

# Association Between the Serotonin Transporter Promoter Polymorphism and Personality Traits in a Primarily Female Population Sample

Benjamin D. Greenberg,<sup>1\*</sup> Qian Li,<sup>1</sup> Frank R. Lucas,<sup>1</sup> Stella Hu,<sup>2</sup> Leo A. Sirota,<sup>2</sup> Jonathan Benjamin,<sup>3</sup> Klaus-Peter Lesch,<sup>4</sup> Dean Hamer,<sup>2</sup> and Dennis L. Murphy<sup>1</sup>

<sup>1</sup>Laboratory of Clinical Science, National Institutes of Mental Health, Bethesda, Maryland

<sup>2</sup>Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

<sup>3</sup>Department of Psychiatry, Ben Gurion University, Beersheva, Israel

<sup>4</sup>Department of Psychiatry, University of Würzburg, Würzburg, Germany

The serotonin transporter (5-HTT) regulates serotonergic neurotransmission and is thought to influence emotion. A 5-HTT-linked polymorphic region (5-HTTLPR) has two common variants, short (s) and long (l). We previously found population and within-family associations between the lower-expressing s allele and neuroticism, a trait related to anxiety, hostility, and depression, on a standard measure (the NEO Personality Inventory, Revised [NEO-PI-R]) in a primarily male population (n=505), and that the s allele was dominant. We investigated this association in a new sample (n=397, 84% female, primarily sib-pairs). The results robustly replicated the 5-HTTLPR neuroticism association, and the dominance of the s allele. Combined data from the two studies (n=902) showed a highly significant association between the s allele and higher NEO Neuroticism both across individuals and within families. Association between genotype and a related measure, Anxiety on the 16PF inventory, was replicated in the new population and within families in the combined sample. Association to another trait, estimated TPQ Harm Avoidance, was not replicated in the new sample but found only within the combined sibship group. Another association found in our original study, between the s allele and lower scores on NEO-PI-R Agreeableness, was also replicated and was more robust in the current and the com-

bined samples. Associations between the functional 5-HTTLPR polymorphism were similar in women and men. These results help to define specific personality features reproducibly associated with 5-HTTLPR genotype. Such associations were strongest for traits defined by the NEO, enhancing the attractiveness of the five-factor personality model in genetic research on complex behavioral dimensions. *Am. J. Med. Genet. (Neuropsychiatr. Genet.)* 96:202–216, 2000.

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## INTRODUCTION

Individual differences in human behavioral predispositions are relatively enduring [Costa and McCrae, 1988]. These continuously distributed personality traits are also substantially heritable, and therefore very likely result from the interplay of genetic variations with environmental influences [Loehlin, 1992]. This possibility has increasingly encouraged investigators to pursue dimensional approaches to neurobehavioral genetics, in addition to the traditional strategy of studying individuals with categorically defined neuropsychiatric disorders [Plomin et al., 1994]. Functional genetic variants affecting components of brain monoamine neurotransmitter systems are a logical starting point for this research.

The midbrain raphe serotonin (5-HT) neurotransmitter system innervates virtually the entire brain [Hansson et al., 1998]. Increasingly, evidence indicates that in addition to its neurotransmitter role, serotonin exerts a major influence in early brain development including cell proliferation, migration, and differentiation [Hansson et al., 1998]. In adult humans and animals, serotonergic neurotransmission is a major modu-

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\*Correspondence to: Dr. Benjamin D. Greenberg, Laboratory of Clinical Science, National Institute of Mental Health, NIH Clinical Center, Bldg. 10/3D-41, Bethesda, MD 20892-1264.

E-mail: bdg@helix.nih.gov

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lator of emotional behavior [Westenberg et al., 1996; Whitaker-Azmitia and Peroutka, 1990]. After release by presynaptic neurons, serotonin's action as a chemical modulator is terminated primarily by reuptake via the serotonin transporter (5-HTT). The 5-HTT thus plays a pivotal role in regulating serotonergic transmission in its numerous terminal fields throughout the brain, including regions crucial to emotional behavior such as cortical and limbic areas involved in sensory processing, motor behavior, emotional experience, and memory [Hensler et al., 1994]. Consistent with this view, the brain 5-HTT is the initial site of action of widely used serotonin reuptake inhibitor (SRI) antidepressant and antianxiety drugs. Similarly, the 5-HTT appears to play an important developmental role influencing brain morphogenesis. Differences in function of the 5-HTT could therefore influence enduring behavioral predispositions (i.e., personality traits) in two ways.

In humans, the 5-HTT is encoded by a single gene on chromosome 17q11.2 [Esterling et al., 1997]. Our group previously described a functional length variation polymorphism in the 5' regulatory region ~1 kb upstream of the transcription initiation site, the 5-HTT-linked polymorphic region or 5-HTTLPR. The polymorphism has two common forms, a long (l) and a short (s) allele. The s variant was associated with reduced 5-HTT gene transcription in a reporter gene construct and in human lymphoblasts, resulting in reduced 5-HT binding sites and reduced 5-HT uptake; it also acted as a dominant allele [Heils et al., 1997; Lesch et al., 1996]. Further biochemical evidence from studies of other cell lines [Heils et al., 1997], postmortem human brain tissue [Little et al., 1998], whole blood serotonin levels [Hanna et al., 1998], and serotonin-containing human platelets [Greenberg et al. 1999] confirmed the initial finding [Lesch et al., 1996] that the s form is associated with reduced 5-HTT expression or function, and, with one possible exception [Hanna et al., 1998], that the s allele is dominant.

In our initial report, the biochemical data were congruent with NEO (NEO Personality Inventory, Revised) personality data from a general population sample of 505 individuals, 93% of whom were male [Lesch et al., 1996]. Individuals with either one or two copies of the short (s) promoter region variant (group S) had significantly greater levels of neuroticism, defined as proneness to negative emotion, including anxiety, hostility, and depression, than those homozygous for the long genotype (group L) in the sample as a whole and also within sibships. Individuals with 5-HTTLPR S genotypes also had significantly decreased Agreeableness as measured by the NEO. In addition, the group S subjects had increased scores on two traits related to neuroticism: Anxiety on the separate 16PF personality inventory, and estimated scores for Harm Avoidance on the Tridimensional Personality Questionnaire [TPQ; Cloninger et al., 1991].

Superficially, association between the better expressed 5-HTTLPR long allele with lower NEO Neuroticism and related traits seemed inconsistent with the known antidepressant and antianxiety effects of serotonin transporter inhibitors (SRIs), and with a re-

port that SRIs lessened negative emotionality in a non-patient sample [Knutson et al., 1998]. However, the 5-HTT is also an important modulator of development of brain regions important in emotional behavior, and hence enduring individual differences in personality could result from genetically influenced differential 5-HTTLPR expression during prenatal life. This conclusion is strongly supported by findings from research in animals. First, mice with a targeted disruption of the 5-HTT displayed enhanced anxiety-related behaviors in two animal models of anxiety, the light-dark box and the zero maze [Wichems et al., 1998]. The second line of evidence comes from studies of rhesus monkeys, a higher primate species that, like humans, has a functional 5-HTTLPR polymorphism [Lesch et al., 1997]. Rhesus infants tested very early in life (at postnatal days 7–30) with either one or two copies of the poorly expressed s allele displayed higher behavioral stress-reactivity on laboratory tests compared with infants with the l allele [Champoux et al., 1999]. Thus, both animal models are consistent with the finding in humans that it is the lower expressed 5-HTT allele that is associated with increased negative emotionality.

Our original findings encouraged ongoing research exploring possible associations between the 5-HTTLPR variants and categorically defined neuropsychiatric disorders, including affective illness [Bellivier et al., 1997; Collier et al., 1996; Rees et al., 1997], panic disorder [Deckert et al., 1997; Matsushita et al., 1997], autism [Cook et al., 1997], obsessive-compulsive disorder [Bengel et al., 2000; Billett et al., 1997; McDougle et al., 1998], schizophrenia [Malhotra et al., 1998], alcoholism [Sander et al., 1997], and Alzheimer's disease [Li et al., 1997; Oliveira et al., 1998].

