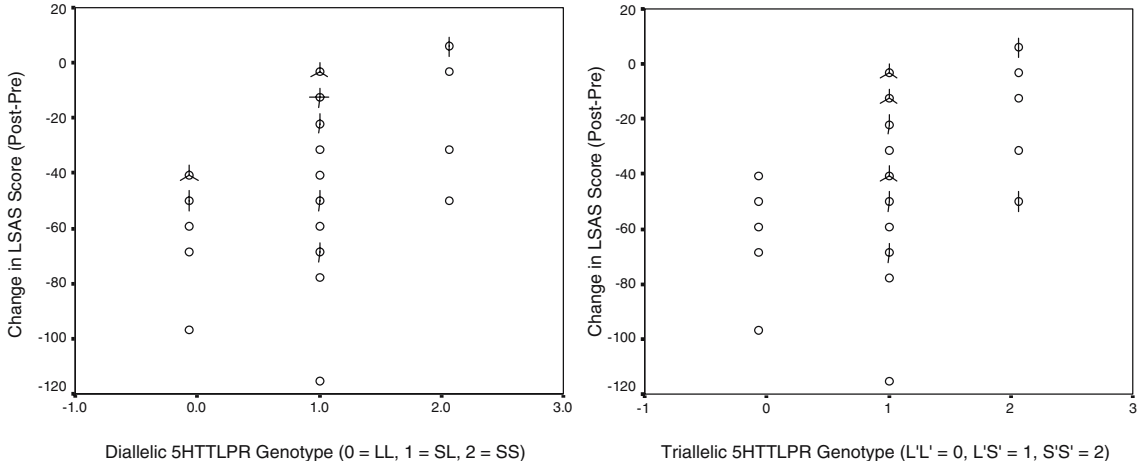

Fig. 2 Relationship of 5HTTLPR genotype (diallelic classification on the *left*; triallelic classification on the *right*) to change in Liebowitz social anxiety scale (LSAS) scores during 12 weeks of SSRI treatment. Larger negative numbers (i.e., at the *bottom* of the graph) indicate the greatest reduction in social anxiety symptoms from pre- to posttreatment. The number of *petals* on each *sunflower* indicates the number of individuals with the graphed value

the findings reached only a statistical "trend", perhaps owing to the small sample size and/or to the loss of information associated with dichotomously assigning response status. Of interest, using two different continuous measures of symptom response, the LSAS and the BSPS variation in 5HTTLPR explained a substantial (~20%) and statistically significant proportion of the variance in outcome. This observation suggests that continuous measures of outcome may be more sensitive to change (which is as expected because they retain more information), and may be more appropriate than dichotomous response measures for use in exploratory pharmacogenomic studies.

A second limitation is that the limited demographic (ethnic and sex) variance in this sample precluded evaluation of possible subgroup differences in response, which should be further explored. And third, the possible impact of variation in other genes (and interactions of these genes with 5HTTLPR) that might contribute to therapeutic response should be investigated.

It remains to be determined whether the resistance to SSRIs associated with the S (or S′) allele can be overcome by using higher doses of an SSRI. Given that the current study used flexible, maximally tolerated doses of SSRI in each patient, it seems unlikely that using higher doses would have been a therapeutic option. It may be that switching to a drug from an entirely different class will be more effective for such individuals. This hypothesis should be tested in future studies. In this regard, much larger clinical trials will be needed to determine whether knowledge of 5HTTLPR genotype can be effectively used to individualize the pharmacological treatment of GSAD.

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