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Serotonin Transporter and GABA(A) Alpha 6 Receptor Variants Are Associated with Neuroticism

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Abstract

Background: A tendency to experience negative affect, as measured by the neuroticism component of the Neuroticism, Extraversion, and Openness Personality Inventory (NEO-PI), is a trait marker for major depression. Epidemiologic studies indicate a strong genetic component, but to date few specific genetic variants have been definitively implicated. A serotonin transporter promoter polymorphism (5-HTTLPR) has been extensively studied in neuroticism and several psychiatric disorders, with inconclusive results. A GABA(A) receptor α6 subunit variant (Pro385Ser) has been associated with alcohol-related traits but has not been studied in neuroticism or depression.

Methods: A total of 384 subjects who completed the NEO-PI were genotyped at 5-HTTLPR and Pro385Ser. Associations between polymorphisms and both alcohol use and personality domains were tested.

Results: The 5-HTTLPR short allele (p = .008) and Pro385Ser Pro allele (p = .003) are associated with higher neuroticism scores. The 5-HTTLPR long allele (p = .006), but not Pro385Ser, is also associated with an increased presence of alcohol use. In addition, there is a nonsignificant suggestion of an interaction: the effect of 5-HTTLPR on neuroticism might be dependent on the Pro385Ser genotype.

Conclusions: These findings support a role for the serotonin transporter and GABA(A) α6 subunit in depression-related traits.

Keywords: Serotonin, γ-aminobutyric acid, association, interaction, NEO-PI, personality, polymorphism, anxiety, depression

The principal function of the serotonin transporter is to remove serotonin from the synapse, returning it to the presynaptic neuron where the neurotransmitter can be degraded or retained for re-release at a later time. Heils *et al.* (1995) identified a functional repeat polymorphism in the promoter region of the serotonin transporter gene (5HTTLPR) where the 14-repeat (s) and 16-repeat (l) variants predominate in Caucasian populations. The same group found that cell lines with at least one 5-HTTLPR s allele produced about half as much of a reporter protein as cell lines homozygous for the l allele. The reduced efficiency of the s allele has been confirmed through messenger ribonucleic acid quantification in cell lines, postmortem human brain, and whole blood serotonin and platelet studies (Hanna *et al.*, 1998; Lesch *et al.*, 1996; Little *et al.*, 1998). 5-HTTLPR subsequently became one of the most extensively investigated polymorphisms in studies of depression and its trait marker, neuroticism. Lesch *et al.* (1996) found that the presence of the 5-HTTLPR s allele was associated with higher mean neuroticism scores. This finding prompted many attempts to replicate the association. In four replication attempts, a sample size similar to that of the original study was used (>350 subjects) (Greenberg *et al.*, 2000; Hamer *et al.*, 1999; Jorm *et al.*, 1998; Mazzanti *et al.*, 1998). One study, carried out by the group that published the original association, robustly confirmed the initial association between 5-HTTLPR and neuroticism (Greenberg *et al.*, 2000). A second study did not confirm the original association but found evidence for linkage between 5-HTTLPR and anxiety-related traits (Mazzanti *et al.*, 1998). The other two studies found no evidence for either association or linkage (Hamer *et al.*, 1999; Jorm *et al.*, 1998). In addition to these larger studies, at least 19 studies with considerably smaller sample sizes have been conducted. Although some of these studies confirmed the original association, most did not (Greenberg *et al.*, 2000; Melke *et al.*, 2001). These results are not surprising, given the lack of statistical power of these studies. Our study investigates the 5-HTTLPR–neuroticism association with the use of a large sample and the same personality inventory that was used in the original study.