In contrast, only two subsequent attempts to replicate the original personality finding have been reported using large populations [Jorm et al., 1998; Mazzanti et al., 1998]. Taken together, two of the three available large studies found evidence congruent with an influence of the 5-HTTLPR on Neuroticism and related traits [Lesch et al., 1996; Mazzanti et al., 1998]. In contrast, a large population study not employing a within-family design [Jorm et al., 1998] did not. Smaller population-based studies have had variable but generally negative results [Ball et al., 1997; Deary et al., 1999; Ebstein et al., 1997; Flory et al., 1999; Gelernter et al., 1998; Katsuragi et al., 1999; Kumakiri et al., 1999; Ricketts et al., 1998]. Interpretation of these studies is complicated by their use of relatively small and in some cases unusual samples, and the lack of within-family controls for possible population stratification artifacts. An additional important issue, which applies to both the large and smaller sample studies, is that the personality trait measures have often differed. For example, the most recent large studies, Mazzanti et al. [1998] and Jorm et al. [1998], used Temperament and Character Inventory (TCI) Harm Avoidance and Eysenck Personality Questionnaire Neuroticism, respectively. Given that the magnitude of 5-HTTLPR personality association is expected to be small based on the prior data [Lesch et al., 1996], it appears crucial that attempts to replicate that finding use the same phenotype definitions.

Prior studies also were unable to determine if the influence of the 5-HTTLPR variants on personality is similar in men and women. The possibility of gender differences in the 5-HTTLPR personality association is raised by evidence of differential modulation of serotonergic neurotransmission in male and female animals [Fischette et al., 1983, 1984; Zhang et al., 1997] and also in women and men [McBride et al., 1990; Nishizawa et al., 1997]. Furthermore, recent findings that estradiol markedly affects expression of the brain 5-HTT in female rats [McQueen et al., 1997], and results from our group that transgenic mice lacking the 5-HTT show gender-related differences in anxiety-like behaviors [Wichems et al., 1998], also suggest that gender might influence this association. Any gender-related differences would be important for research on the possible influences of the 5-HTTLPR on depression- and anxiety-related neuropsychiatric disorders, which are generally more prevalent in women than men [Regier et al., 1993].

We therefore recruited a new population ( $n = 397$ ) that was 84% female, again primarily sibships, to investigate the influence of the 5-HTTLPR on personality. We hypothesized that the 5-HTTLPR would again be associated with differences in the traits of Neuroticism, Agreeableness, Anxiety, and estimated Harm Avoidance. In view of the evidence above, we also hypothesized that the magnitudes of some of the 5-HTTLPR personality trait associations might differ in men and women. The results supported the first hypothesis but not the second; the association between the 5-HTT promoter region sequence variation and personality traits was replicated, with generally comparable 5-HTTLPR-related personality differences in women and men. The 5-HTTLPR trait associations were most strongly replicated for the traits defined by the NEO, based on the five-factor model of personality, supporting the primary use of this model in research on genetic influences on quantitative behavioral traits.

## SUBJECTS AND METHODS

### Subjects

The new study sample included 397 individuals who were recruited by advertisements for same-sex sibling pairs from the general population for two ongoing National Institutes of Health (NIH)-Institute Review Board (IRB)-approved protocols [Lesch et al., 1996; Sabol et al., 1999]. This was intended from the outset as a study of a general population sample; the only inclusion criteria were that the subjects were over age 18 and gave written informed consent for this National Institute of Mental Health (NIMH)- and National Cancer Institute (NCI)-IRB-approved study. No attempt to screen potential participants for psychopathology was made. Because the personality data and DNA sampling were almost always completed in one in-person session, the attrition rate was extremely low, approximately 2–3% of those enrolled. There were 84% women and 16% males, and the average age was  $28.6 \pm 12.4$  years (range 18–76 years). Most subjects were female siblings (of 394 total siblings from 215 independent families). The ethnic composition of the new sample is shown in Table I. The original sample of 505 individu-

TABLE I. Genotype Frequencies Stratified by Ethnic Group

		l/l	l/s	s/s	Total
New sample					
White/Non-Hispanic	n	88	152	42	282
	%	31.2	53.9	14.9	71.0
Asian/Pacific Islander	n	4	16	9	29
	%	13.8	55.2	31.0	7.3
Hispanic/Latino	n	5	11	8	24
	%	20.8	45.8	33.3	6.0
African Amer/Black	n	14	18	3	35
	%	40.0	51.4	8.6	8.8
Other	n	3	20	4	27
	%	11.1	74.1	14.8	6.8
Total	n	113	217	66	397
	%	28.7	54.7	16.6	100
Combined samples					
White/Non-Hispanic	n	236	372	113	721
	%	32.7	51.6	15.7	79.9
Asian/Pacific Islander	n	8	21	22	51
	%	15.7	41.2	43.1	5.7
Hispanic/Latino	n	10	22	16	48
	%	20.8	45.8	33.3	5.3
African Amer/Black	n	19	22	5	46
	%	41.3	47.8	10.9	5.1
Other	n	4	27	5	36
	%	11.1	75.0	13.9	4.0
Total	n	277	464	161	902
	%	30.7	51.4	17.8	100

als, who were recruited for studies on personality and sexual orientation, consisted of 92.5% males and 7.5% females; average age was  $31.3 \pm 11.1$  years (range 18–72 years) [Lesch et al., 1996]. That sample included 459 siblings from 210 families. The combined sample consisted of 902 individuals (88% of whom were sibling pairs) with 59% males and 41% females, and an average age of  $30.1 \pm 11.7$  (range 18–76) years; the ethnic composition is shown in Table I.

### Personality Assessment

The NEO, Form S [NEO; Costa and McCrae, 1992], was the primary psychometric instrument, as in our previous study [Lesch et al., 1996]. The advantages of the NEO, an implementation of the five-factor model of personality based on trait descriptions in natural language, include high retest reliability, longitudinal stability, and a robust factor structure validated in a variety of populations and cultures [McCrae and Costa, 1990]. In addition, in our first study [Lesch et al., 1996] NEO Neuroticism showed a much more significant association to 5-HTTLPR genotype ( $P = 0.002$ ) than either of the related trait measures, Anxiety on the 16PF or estimated Harm Avoidance on the TPQ ( $P = 0.023$  for each of these additional measures). The NEO postulates five major personality domains, each consisting of six related facets, allowing investigators to focus on aspects of personality within broad domains that might be more strongly influenced by particular genetic variants.

Also as in our prior study, Cattell's self-report 16PF inventory [16PF, 4th edition; Cattell, 1989] was used as a secondary, independent instrument. The 16PF uses a factor-analytically derived model in which five second-order factors are derived from 16 core traits. In addition, we estimated scores for the temperament fac-

tors of a third inventory, the TPQ [Cloninger et al., 1991], from the NEO data using weighted regression equations [Lesch et al., 1996].

Average ( $\pm$ SD) personality scores were as follows. In the new sample: NEO-Neuroticism,  $58.88 \pm 11.61$ ; NEO-Agreeableness,  $47.49 \pm 11.53$ ; 16PF-Anxiety,  $6.26 \pm 1.74$ ; TPQ estimated Harm Avoidance,  $51.66 \pm 9.77$ . In the combined samples: NEO Neuroticism,  $56.88 \pm 11.83$ ; NEO Agreeableness,  $47.07 \pm 11.42$ ; 16PF Anxiety,  $6.36 \pm 1.75$ ; TPQ Estimated Harm Avoidance,  $50.00 \pm 9.98$ .