The γ-aminobutyric acid (GABA) system has been repeatedly implicated in anxiety- and depression-related traits (Petty, 1995). Cerebrospinal fluid and occipital cortex GABA concentrations are reduced in depressed patients (Sanacora *et al.*, 1999; Sanacora *et al.*, 2000). In addition, occipital cortex GABA concentrations are increased after selective serotonin reuptake inhibitor administration (Sanacora *et al.*, 2002). Furthermore, postmortem studies suggest that mood disorders are associated with a reduced number of GABAergic neurons in the frontal cortex (Heinz *et al.*, 2001). The GABA(A) receptor is the site of action for certain anxiolytic drugs, including benzodiazepines and barbiturates, providing additional evidence for a role for the receptor in neuroticism. The Pro385Ser polymorphism is a Pro to Ser variant located in the α6 subunit of the GABA(A) receptor. Iwata *et al.*, (1999) reported an association between this variant and benzodiazepine sensitivity. In addition, Schuckit *et al.*, (1999) reported an association between Pro385Ser and low response to alcohol. Pro385Ser however, has yet to be investigated in personality traits, depression, or anxiety. In this study, we investigated the role of this polymorphism in Neuroticism, Extraversion, and Openness Personality Inventory (NEO-PI) personality traits, including neuroticism. To explore possible nonadditive genetic effects, we tested for potential statistical interactions between

all variants that individually associate with neuroticism. In a previous report, a coding brain-derived neurotrophic factor (BDNF) variant was found to be associated with neuroticism in this sample (Sen *et al.*, 2003). Thus, we explore interactions between variants that associate with neuroticism in this study, as well as potential interactions between each associated variant and BDNF with respect to neuroticism.

Materials and Methods

Subjects

The 242 female and 177 male subjects are from 257 families participating in the Family Blood Pressure Program at the Tecumseh, Michigan Site (Thiel *et al.*, 2003). Subjects gave informed consent, and the study has been approved by the University of Michigan Medical School institutional review board. Ninety-nine percent of the subjects are non-Hispanic Caucasians. Family eligibility in this study required an available proband between 25 and 40 years of age with a systolic blood pressure in the upper 15% of the blood pressure distribution and a sibling willing to participate. Parents and additional siblings of probands were required when available.

5-HTTLPR genotyping

Primers SERT1 (5′-ATGCCAGCACCTAACCCCTAATGT-3′) and SERT2 (5′-GGACCGCAAGGTGGGCGGGA-3′) were used to amplify a product that was 375 base pair (bp) product for the 14-repeat (s) allele and a 419 bp product for the 16-repeat (l) allele (Gelernter *et al.*, 1998). A PTC 100 thermal cycler (MJ Research, Watertown, Massachusetts) was used for deoxyribonucleic acid (DNA) amplification. Amplification reactions were performed in a total volume of 20 μL, containing approximately 50 ng of genomic template, 1 μmol/L of each primer, 200 μmol/L deoxynucleoside triphosphate (dNTP), 2 μL 10× Opti-Prime Buffer #6 (Stratagene, La Jolla, California) and 1 unit of *Taq* polymerase. The polymerase chain reaction (PCR) cycling conditions consisted of an initial denaturation for 2 min at 94°C, followed by 35 cycles of 94°C for 1 min, 60°C for 2 min, and 72°C for 2 min, and a final extension at 72°C for 4 min. Polymerase chain reaction products were electrophoresed on a 2% agarose gel and visualized under ultraviolet light with the Gel-Star nucleic acid gel stain (BioWhittaker Molecular Applications, Rockland, Maine).

Originally, an alternate set of primers, STPR3 (5′-GGCGTTGCCGCTCTGAATTGC-3′) and STPR5 (5′-GAGGGACTGAGCTGGACAACCCAC-3′), was used for amplification (Greenberg *et al.*, 2000). These primers only amplified from the DNA of approximately 70% of subjects. In addition, in eight cases the genotypes produced were inconsistent with simple Mendelian inheritance, and the frequency of the genotypes produced with these primers did not fit into Hardy-Weinberg equilibrium. Upon reanalysis with the SERT1 and SERT2 primers, 91% of samples were successfully genotyped, and there were no Mendelian errors or deviation from Hardy Weinberg equilibrium. Comparison of the two methods showed that amplification with the STPR3 and STPR5 primers erroneously classified a subset of heterozygous l/s subjects as s/s.