**Genotyping**

Genomic DNA was isolated from whole blood using Puregene DNA Isolation Kits (Gentra Systems, Inc, Minneapolis, MN). Polymerase chain reaction (PCR)-based genotyping for the 5-HTTLPR was performed as previously described [Lesch et al., 1996] with minor modifications [Greenberg et al., 1999]. The 5-HTT regulatory gene region was amplified using oligonucleotide primers 5'-GGCGTTGCCGCTCTGAATGTC (corresponding to 5-HTTLPR nucleotide positions from -1,416 to -1,397) and 5'-GAGGGACTGAGCTG-GACAACCAC (from -910 to -889 of the 5-HTTLPR) [Heils et al., 1996]. PCR was performed in a 25- $\mu$ L volume containing approximately 50 ng of genomic template; 0.35 mM of each primer; 150 mM of each dATP, dCTP, and dTTP; 75 mM each of dGTP and 7'-deaza-dGTP; 1.6 units of Taq polymerase (AmpliTaq; Perkin-Elmer); 1.5 mM  $MgCl_2$ ; 10 mM Tris-HCL (pH 8.30); 50 mmol/L KCl; and 0.001% gelatin. The Taq polymerase was bound to TaqStart antibody (Clontech, Palo Alto, Calif.) in a molar ratio of 1:28 (Taq polymerase: antibody) before it was added to the reaction mixture to produce a "hot start" PCR. Samples were processed in a Perkin Elmer GeneAmp PCR system 9600 by two touchdown cycles (95°C for 30 sec, 63°C for 30 sec, 72°C for 1 min, 95°C for 30 sec, 62°C for 30 sec, and 72°C for 1 min), followed by 43 cycles with denaturation at 95°C for 30 sec, annealing at 61°C for 30 sec, and extension at 72°C for 1 min. The reaction was ended by incubation at 72°C for 5 min.

The PCR products were resolved in a 2% agarose gel containing 0.5  $\mu$ g/mL ethidium bromide in TAE buffer (40 mmol/L Tris-Acetate, 1 mmol/L EDTA, pH 8.0). The gel underwent electrophoresis at 120 volts for approximately 40 min. Each gel contained one lane of a 50-bp ladder to identify the 484/528-bp fragment of the 5-HTTLPR. Bands were visualized by ultraviolet illumination.

Table I shows the 5-HTTLPR genotype frequencies in the new and combined samples stratified by ethnic

group. The genotype frequencies in the new and previous samples were not significantly different ( $\chi^2 = 2.94$ ,  $P = 0.23$ ) [Lesch et al., 1996]. As expected [Gelernter et al., 1999], there were significant differences in genotype frequencies in the different ethnic groups in both the new sample ( $\chi^2 = 19.7$ ,  $P = 0.01$ ) and the combined samples ( $\chi^2 = 45.9$ ,  $P < 0.000001$ ).

**Statistical Analyses**

Statistical analyses were performed using SPSS and SAS software for Windows®. The NEO questionnaire results are given as T scores, which are standardized to have a mean  $\pm$ SD of  $50 \pm 10$  in the normative population. The 16PF data are reported in Sten score units, which are standardized to have a mean  $\pm$ SD of  $5.5 \pm 1$ . Estimated TPQ scores were calculated from the NEO data by weighted regression equations and normalized to T scores [Lesch et al., 1996].

The current and previous data showed no significant differences in personality test scores for individuals with l/s and s/s genotypes but did show differences for the l/l genotype. Therefore, in accord with our previous study [Lesch et al., 1996] and the multitissue data reviewed in the Introduction, the analyses presented in the article were performed by dichotomizing the genotypes into two groups: group S for s/s combined with l/s genotypes, and group L for l/l genotypes. Population associations were initially analyzed by one-way analysis of variance (ANOVA) comparing the S and L genotype groups; these significance levels are designated as P. Because there was some cross correlation of the personality test scores and genotype with demographic variables (see below), the significance of the associations was also tested after inclusion of sex, age, and ethnic group as covariates in the analysis of variance (ANCOVA); these significance levels are referred to as P\*. The Pearson correlations of genotype (frequency of s alleles) with demographic variables were  $r = .04$  ( $P = .39$ ) for sex in the new sample,  $r = .03$  ( $P = .39$ ) for sex in the combined samples,  $r = -.11$  ( $P = .02$ ) for age in the new sample, and  $r = -.07$  ( $P = .02$ ) for age in the combined samples. The association of genotype with ethnic group is shown in Table I. The Pearson correlations of personality trait scores with sex and age in the new and combined samples are shown in Table II. Personality trait scores were not significantly associated with ethnic group in either the new sample or combined samples. Significance levels for population associations are reported as two-tailed probabilities without correction for multiple testing since there was a strong prior hypothesis.

TABLE II. Correlations of Personality Traits With Sex and Age

	New sample (n = 397)		Combined sample (n = 902)	
	Sex	Age	Sex	Age
NEO-neuroticism	.1058*	-.2052**	.1660**	-.1591**
NEO-agreeableness	.1950**	.2591**	.1330*	.2637**
16PF-anxiety	-.0542	.0022	-.0561	-.0003
TPQ estimated-HA	.0940	-.0592	.1420**	-.0191

\* $P < 0.05$ .  
\*\* $P < 0.01$ .

Possible effects of gender on genotype-phenotype associations were tested by (i) including sex as the independent variable in a Fisher Z test and (ii) including sex as a between-subjects factor in ANOVA and ANCOVA to determine both main effects and potential sex  $\times$  genotype interactions. Possible confounding effects of ethnic group on genotype-phenotype associations were tested by comparing the results obtained for the white non-Hispanic subjects (the largest subgroup,  $n = 721$ ) with the results for the entire combined sample. For simplicity of presentation and comparison, these results are presented in Table III as Pearson correlations in which genotype was dichotomized as 1 for the L group and 2 for the S group; significance levels for a Pearson correlation and ANOVA are the same under these conditions.

Within-pedigrees analysis used those sibling pairs that were discordant for 5-HTTLPR group L (l/l genotype) versus S (l/s or s/s genotype) to distinguish population stratification from genetic transmission. The sibling differences in scores for NEO Neuroticism and Agreeableness, 16 PF Anxiety, and estimated TPQ Harm Avoidance were determined by paired-sample t-tests using a conservative correction for the nonindependence of pairs from the same family [t\*; Lesch et al., 1996]. Because the directions of the differences were predicted in advance from the population results, one-sided tests of significance were employed.

The power of the new sample to replicate the previously observed population associations at a two-sided significance level of 0.05 was determined using the standard equation for the power of an F test. The power of the within-family analysis of the complete sample of

5-HTTLPR-discordant sib-pairs to detect the effect sizes found in the total population at a one-sided significance level of 0.05 was determined by the corresponding equation for a paired-sample t-test [Cohen, 1988]. The significance (Pz) of differences between population association effect sizes in the new and previous samples were determined by using population as the independent variable in a Fisher Z test. The significance (Pt) of differences between population and sib-pair effect sizes was determined by a two-sample t-test [Zar, 1996]. (Complete tables of the S-L differences for the 16PF primary factors and estimated TPQ factors for the new sample are available from the authors by request.)

## RESULTS

### Replication of 5-HTTLPR s Allele Associations to Higher Neuroticism and Lower Agreeableness on the NEO

We previously found that individuals carrying one or two short 5-HTTLPR alleles (group S) had significantly higher scores for NEO Neuroticism and lower scores for Agreeableness than did individuals carrying only long alleles (group L) in a large, predominantly male cohort [Lesch et al., 1996]. The effect sizes (in standard deviation units, D) were  $D = 0.29$  for Neuroticism and  $D = -0.19$  for Agreeableness. Power to detect effects of this size in the new sample of 397 mainly female subjects was 75% for Neuroticism and 40% for Agreeableness.

Table IV (top) shows that the association of the 5-HTTLPR s allele with increased Neuroticism was robustly replicated in the new sample ( $P = 0.006$ ). As in the previous study, the short allele appeared to be dominant; the scores for l/s and s/s genotypes did not differ significantly but were higher than for l/l genotype individuals. The effect size ( $D = 0.31$ ) in the new sample was not significantly different from the previous one of 0.29 ( $Z = 0.1$ ,  $Pz = 0.9$ ). The association between the 5-HTTLPR and Neuroticism remained significant ( $F = 3.9$ ,  $P^* < 0.05$ ) after NEO scores were corrected for age, sex, and ethnic group by multiple regression.