Pro385Ser genotyping

Two primers within the GABA A receptor α 6 (GABRA6) subunit gene, GABRA61, 5′-AGGCCAATAAAGTGCTCACG-3′, and GABRA62, 5′-TTTACTGGTGCCTCCAAAGG-3′, were used to amplify a 92 bp PCR product from genomic DNA flanking the single nucleotide polymorphism 1236C>T (Pro385-Ser). For genotyping, two molecular beacons were designed, one specific for the "C" allele and 5′ labeled with a green fluorophore (FAM) (5′-/56-FAM/AGCAGCAACCTGTCACACCCCCACCACTGCTGCT/3Dab/-3′) and the other specific for the "T" allele and 5′ labeled with a red fluorophore (Texas red) (5′-/5TexRd-XN/CTCCGGTCACCTGTCACATCCCCACCACACCGGAG/3Dab/-3′). Both oligonucleotides were 3′ labeled with the quencher DABCYL. Molecular beacons were purchased from Integrated DNA Technologies, Coralville, Iowa. Genotyping was performed during real-time PCR in an iCycler Thermal Cycler (BioRad, Hercules, California). Amplification reactions were performed in a total volume of 25 μL containing 10 pmol each of the sense and antisense primers, 250 μmol/L dNTP, .34 μmol/L of each of the molecular beacons probes, 1 unit of *Taq* polymerase, and approximately 20 ng of genomic DNA. The PCR conditions were as follows: initial denaturation for 3 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 56°C for 1 min, and 72°C for 30 sec. Fluorescence was measured during annealing temperature (56°C). To verify the specificity of the molecular beacons as a single nucleotide polymorphism genotyping method, we analyzed a different subset of 56 samples through both molecular beacons and *Fok* I digestion. No discrepancies were found between the two methods.

Questionnaire

Personality traits were assessed with the NEO-PI. This inventory, consisting of 181 questions, assesses subjects on five global personality domains and breaks down three of these domains (neuroticism, extraversion, and agreeableness) into six facets each. The NEO-PI is a well-established inventory constructed through factor analytic strategies. This inventory also provides high test–retest reliability and longitudinal stability (Costa and McCrae, 1997).

Data to make diagnoses of alcohol abuse and dependence were not collected in this sample; however, subjects were asked questions regarding alcohol use. Specifically, subjects were asked to answer yes or no to the question: "Have you ever regularly consumed alcohol in your life?" 62.7% of subjects answered yes to this question. The presence of associations between genetic markers and subjects' response to this question were also explored.

Statistical analysis

The presence of association was determined with the QTDT Program version 2.1 (available at http://www.sph.umich.edu/statgen/abecasis/QTDT/). This program was not used to test for association through a transmission disequilibrium test. Instead, QTDT was used to test for association while accounting for familial resemblance due to kinship and linkage (Abecasis *et al.*, 2000a, 2000b). The presence of a statistical interaction between the effect of two variants on neuroticism were assessed through linear regression in SPSS 10.0 (SPSS, Chicago, Illinois). The regression was performed on neuroticism with three independent factors: locus 1 genotype, locus 2 genotype, and the product of the two genotypes as an interaction term. Familial resemblance was not considered in testing for interactions.

Results

We determined genotypes for 419 subjects at the 5-HTTLPR polymorphic site. Allele frequencies in this sample (adjusted for familial correlations) were S = .42 and L = .58.