The association of 5-HTTLPR s genotypes with decreased Agreeableness found initially [Lesch et al., 1996] was also clearly replicated in the new sample ( $P = 0.0008$ ; Table IV, top). And, as previously observed, the short allele was dominant to the long allele in the new sample. The effect size of  $D = -0.38$  in the new sample was greater but not significantly different from that in the prior study ( $D = -0.19$ ;  $Z = 1.3$ ,  $Pz = 0.2$ ). The negative association between the 5-HTTLPR and Agreeableness remained significant ( $F = 9.1$ ,  $P^* = 0.003$ ) after correcting for age, sex, and ethnic group.

As in the previous study, the 5-HTTLPR was not significantly associated with the other NEO domains of Extraversion or Openness (Table IV, top). There was, however, a negative association between S genotypes and Conscientiousness ( $P = 0.01$ ) in the new sample, and this remained significant after correcting for sex, age, and ethnic group ( $F = 4.6$ ,  $P^* = 0.03$ ). Although association to Conscientiousness was not found in the previous study, there was a trend in that direction, and the effect size of  $D = -0.09$  found there was not sig-

TABLE III. Correlations of the 5-HTTLPR With Personality Traits in Total Combined Sample Compared With White Non-Hispanic Subjects

Pearson correlation	Total sample (N = 902)	White Non-Hispanic (N = 721)
NEO Traits		
N	.1403**	.1525**
E	-.0451	-.0729
O	.0133	-.0022
A	-.1221**	-.1185**
C	-.0809*	-.0962*
NEO Neuroticism Facets		
N1	.0737*	.0840*
N2	.1458**	.1385**
N3	.1437**	.1490**
N4	.0605	.0787*
N5	.0994**	.1112**
N6	.0749*	.0908*
16 PF Personality Factors		
Extraversion	-.0198	-.0340
Anxiety	.1044**	.1134**
Tough-mindedness	.0177	-.0080
Independence	.0687*	.0622
Self-Control	-.0507	-.0650
Estimated TPQ Factors		
Novelty seeking	.0403	.0316
Harm avoidance	.0733*	.0900*
Reward dependence	-.0241	-.0239
Persistence	-.0251	-.0270

\* $P < 0.05$ .

\*\* $P < 0.01$ .

TABLE IV. Population Association Between the 5-HTTLPR and NEO Personality Traits\*

Genotype	NEO T-scores by genotype				F	S-L	P	P*
	l/l	l/s	s/s	l/s & s/s				
	(Group L)			(Group S)				
New data								
Number per group	114	217	66	283				
Neuroticism								
Mean	56.29	60.17	59.02	59.90	7.66	3.61	0.0059	0.04
AD	11.65	11.21	12.25	11.45				9
Extraversion								
Mean	56.05	55.25	54.08	54.97	0.72	ns	0.396	0.12
SD	10.62	11.24	12.26	11.48				0
Openness								
Mean	55.17	57.87	57.26	57.73	3.64	ns	0.057	0.19
SD	11.43	11.57	11.46	11.52				1
Agreeableness								
Mean	50.64	46.14	46.57	46.23	11.39	-4.41	0.0008	0.00
SD	10.06	11.52	13.09	11.86				3
Conscientiousness								
Mean	45.41	42.42	40.46	42.01	6.05	-3.40	0.0144	0.03
SD	11.93	12.21	11.65	12.10				3
Combined data								
Number per group	277	464	161	625				
Neuroticism								
Mean	54.38	58.18	57.38	57.98	17.78	3.60	0.0000	0.000
SD	11.65	11.78	11.67	11.75				8
Extraversion								
Mean	54.97	54.22	52.94	53.89	1.80	ns	0.180	0.054
SD	10.64	11.25	12.26	11.28				
Openness								
Mean	57.66	58.10	57.65	57.99	0.15	ns	0.696	0.860
SD	11.67	11.39	11.47	11.40				
Agreeableness								
Mean	49.16	46.15	46.13	46.14	13.19	-3.02	0.0003	0.000
SD	10.53	11.40	12.53	11.69				7
Conscientiousness								
Mean	45.56	43.76	42.56	43.46	5.61	-2.10	0.0181	0.077
SD	11.31	12.49	11.68	12.29				

\*Mean scores and standard deviations for the five major traits measured by the NEO Personality Inventory, Revised, in the current sample ( $n = 397$ ) and the combined population ( $n = 902$ ) stratified by genotype and genotype group. F = one-way ANOVA statistic; S-L = mean score for S genotypes (s/s and l/s) minus mean score for L genotypes (l/l); P = two-tailed significance; P\* = two-tailed significance level after including sex, age, and ethnic group as covariates in ANCOVA.

nificantly different from the effect size of  $D = -0.28$  found in the new sample ( $Z = 1.3$ ,  $P_z = 0.2$ ).

Table IV (bottom) shows the association results for the combined old and new samples, totaling 902 subjects (59% male). There were significant associations of 5-HTTLPR S genotypes with higher Neuroticism ( $D = 0.31$ ), lower Agreeableness ( $D = -0.38$ ), and lower Conscientiousness ( $D = -0.28$ ). After correcting for sex, age, and ethnic group, the associations with Neuroticism ( $F = 11.8$ ,  $P^* = 0.0008$ ) and Agreeableness ( $F = 11.9$ ,  $P^* = 0.0007$ ) remained significant, whereas the association with Conscientiousness did not ( $F = 3.1$ ,  $P^* = 0.08$ ). Since the five NEO personality domains are not strictly orthogonal but are appreciably [Costa and McCrae, 1993], the significance of the 5-HTTLPR associations to each domain was tested after controlling for scores on the other four domains by partial correlation. This analysis showed that the associations with Neuroticism ( $r = 0.09$ ,  $P = 0.01$ ) and Agreeableness ( $r = -0.1$ ,  $P = 0.006$ ) remained significant, while the association with Conscientiousness became nonsignificant ( $r = -0.02$ ,  $P = 0.5$ ). The weak association of genotype with Conscientiousness thus appears to be pri-

marily due to contributions from other personality traits. Taken together, these results show that there is a reproducible association of 5-HTTLPR genotype with both Neuroticism and Agreeableness, and that these associations are not confounded by demographic variables or by correlations between personality domains.

#### 5-HTTLPR s Allele Associations to NEO Neuroticism and Agreeableness Subscales

Because the 5-HTTLPR was reproducibly associated with Neuroticism and Agreeableness, the next analysis focused on the subscales ("facets") comprising these two domains. In the previous study, 5-HTTLPR s genotypes were positively associated with the Neuroticism facets of Anxiety ( $D = 0.21$ ), Angry Hostility ( $D = 0.30$ ), Depression ( $D = 0.25$ ), and Impulsiveness ( $D = 0.25$ ), and negatively associated with the Agreeableness facets of Straightforwardness ( $D = -0.19$ ) and Compliance ( $D = -0.21$ ). The current sample had between 39 and 78% power to detect effects of this magnitude.

Table V (top) shows that the associations with the

Neuroticism facets of Angry Hostility and Depression were replicated with significance levels of  $P = 0.004$  and  $P = 0.001$ , respectively. The effect sizes were indistinguishable from those observed in the previous sample (for Angry Hostility,  $D = 0.32$ ,  $Z = 0.1$ ,  $Pz = 0.9$ ; for Depression,  $D = 0.38$ ,  $Z = 0.8$ ,  $Pz = 0.4$ ), and both associations remained significant after correcting for sex, age, and ethnic group (for Angry Hostility,  $F = 4.3$ ,  $P^* = 0.04$ ; for Depression,  $F = 8.2$ ,  $P^* = 0.004$ ). Although the previously observed associations for Anxiety and Impulsiveness were not replicated at the two-sided  $P < 0.05$  level of significance in the present study, there was a trend in the expected direction for both facets, and the effect sizes did not vary significantly across studies (for Anxiety,  $D = 0.07$ ,  $Z = 1.0$ ,  $Pz = 0.3$ ; for Impulsiveness,  $D = 0.17$ ,  $Z = 0.6$ ,  $Pz = 0.6$ ).

The negative associations with both Agreeableness

facets were also replicated, with significance levels of  $P = 0.001$  for Straightforwardness and  $P = 0.007$  for Compliance (data not shown). The effect sizes were not significantly different from those observed previously (for Straightforwardness,  $D = -0.36$ ,  $Z = 1.1$ ,  $Pz = 0.3$ ; for Compliance,  $D = -0.30$ ,  $Z = 0.6$ ,  $Pz = 0.6$ ), and both associations remained significant after correcting for sex, age, and ethnic group (for Straightforwardness,  $F = 8.0$ ,  $P^* = 0.005$ ; for Compliance,  $F = 4.2$ ,  $P^* = 0.04$ ). The new sample also showed an association with Trust that was significant both for the raw data ( $D =$ ,  $P = 0.0001$ ) and after correcting for sex, age, and ethnic group ( $F = 11.2$ ,  $P^* = 0.0009$ ). Although trust was not significantly associated with the 5-HTTLPR in the previous study, there was a trend in the same direction ( $D = -0.16$ ), and the results across study groups were not significantly different ( $Z = 1.7$ ,  $Pz = 0.08$ ).

The associations to the NEO Neuroticism facets for

TABLE V. Association of 5-HTTLPR and NEO Neuroticism Facets\*

Genotype	NEO T-scores by genotype				F	S-L	P	P*
	l/l (Group L)	l/s	s/s	l/s & s/s (Group S)				
New data								
Number per group	114	217	66	283				
N1-Anxiety								
Mean	56.77	58.00	56.06	57.55	0.35	ns	0.552	0.787
SD	12.11	11.66	11.10	11.54				
N2-Angry hostility								
Mean	53.36	57.55	55.90	57.18	8.59	3.82	0.0036	0.038
SD	11.19	12.03	11.23	11.85				
N3-Depression								
Mean	54.11	58.52	57.65	58.32	11.74	4.21	0.0007	0.004
SD	10.87	10.95	11.61	11.10				
N4-Self consciousness								
Mean	52.95	54.69	54.74	54.70	1.89	ns	0.170	0.380
SD	11.17	11.68	11.15	11.54				
N5-Impulsiveness								
Mean	55.27	57.85	54.87	57.17	2.26	ns	0.133	0.266
SD	11.09	11.20	11.71	11.37				
N6-Vulnerability								
Mean	55.12	57.89	56.50	57.56	3.36	ns	0.068	0.283
SD	11.56	11.60	13.38	12.04				
Combined data								
Number per group	277	464	161	625				
N1-Anxiety								
Mean	54.22	56.54	54.84	56.10	4.88	1.88	0.0275	0.137
SD	11.98	11.59	11.70	11.63				
N2-Angry hostility								
Mean	51.66	55.50	54.87	55.34	19.39	3.68	0.0000	0.0004
SD	11.04	11.89	11.53	11.79			1	8
N3-Depression								
Mean	53.08	56.83	56.16	56.66	18.86	3.58	0.0000	0.0002
SD	10.98	11.58	11.54	11.56			1	1
N4-Self consciousness								
Mean	52.98	54.40	54.76	54.50	3.29	ns	0.070	0.127
SD	11.12	11.84	11.42	11.73				
N5-Impulsiveness								
Mean	54.60	57.22	56.36	57.00	8.88	ns	0.003	0.009
SD	11.07	11.04	11.33	11.11				
N6-Vulnerability								
Mean	53.00	55.32	53.73	54.90	5.04	1.9	0.0250	0.178
SD	11.30	11.87	11.87	11.89				

\*Mean scores and standard deviations for the six Neuroticism facets measured by the NEO Personality Index, Revised, in the current sample ( $n = 397$ ) and the combined population ( $n = 902$ ) stratified by genotype and genotype group. F = one-way ANOVA statistic; S-L = mean score for S genotypes (s/s/ and l/s) minus mean score for L genotypes (l/l); P = two-tailed significance; P\* = two-tailed significance level after including sex, age, and ethnic group as covariates in ANCOVA.

the combined populations are shown in Table V (bottom). The S genotype group showed increased scores for Anxiety ( $D = 0.16, P = 0.03$ ), Angry Hostility ( $D = 0.32, P = 0.00001$ ), Depression ( $D = 0.31, P = 0.00001$ ), Impulsiveness ( $D = 0.22, P = 0.003$ ), and Vulnerability ( $D = 0.16, P = 0.03$ ). After correcting for sex, age, and ethnic group, the associations for Angry Hostility ( $F = 13.3, P^* = 0.0004$ ), Depression ( $F = 13.9, P^* = 0.0002$ ), and Impulsiveness ( $F = 6.9, P^* = 0.009$ ) remained significant, whereas those for Anxiety ( $F = 2.2, P^* = 0.14$ ) and Vulnerability ( $F = 1.8, P^* = 0.18$ ) did not. For NEO Agreeableness (data not shown), the S genotype group had decreased scores for three facets, all of which remained significant after correction for sex, age, and ethnic group: Trust ( $D = -0.27, P = 0.001, P^* = 0.002$ ), Straightforwardness ( $D = -0.25, P = 0.001, P^* = 0.0005$ ), and Compliance ( $D = -0.26, P = 0.0004, P^* = 0.005$ ). In summary, four of the six NEO facet associations observed in the original sample were replicated in the new sample, and there were no significant differences between the two populations for any of the facets analyzed.

**Replication of Association to 16PF Anxiety**

We previously found that 5-HTTLPR group S genotypes were associated with increased scores on Anxiety ( $D = 0.21$ ), the closest 16PF analog of NEO Neuroticism, including its constituent traits of Suspiciousness ( $D = 0.28$ ) and Tension ( $D = 0.33$ ). The new sample had 54 to 87% power to detect effects of this size.

Table VI shows that the association to 16PF Anxiety was replicated in the new sample ( $P = 0.02$ ). This association remained significant after correcting for sex, age, and ethnic group ( $F = 4.6, P^* = 0.03$ ), and the effect size of  $D = 0.26$  did not differ significantly from that observed in the previous sample ( $Z = 0.26, Pz = 0.8$ ). In the combined samples, 5-HTTLPR S genotypes were associated with increased Anxiety at a significance level of  $P = 0.002$  for the raw data and  $P^* = 0.002$  ( $F = 9.5$ ) for the demographically corrected data. An additional association, with the factor of Control in the new sample ( $P = 0.02$ ), was not significant in the total sample ( $P = .14$ ). An association with the factor of Independence in the combined sample ( $P = 0.04$ ) was not significant in either of the split samples and did not

TABLE VI. Population Association Between the 5-HTTLPR and 16PF Personality Factors\*