Table 1. NEO-PI Domain Scores as a Function of 5-HTTLPR and Pro385Ser Genotypes

	Genotype	*n*	Mean	SEM	Regression *p* value (2-tailed)	*t* test s dominant	*t* test l dominant	Regression *F* statistic
5-HTTLPR								
Neuroticism	s/s	83	87.8	2.07	.008	.006	.024	9.00
	s/l	183	84.5	1.54				
	l/l	149	79.9	1.59				
Extraversion	s/s	82	105.3	1.94	*ns*	*ns*	*ns*	1.45
	s/l	183	109.2	1.28				
	l/l	149	108.6	1.36				
Openness	s/s	82	104.1	1.77	*ns*	*ns*	*ns*	.00
	s/l	183	104.4	1.26				
	l/l	149	104.1	1.38				
Conscientiousness	s/s	83	48.0	.88	*ns*	*ns*	*ns*	.00
	s/l	184	48.6	.62				
	l/l	151	48.3	.67				
Agreeableness	s/s	83	45.4	.72	.049	*ns*	.007	4.88
	s/l	183	47.6	.49				
	l/l	151	47.7	.56				
Alcohol Use	s/s	57	.19	.05	.006	.013	.016	8.43
	s/l	124	.29	.04				
	l/l	109	.40	.05				
Pro385Ser								
Neuroticism	Pro/Pro	340	85.15	1.11	.003			10.04
	Pro/Ser	51	75.67	2.27				
Extraversion	Pro/Pro	340	108.07	.95	*ns*			1.97
	Pro/Ser	51	111.73	2.28				
Openness	Pro/Pro	339	103.63	.93	*ns*			2.12
	Pro/Ser	51	107.39	2.10				
Conscientiousness	Pro/Pro	343	48.25	.45	*ns*			.31
	Pro/Ser	52	48.19	1.08				
Agreeableness	Pro/Pro	342	47.16	.35	*ns*			1.13
	Pro/Ser	52	48.18	.93				
Alcohol Use	Pro/Pro	236	.31	.03	*ns*			1.27
	Pro/Ser	35	.40	.08				

NEO-PI, Neuroticism, Extraversion, and Openness Personality Inventory; 5-HTTLPR, serotonin transporter promoter polymorphism.

These frequencies are consistent with reported values for samples with similar ethnic compositions: S = .45; L = .55 (Lesch *et al.*, 1996).

In this sample, presence of the short allele is associated with a significantly higher mean neuroticism score ($p = .004$). In addition, the presence of the l allele was associated with lower mean agreeableness ($p = .007$). 5-HTTLPR alleles were not associated with the other three personality domains (Table 1). Of the six facets of neuroticism, 5-HTTLPR was associated with anxiety (N1), hostility (N2), depression (N3), and self consciousness (N4), but not with impulsiveness (N5) or vulnerability (N6) (Table 2). 5-HTTLPR was not associated with any of the 12 facets that compose the domains extraversion and openness. In the version of the NEO-PI used in this study, no facet scores for the agreeableness and conscientiousness domains were available. We also found an association between the 5-HTTLPR l allele and an increase in the presence of regular alcohol use ($p = .006$) (Table 1). A linear regression analysis showed no correlation between regular alcohol use and neuroticism in this sample ($p = .556$).

Because previous studies are inconsistent concerning the dominance pattern at this locus (Greenberg *et al.*, 2000; Melke *et al.*, 2001), we did not assume any specific dominance pattern when testing association. For neuroticism, the s/s and l/s genotype groups did not differ significantly from each other, whereas both differed significantly from the l/l genotype group. For agreeableness, the l/l and s/l groups did not differ significantly from each other, whereas both groups differed significantly from the s/s group. For alcohol use, the s/s and l/l genotype groups differed significantly from each other, but neither homozygote group differed significantly from the s/l group.

A total of 391 subjects were genotyped at the Pro385Ser polymorphism of GABRA6. Allele frequencies at this site (adjusted for familial correlations) were Pro = .94 and Ser = .06. This is consistent with reported allele frequencies (Pro = .96, Ser = .04; (Iwata *et al.*, 1999). In our sample, there were no Ser/Ser subjects. The Pro/Ser genotype was significantly associated with lower mean neuroticism but not with the other four personality domains (Table 1). Of the six facets of neuroticism, Pro385Ser was associated with anxiety (N1), hostility (N2), depression (N3), self consciousness (N4), and vulnerability (N6) (Table 2). In addition, Pro385Ser was associated with the extraversion facet called positive emotions (E6) and the openness facet called ideas (O5). Pro385Ser showed no association with the presence of regular alcohol use.