Genotype	16PF Sten scores by genotype				F	S-L	P	P*
	l/l (Group L)	l/s	s/s	l/s & s/s (Group S)				
New data								
Number per group	106	210	66	276				
Extraversion								
Mean	5.81	5.47	5.38	5.45	2.06	ns	0.152	0.370
SD	2.01	2.27	2.28	2.27				
Anxiety								
Mean	5.93	6.40	6.34	6.39	5.29	0.46	0.022	0.033
SD	1.78	1.69	1.81	1.71				
Tough-mindedness								
Mean	5.83	5.68	5.56	5.65	0.73	ns	0.394	0.330
SD	1.76	1.85	1.71	1.81				
Independence								
Mean	6.47	6.82	6.76	6.81	1.61	ns	0.205	0.584
SD	2.12	2.41	2.43	2.41				
Control								
Mean	5.14	4.72	4.25	4.61	5.39	-0.53	0.021	0.032
SD	2.01	1.98	1.93	1.97				
Combined data								
Number per group	269	456	160	616				
Extraversion								
Mean	5.34	5.32	5.03	5.25	0.34	ns	0.557	0.202
SD	2.06	2.31	2.25	2.29				
Anxiety								
Mean	6.08	6.50	6.41	6.48	9.73	0.40	0.002	0.002
SD	1.76	1.73	1.76	1.73				
Tough-mindedness								
Mean	5.13	5.15	5.38	5.21	0.28	ns	0.599	0.399
SD	1.91	1.98	1.92	1.96				
Independence								
Mean	6.52	6.90	6.73	6.85	4.19	0.33	0.041	0.090
SD	2.12	2.24	2.43	2.29				
Self control								
Mean	4.76	4.60	4.41	4.55	2.28	ns	0.137	0.177
SD	1.86	1.93	1.92	1.93				

\*Mean scores and standard deviations for the five second-order personality factors measured by the 16PF in the current sample (n = 397) and the combined population (n = 902) stratified by genotype and genotype group. F = one-way ANOVA statistic; S-L = mean score for S genotypes (s/s/ and l/s) minus mean score for L genotypes (l/l); P = two-tailed significance; P\* = two-tailed significance level after including sex, age, and ethnic group as covariates in ANCOVA.



survive correction for demographic variables ( $F = 2.9$ ,  $P^* = 0.09$ ).

Analysis of the constituent traits of 16PF Anxiety showed that the prior association with Suspiciousness was replicated ( $P = 0.02$ ), although, while indistinguishable from that in the prior sample ( $D = 0.26$ ,  $Z = 0.2$ ,  $P_z = 0.8$ ), it became nonsignificant after demographic correction ( $F = 0.94$ ,  $P^* = 0.3$ ). The previously observed association to the Anxiety constituent trait of Tension was neither replicated nor repudiated in the new sample ( $D = 0.07$ ,  $P = 0.5$ ,  $Z = 1.8$ ,  $P_z = 0.08$ ). However, the new sample did show a negative association with a different core constituent of Anxiety, C (calm and stable versus easily upset), which was significant both for the raw ( $D = 0.26$ ,  $P = 0.02$ ) and corrected ( $F = 5.4$ ,  $P^* = 0.02$ ) data and was not significantly different from the previous study ( $Z = 1.7$ ,  $P_z = 0.08$ ). In the combined sample, 5-HTTLPR S genotypes were significantly associated with lowered levels of C ( $P = 0.04$ ,  $P^* = 0.04$ ), increased Suspiciousness ( $P = 0.0001$ ,  $P^* = 0.003$ ), and increased Tension ( $P = 0.004$ ,  $P^* = 0.001$ ). Thus, the association between the 5-HTTLPR and the second-order factor of Anxiety was reproducible, but there were some differences across studies in its constituent core traits.

#### Estimated TPQ Scores

The third method of personality assessment was based on Cloninger's TPQ, which postulates four broad

domains of heritable personality or temperament. As previously, TPQ scores were not actually measured, but rather were estimated from the NEO data by using weighted regression equations [Lesch et al., 1996]. In the previous study, we found that 5-HTTLPR S genotypes were associated with increased scores for estimated Harm Avoidance with an effect size of  $D = 0.19$ . Although the new sample had 47% power to detect such an effect, no association between the 5-HTTLPR and Harm Avoidance was found ( $D = 0.06$ ,  $P = 0.6$ ,  $Z = 0.9$ ,  $P_z = 0.4$ ; Table VII). There was an association between the 5-HTTLPR and Novelty Seeking in the new sample ( $D = 0.25$ ,  $P = 0.04$ ,  $Z = 1.7$ ,  $P_z = 0.1$ ), but this became nonsignificant after correcting for sex, age, and ethnic group ( $F = 2.4$ ,  $P^* = 0.12$ ). The combined sample showed association only to Harm Avoidance ( $D = 0.15$ ,  $P = 0.04$ ); this association was not significant ( $F = 4.4$ ,  $P^* = 0.07$ ) after correcting for the demographic variables.

#### Within Family Association

The current sample, like the previous one, consisted largely of sib-pairs, thereby allowing family-based association analysis using the discordant genotype sib-pair method [Lesch et al., 1996]. The new study population contained just 43 sib-pairs that were discordant for the L and S genotypes of the 5-HTTLPR, which gave <20% power to detect associations equivalent to those

TABLE VII. Population Association Between the 5-HTTLPR and Estimated Tridimensional Personality Questionnaire (TPQ) Factors\*

Genotype	Estimated TPQ T-scores by genotype				F	S-L	P	P*
	l/l (Group L)	l/s	s/s	l/s & s/s (Group S)				
New data								
Number per group	90	178	53	231				
Novelty seeking								
Mean	48.23	50.54	51.46	50.75	4.19	2.52	0.042	0.118
SD	9.88	10.08	9.51	9.94				
Harm avoidance								
Mean	51.23	52.01	51.23	51.83	0.25	ns	0.617	0.800
SD	10.21	9.62	8.94	9.45				
Reward dependence								
Mean	52.17	51.57	49.49	51.10	0.69	ns	0.407	0.179
SD	9.89	10.33	11.68	10.66				
Persistence								
Mean	52.62	51.44	48.55	50.77	2.18	ns	0.141	0.056
SD	9.55	10.16	10.58	10.30				
Combined data								
Number per group	251	421	146	567				
Novelty seeking								
Mean	49.40	50.10	50.75	50.27	1.33	ns	0.250	0.549
SD	9.09	10.38	10.37	10.37				
Harm avoidance								
Mean	48.90	50.66	50.00	50.49	4.40	1.59	0.036	0.073
SD	10.01	10.08	9.64	9.97				
Reward dependence								
Mean	50.35	50.24	48.68	49.48	0.47	ns	0.492	0.264
SD	9.49	10.13	10.44	10.22				
Persistence								
Mean	50.38	50.34	48.37	49.83	0.51	ns	0.474	0.348
SD	9.42	10.18	10.33	10.25				

\*Estimated mean scores and standard deviations for the four temperament factors of the TPQ in the current sample ( $n = 397$ ) and the combined population ( $n = 902$ ) stratified by genotype and genotype group. F = one-way ANOVA statistic; S-L = mean score for S genotypes (s/s/ and l/s) minus mean score for L genotypes (l/l); P = two-tailed significance; P\* = two-tailed significance level after including sex, age, and ethnic group as covariates in ANCOVA.

observed in the population at large. The combined new and previous populations contained a total of 125 5-HTTLPR discordant sib-pairs from 105 families, giving a power of 32 to 70% to detect such associations. The analysis focused on the broadly defined personality domains rather than on individual subscales, and the results were conservatively corrected for the nonindependence of sib-pairs from the same family [Lesch et al., 1996].

Table VIII (top) shows that, as anticipated from the small number of genotype-discordant pairs, the results for the new sample were not significant. The results for the combined samples were more informative (Table VIII bottom). For NEO Neuroticism, probands with group S genotypes scored significantly higher than their siblings, with group L genotypes at a one-sided significance level of  $P = 0.012$ . As in the previous study, the effect size within sibships was at least as large as in the total population (one-sided  $t = -0.1$ ,  $Pt = 0.5$ ). For NEO Agreeableness, the association within sibships was not significant ( $P = 0.11$ ); however, there was a trend in the expected direction, and hence the result in sibships was not significantly weaker than the result in the population at large ( $t = 1.0$ ,  $Pt = 0.16$ ). For 16PF Anxiety, the association to the 5-HTTLPR was significant in sibships ( $P < 0.05$ ) and not significantly different from that in the total population ( $t = -0.1$ ,  $Pt = 0.5$ ). The association between 5-HTTLPR genotype and estimated TPQ Harm Avoidance was also significant in sibships ( $P = 0.02$ ) and as strong as in the population at large ( $t = -0.9$ ,  $Pt = 0.8$ ). These results show no significant evidence for population stratification for any of the personality domains ana-

lyzed and, in the case of NEO Neuroticism and 16PF Anxiety, significant evidence in favor of genuine transmission.

**Gender and Demography**

As described above, the present findings in a primarily female population replicated the significant associations between 5-HTTLPR genotype and NEO Neuroticism and Agreeableness and 16PF Anxiety previously found in a primarily male sample. Although the association with estimated TPQ Harm Avoidance previously found was not significant here, it was in the same direction. This pattern of findings suggests that gender differences do not play a major role in the 5-HTTLPR personality association. To more fully assess this possibility, we tested for gender-related differences using the combined sample from the present and the prior study, consisting of 532 men and 370 women. As expected from many studies, gender by itself had significant main effects on several of the traits studied here (Table II); women were, on average, more neurotic, more agreeable, and more harm avoidant than men. However, Fisher Z and two-way ANOVA tests showed that there were no significant effects of gender on any of the genotype-trait associations found in the population at large; that is, the genotype  $\times$  gender interaction term was insignificant in all analyses. Thus, while the associations themselves were replicated, the data provided no evidence of major qualitative differences in the influence of the 5-HTTLPR polymorphism on personality in women compared with men.

Possible effects of ethnic group were examined in

TABLE VIII. Within-Family Association Between the 5-HTTLPR and Personality Traits\*

	Standardized scores in genotype-discordant sib-pairs				
	L sibling	S sibling	S-L	T*	P
New data					
NEO Neuroticism					
Mean	56.04	58.60			
SD	10.88	11.84	2.56	0.97	0.168
NEO Agreeableness					
Mean	51.02	47.28			
SD	9.71	10.67	-3.70	1.64	0.054
16PF Anxiety					
Mean	6.09	6.28			
SD	1.83	1.64	0.19	0.51	0.308
TPQ Estimated HA					
Mean	18.31	17.45			
SD	10.36	7.96	-0.86	0.38	0.353
Combined data					
NEO Neuroticism					
Mean	54.80	58.54			
SD	11.76	12.20	3.74	2.30	0.012
NEO Agreeableness					
Mean	49.67	48.08			
SD	9.79	10.52	-1.6	1.21	0.115
16PF Anxiety					
Mean	6.30	6.71			
SD	1.79	1.78	0.41	1.71	0.045
TPQ Estimated HA					
Mean	16.00	18.90			
SD	10.40	9.44	2.89	2.06	0.021

\*NEO = NEO Personality Inventory, Revised; TPQ = Tridimensional Personality Questionnaire; 16 PF = 16 PF Personality Inventory.

light of Gelernter et al.'s [1998] finding of a significant genotype  $\times$  ethnic group interaction for the association between the 5-HTTLPR and neuroticism and the well-known hazards of ethnic admixture. We analyzed the combined data set for possible genotype  $\times$  ethnic group interactions, but none were found for any of the traits of interest (data not shown). We also compared genotype-phenotype associations in the complete data set to those in the White non-Hispanic subjects, who comprised the largest subgroup in our sample ( $n = 721$ ). Table III shows that the associations in the White non-Hispanic group were as strong as those in the entire population for every trait of interest. This provides further evidence that the observed population-based associations are not artifacts of population stratification.

As expected from population surveys, several of the personality measures studied here were modestly correlated with age. There was also an unexpected correlation between age and genotype, highlighting the complications of using a broad age range of subjects (18–76 years) in this type of study; thus, possible age and age  $\times$  genotype interaction effects may be a useful focus for future studies.

Finally, we analyzed the effect of sample source by two-way ANOVA using membership in the old or new sample as a factor. These analyses showed that the observed genotype-phenotype associations were indistinguishable in the two samples.

## DISCUSSION

The results of the present study, in a primarily female sample, robustly replicate the earlier finding of associations in a predominantly male cohort between the 5-HTTLPR *s* allele and the broad NEO personality domains of increased Neuroticism and decreased Agreeableness [Lesch et al., 1996]. The replicated association of the 5-HTTLPR with NEO Neuroticism and related personality dimensions is in accord with the clear and consistent evidence that this polymorphism is functional. Recent studies of postmortem human brain tissue [Little et al., 1998], whole blood serotonin levels [Hanna et al., 1998], and human platelets [Greenberg et al., 1999], as well as earlier studies using cell culture models [Heils et al., 1996, 1997; Lesch et al., 1996], all show that the *s* allele is associated with reduced 5-HTT expression and/or function. As previously, and in agreement with these gene expression studies, the *s* allele was dominant to the *l* allele in its influence on personality. The effect sizes for the 5-HTTLPR personality associations, which were comparable in the two populations, indicate that this polymorphism has a modest influence on these behavioral predispositions of approximately 0.30 standard deviation units. The results are consistent with the view that the influence of a single, common polymorphism on continuously distributed traits is likely to be small in humans as well as different quantitative characteristics in other species [Plomin et al., 1994].

Further evidence for the role of the serotonin transporter in behavioral dimensions similar to those described in the present investigation comes from a pharmacological experiment. Knutson et al. [1998] reported

that long-term inhibition of the 5-HTT by the SRI antidepressant paroxetine reduced indices of hostility through a more general decrease in negative affect, a personality dimension related to neuroticism. The same individuals also demonstrated an increase in directly measured social cooperation after paroxetine treatment [Knutson et al., 1998], an interesting finding in light of the replicated association between 5-HTTLPR genotype and agreeableness. That a drug which inhibits the 5-HTT lessened negative emotionality and increased social cooperation appears to conflict with findings that the 5-HTTLPR long allele, which confers greater 5-HTT expression, is associated with lower NEO Neuroticism and higher NEO Agreeableness. However, since it is very likely that the 5-HTT plays an important role in brain development that is different from its function regulating neurotransmission in the adult, this inconsistency may be more apparent than real. For example, animal studies find that the 5-HTT gene is expressed in some brain regions critical to emotional behavior during fetal development but not later in life [Hansson et al., 1998]. This apparent role of the 5-HTT as a developmental modulator, which is likely to be influenced by the variation in the 5-HTTLPR 5' regulatory region, could result in enduring effects on behavioral predispositions.

## Reproducible 5-HTTLPR— Personality Associations

Analysis of the NEO subscales helps to define the specific aspects of personality that are reproducibly associated with 5-HTTLPR genotype. For the NEO Neuroticism subscales, the new sample demonstrated significant associations between 5-HTTLPR-*S* genotypes and the facets of increased Depression and Angry Hostility, two of the three facets that showed the most significant associations in the prior sample [Lesch et al., 1996]; by contrast, the NEO Neuroticism Anxiety facet, which was associated with genotype in the first study at a weaker significance level, was not significantly associated with 5-HTTLPR genotype in the new cohort. With regard to the NEO Agreeableness subscales, the previously observed associations of 5-HTTLPR-*S* genotypes with decreased Straightforwardness and Compliance were robustly replicated, and an additional association with low Trust also was found. It therefore appears more accurate to state that the functional 5-HTTLPR promoter polymorphism is associated with traits of negative emotionality related to interpersonal hostility and depression. The relationship between these two aspects of negative emotionality is not unexpected in view of the previously observed negative correlation between Angry Hostility, a facet of Neuroticism, and Agreeableness ( $R = -0.48$ ; [Costa and McCrae, 1992]), indicating that both measures assess a behavioral predisposition toward uncooperative interpersonal behavior. In this regard it is of interest that a new study in a large sample [ $n = 634$ ; Hamer et al., unpublished data] using the TCI [Cloninger et al., 1993], an expansion of the TPQ, found that the 5-HTTLPR *S* genotypes were most strongly, and negatively, associated with the TCI trait of cooperativeness

( $P = 0.00008$ ). These findings provide converging evidence that the 5-HTTLPR influences individual differences in social cooperation.