A total of 366 subjects were successfully genotyped at both the 5-HTTLPR and Pro385Ser sites. In the presence of the Pro/Pro genotype, 5-HTTLPR was strongly associated with neuroticism (genotype: mean ± SEM) (s/s: 90.98 ± 2.61; l/s: 86.75 ± 1.79; l/l: 80.07 ± 1.69; $p = .0004$). In the presence of the alternate Pro/Ser genotype, 5-HTTLPR showed no association with neuroticism (s/s: 76.38 ± 3.75; l/s: 75.43 ± 3.70; l/l: 75.27 ± 4.28; $p = .87$). These results are illustrated in Figure 1. In a lin-

Table 2. Neuroticism Facet Scores by 5-HTTLPR and Pro385Ser Genotype

	Genotype	*n*	Mean	SEM	Regression *p* value (2-tailed)	*t* test s dominant	*t* test l dominant	Regression *F* statistic
5-HTTLPR								
N1–Anxiety	s/s	83	16.12	.52	.032	.049	.044	6.13
	s/l	184	15.19	.36				
	l/l	151	14.48	.40				
N2–Hostility	s/s	83	12.65	.49	.052	*ns*	*ns*	3.82
	s/l	183	12.21	.34				
	l/l	151	11.51	.35				
N3–Depression	s/s	82	14.71	.59	.008	.010	.043	9.31
	s/l	184	13.90	.42				
	l/l	150	12.53	.44				
N4–Self-Consciousness	s/s	83	15.94	.47	.045	.015	*ns*	7.34
	s/l	182	15.34	.35				
	l/l	150	14.38	.34				
N5–Impulsiveness	s/s	83	16.93	.41	*ns*	*ns*	*ns*	.76
	s/l	183	16.69	.27				
	l/l	149	16.48	.33				
N6–Vulnerability	s/s	83	11.34	.41	*ns*	*ns*	*ns*	2.96
	s/l	183	11.14	.29				
	l/l	151	10.51	.31				
Pro385Ser								
N1–Anxiety	Pro/Pro	342	15.56	.27	.015			7.04
	Pro/Ser	52	13.64	.60				
N2–Hostility	Pro/Pro	341	12.21	.24	.025			6.59
	Pro/Ser	52	10.55	.51				
N3–Depression	Pro/Pro	340	14.02	.30	.004			8.52
	Pro/Ser	51	11.62	.68				
N4–Self-Consciousness	Pro/Pro	341	15.30	.25	*ns*			3.89
	Pro/Ser	52	13.99	.52				
N5–Impulsiveness	Pro/Pro	342	16.86	.20	*ns*			1.71
	Pro/Ser	51	16.12	.53				
N6–Vulnerability	Pro/Pro	343	11.16	.21	.006			7.23
	Pro/Ser	52	9.64	.40				

5-HTTLPR, serotonin transporter promoter polymorphism.

ear regression, the addition of the interaction term (5HTTLPR × Pro385Ser) was not significant (p = .22).

In a previous report, we described an association between a BDNF polymorphism and neuroticism in this sample. There is no evidence of a statistical interaction between BDNF and either 5-HTTLPR (p = .56) or GABRA6 (p = .45) (graphs not shown but available upon request).

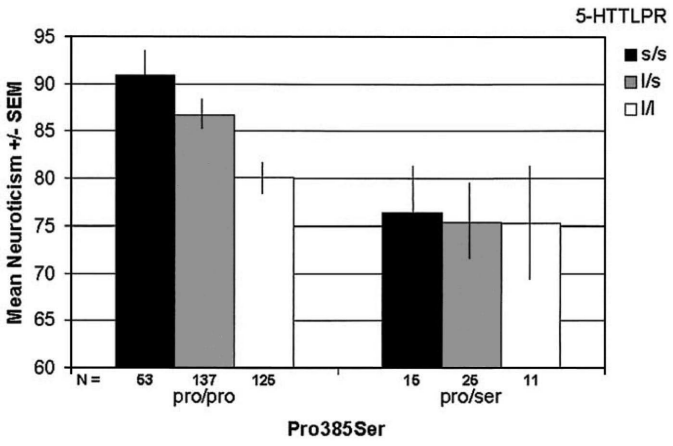

Figure 1. Neuroticism scores by 5-HTTLPR and Pro385Ser genotypes in tandem. 5-HTTLPR, serotonin transporter promoter polymorphism.

Discussion

This study represents the first large-scale replication of the association between 5-HTTLPR and neuroticism described by a group other than Lesch *et al.* (1996). In our sample, 5-HTTLPR accounts for 2%–3% of the overall variance and 4%–5% of the genetic variance in neuroticism score. This is a smaller effect size than Lesch *et al.* (1996) found in the original study (overall variance: 3%–4%; genetic variance: 7%–9%), consistent with the finding that original reports of an association tend to overestimate the true effect size (Ioannidis *et al.*, 2001).

There are several reasons why some previous studies might not have found an association between 5-HTTLPR and neuroticism. The most important is that the majority of studies investigating this association used samples that were too small to reliably detect this effect. Even with the effect size reported in the original study, power analysis shows that a sample of at least 350 subjects is necessary to obtain 80% statistical power at a .05 significance level (Flory *et al.*, 1999). Of the 23 studies, 17 show associations in the predicted direction, indicating higher anxiety-related personality trait scores for subjects with the 5-HTTLPR s allele. In addition, whereas eight studies found significant evidence for an association between the 5-HTTLPR s allele and higher trait scores, not a single study found an association between the alternate 5-HTTLPR l allele and higher trait scores, arguing against a chance distribution. Of the studies in which a sample greater than 350 subjects was

used, all three in which the NEO-PI was used found an association (our group used the NEO-PI; Lesch *et al.*, [1996] and Greenberg *et al.*, [2000] used the NEO-PI-R). In the three studies involving more than 350 subjects in which an association was not found, personality inventories other than the NEO-PI were used (Mazzanti *et al.*, [1998] used the Tridimensional Personality Questionnaire; Hamer *et al.*, [1999] used the Temperament and Character Inventory; Jorm *et al.*, [1998] used the Eysenck Personality Questionnaire). Thus, 5-HTTLPR might be more closely associated to NEO-PI neuroticism than to other anxiety-related traits. In addition, our study, along with the two other large-scale positive association studies, used samples composed primarily of Caucasians not selected on the basis of psychopathology (Lesch *et al.*, 1996; Greenberg *et al.*, 2000); whether these findings are valid in other populations is still unknown. This report is also the first confirmation of an association between 5-HTTLPR and agreeableness by a group other than Lesch and colleagues. Our findings on agreeableness are in line with a recent study reporting significant covariance between agreeableness and neuroticism, 10% of which is attributable to the 5-HTTLPR locus (Jang *et al.*, 2001).

The direction of association between 5-HTTLPR and neuroticism is the same in all studies finding significant association (s allele associates with higher mean neuroticism); however, which allele seems dominant differs among studies (Greenberg *et al.*, 2000; Melke *et al.*, 2001; this report). Similarly, the dominance pattern among expression studies is also inconsistent (Du *et al.*, 1999; Hanna *et al.*, 1998; Heils *et al.*, 1995). In our study, apparent dominance of alleles at the 5-HTTLPR locus also shows different patterns for the different traits we analyzed in the same population sample. This heterogeneity in dominance patterns argues against complete dominance of either allele at this site, but further studies are warranted. Assuming the promoter variants affect expression, one would expect heterozygotes to show an intermediate phenotype, although not necessarily exactly at the midpoint. There are many reasons why not all studies show codominance. One possibility would be ceiling effects (heterozygotes and homozygotes showing close to maximal or close to the minimal scores) in a continuous scale such as the NEO-PI. Threshold effects on some but not all traits might also play a role.

In addition, we also report an association between the 5-HTTLPR l allele and an increased presence of regular alcohol use. Schuckit *et al.* (1999) reported an association between the same allele and increased alcohol dependence. Regular alcohol use and alcohol dependence are distinct traits, so this study is not a strict replication of the findings presented by Schuckit et al; however, these two sets of results are likely related and should be explored further. Given that there is no association between neuroticism and regular alcohol use in this sample, the associations between 5-HTTLPR and these two traits are likely independent.

We also report an association between the Pro385Ser Pro/Ser genotype and decreased neuroticism in this sample. Even though the two genotype groups differ markedly in mean neuroticism score, Pro385Ser accounts for only approximately 2% of the overall neuroticism variation and 4% of the genetic neuroticism variation in this sample, owing to the relatively low frequency of the Ser allele. This represents the first report of an association between Pro385Ser and a depression related trait. Schuckit *et al.* (1999) found that the Pro385Ser Pro/Ser genotype and the 5-HTTLPR l/l genotype associated with low response to alcohol and increased alcohol dependence. We find that the same two alleles at these loci are associated with lower neuroticism. Further studies are indicated to confirm the role of Pro385Ser in depression related traits, both individually and together with 5-HTTLPR.

The association between 5-HTTLPR and neuroticism was not corrected for multiple testing because it was designed as a replication of previously reported results. When a Bonferonni correction for the five NEO-PI domains is performed for Pro385Ser, the association with neuroticism remains significant. Because the neuroticism facets are strongly associated with each other, it is difficult to assess how to correct for further multiple testing.

Population stratification in studies such as ours can lead to false-positive associations (Lander and Schork, 1994). To assess this possibility, we used the program STRUCTURE, designed to infer population structure using genotypes from numerous unlinked markers as genomic controls (Pritchard and Rosenberg, 1999). Weak evidence for clustering into two groups was found (p = .024; Theil and Schork, personal communication, February 15, 2002; data available upon request). For population structure to affect the results of an association test, the allele frequencies at the relevant loci and the trait score must differ between the two clusters. In our sample, there were no significant differences between the clusters in either 5-HTTLPR allele frequency (p = .763), Pro385Ser allele frequency (p = 1.00), or neuroticism (p = .440). Thus, these reported results are unlikely to be the product of population stratification. An additional concern for this study is that the sample was drawn from families with a moderately hypertensive proband. There is no association in this sample between blood pressure and either 5-HTTLPR or Pro385Ser and only a weak association with neuroticism (p = .049). Nonetheless, the hypertensive status of the sample should be noted.

In addition to the individual associations between 5- HTTLPR and Pro385Ser with neuroticism, we also find suggestive evidence for an interaction between the two loci with reference to neuroticism. Specifically, the effect of 5-HTTLPR on neuroticism seems to be restricted to Pro385Ser Pro/Pro subjects. The interaction term did not achieve statistical significance in a linear regression analysis. This might be due to a lack of statistical power, in part because the Pro385Ser Pro/Ser group is relatively small (n = 51). The sample size needed to detect a significant statistical interaction is substantially larger than the sample needed to detect a simple association (Wahlsten, 1990). Although chance can of course not be excluded, it should be noted that this suggestive interaction is similar to one found by Schuckit *et al.* (1999). These authors found that all four subjects in their sample with both the 5-HTTLPR l/l genotype and the Pro/Ser genotype developed alcohol dependence and demonstrated a low level of response to alcohol. Given that Schuckit et al found a similar nonsignificant interaction in the same direction, the nature of this interaction with respect to neuroticism and alcoholism warrants further study in samples with large statistical power.

Little biological evidence directly connects the GABA A receptor α6 subunit and the serotonin transporter. There is evidence however, of interplay between the GABA and serotonin systems that might shed light on this statistical interaction. The majority of serotonergic axons in the brain synapse on cortical GABAergic interneurons (Smiley and Goldman-Rakic, 1996). In addition, recent evidence suggests that the convergence of serotonergic and dopaminergic neurons on GABAergic neurons might be important in the pathology of mood disorders (Benes and Berretta, 2001). The findings of Sanacora *et al.* (2002), that inhibitors of the serotonin transporter affect cortical GABA con-

centrations, provides further evidence of a strong connection between the two neurotransmitter systems. The interplay between the two systems should be explored further to determine whether the suggestive interaction between 5-HTTLPR and Pro385Ser has a meaningful biological basis.

Neuroticism is a strong marker for vulnerability to depression (Duggan *et al.*, 1995; Kendler *et al.*, 1993). Characteristics of neuroticism make it particularly useful for genetic studies of depression. The reported heritability of neuroticism is 40%–50% (Jang *et al.*, 1996; Lake *et al.*, 2000), equal to or greater than heritibility estimates for depression (36%) (Kendler and Prescott, 1999). Furthermore, approximately 70% of the correlation between neuroticism and depression risk is due to shared genetic risk factors (Kendler *et al.*, 1993). Neuroticism is also a quantitative trait and stable through adulthood (Costa and McCrae, 1988). Our finding of associations between two polymorphisms and neuroticism suggest that these loci might contribute to the risk for depression.

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