The Anxiety factor of the 16PF personality inventory, the closest 16PF analog of NEO Neuroticism, also showed the same association with 5-HTTLPR genotype previously observed in the mostly male population [Lesch et al., 1996]. The failure to replicate population association of the 5-HTTLPR with estimated Harm Avoidance was the exception to the overall pattern of replication of results from our prior study [Lesch et al., 1996]. This may in part be due to the fact that the regression equation used to estimate Harm Avoidance scores from the NEO data puts more weight on Extraversion and Openness, two traits that are not significantly influenced by the 5-HTTLPR, than it does on Agreeableness, which is significantly associated with 5-HTTLPR genotype (estimated Harm Avoidance =  $.74*N - .32*E - 27*O + .05*A + .11*C$ ). Moreover, a recent study using actual TCI questionnaire scores obtained from a large population found that the 5-HTTLPR was only weakly associated with Harm Avoidance (Hamer et al., unpublished data).

#### **Within Family 5-HTTLPR— Personality Associations**

Our present and prior studies used within-family designs to control for population stratification artifacts. Specifically, we employed a genotype-discordant sib-pair test that is a combined measure of linkage and association (i.e., a linkage/disequilibrium test) and, is therefore, immune to population stratification. The associations of the 5-HTTLPR with NEO Neuroticism and 16PF Anxiety remained significant in a within-family analysis of the 125 sibships discordant for genotype from both general population samples, reflecting a genuine genetic influence rather than an artifact of ethnic admixture. The results for NEO Agreeableness, however, were inconclusive. Somewhat surprisingly, the within-family results for estimated Harm Avoidance were positive, even though population association was not apparent in the new sample and was weak in the combined population. A similar discrepancy between within-family and population-based results was observed in a study of 655 subjects who were primarily Finnish alcoholic probands and their relatives. This study [Mazzanti et al., 1998] found significant sib-pair linkage between the 5-HTTLPR and two subscales of Harm Avoidance but no evidence for allelic association. Mazzanti et al. [1998] suggest that the 5-HTTLPR might be in linkage disequilibrium with a different, still unidentified polymorphism that influences serotonin transporter expression or function; however, this would not explain our observation that allelic association was observed for NEO and 16PF scores in the same sample for which estimated Harm Avoidance did not show allelic association. An alternative interpretation is that within-family analysis is more sensitive than population-based analysis for Harm Avoidance because an unusually high proportion of the variance in this measure is shared by siblings.

#### **Effects of Gender**

Several lines of evidence, noted in the Introduction, demonstrate gender-related differences in serotonin system functioning in humans and in animals. These findings include effects of gonadal steroids on 5-HTT expression in rat brain and differences in fear-related behaviors in male and female 5-HTT knockout mice. Although such evidence provides a theoretical basis for possible gender-related differences in the 5-HTTLPR personality association, we found that the 5-HTTLPR polymorphism has a qualitatively similar influence in men and women. The present results do, however, suggest a possibly stronger association between 5-HTTLPR-S genotypes and a predisposition to lower agreeableness and related traits in women. This possibility would be a useful focus for further studies.

#### **Comparison With Other Studies**

As discussed above, a large within-family study found linkage between the 5-HTTLPR and Harm Avoidance [Mazzanti et al., 1998]. The only other study to use a large sample ( $n = 759$ ) used a population-based design and found no association between the 5-HTTLPR and the Eysenck Personality Questionnaire-Revised (EPQ-R) measure of Neuroticism [Jorm et al., 1998]. Although the NEO and the Eysenck Neuroticism scales are correlated in the range of 0.75 [McCrae and Costa, 1985], differences in these measures leave a large proportion of the variance measured by one questionnaire unexplained by the other. This amount of variance, approximately 40%, which is in fact very close to estimates of the entire heritable component of neuroticism [Jang et al., 1996; Loehlin, 1992], could be critical in assessing the influence of genetic variants of small effect such as the 5-HTTLPR. Thus, although this study may have had adequate statistical power to replicate an association between genotype and neuroticism, the use of a different personality inventory makes direct comparisons of the findings problematic. An additional important difference between the five-factor NEO and the three-factor EPQ is that the NEO Agreeableness domain, which shows a replicated association to 5-HTTLPR genotype, is not measured separately by the EPQ-R, but is instead a component of the Psychoticism dimension [Goldberg, 1993]. It is possible that the use of a composite trait such as Psychoticism might obviate detection of a small 5-HTTLPR influence on traits related to hostility and social cooperation. A final major concern is the lack of a within-family design in that study, raising the possibility of artifacts due to admixture.

Other attempts to detect associations between the 5-HTTLPR and personality traits have been complicated by the use of small sample sizes, heterogeneous subject populations, and differing methods of personality assessment. Three studies have used sample sizes of 120 or fewer subjects [Ball et al., 1997; Ebstein et al., 1997; Ricketts et al., 1998], which greatly increases the risk of type II error in association studies [Suarez et al., 1995]. Even the largest of these studies [Ebstein et al., 1997] had a power of only 25% to detect a modest effect size comparable to the association with estimated

Harm Avoidance we originally reported [D = 0.19, Lesch et al., 1996]. It is therefore unsurprising that one study found association between the 5-HTTLPR and TPQ Harm Avoidance [Ricketts et al., 1998], one failed to find that association [Ebstein et al., 1997], and a third failed to find association between 5-HTTLPR genotype and peer-rated NEO Neuroticism [Ball et al., 1997], a measure found earlier to be only modestly correlated with self-ratings of the same trait [ $r = 0.36$ ; Costa and McCrae, 1992].

Another study [Gelernter et al., 1998] obtained trait measures using the abbreviated 60-item NEO-Five Factor Inventory (NEO-FFI; versus 240 items in the full NEO) in an ethnically heterogeneous population of substance-dependent patients and controls ( $n = 185$ ). Although a main effect of 5-HTTLPR genotype was not found, there was a significant interaction between genotype and ethnicity. The subset of European-American subjects, which most closely resemble the population studied here, showed an association of 5-HTTLPR S genotypes with increased neuroticism on the abbreviated NEO-FFI, with an effect size of approximately 0.22, indistinguishable from that in the present study. The NEO-FFI does not include facet scales, and data for the other four NEO traits were not reported [Gelernter et al., 1998]. The subject selection in two other studies [Ball et al., 1997; Deary et al., 1999] was unusual in that subjects at the high or low ends of the distribution for Neuroticism were selected on the assumption that the 5-HTTLPR affects the trait uniformly across its distribution. However, an analysis of our data [Sirota et al., 1999] shows instead that the contribution of the 5-HTTLPR to NEO Neuroticism is greatest in the central range of the distribution and actually decreases at the extremes. This illustrates the need to obtain genotypes from individuals across the distribution of a continuous trait, and suggests caution in the use of unusual subject populations in attempting to establish genetic influences on traits that are continuously distributed in the population.

Difficulties in interpretation of population-based association studies due to ethnic differences in serotonin transporter gene allele frequencies are raised by other recent studies. One found no association between the 5-HTTLPR and NEO Neuroticism in a sample of 191 Japanese medical staff and students, or between 5-HTTLPR genotype and TCI Harm Avoidance in a subsample [ $n = 144$ ; Kumakiri et al., 1999]. In addition to its relatively small sample size, a major difficulty with this study is that the frequency of the l/l genotype (group L as defined in the present study) was only 6% of the total population, giving very low statistical power to detect a genotype-related difference if one existed. A recent study of association between the 5-HTTLPR and TPQ Harm Avoidance in a Japanese population is also difficult to interpret due to differences in genotypes related to ethnicity [Katsuragi et al., 1999]. In that case, the small sample ( $n = 101$ ) had a frequency of l/l genotypes of only 4%, again due to ethnic differences.

Finally, another recent investigation of possible associations between personality traits and the 5-HTTLPR used subjects from a study of the relationship

between serum cholesterol and mood and neurobehavioral functioning [Flory et al., 1999]. Those investigators found no association between the 5-HTTLPR and NEO Neuroticism and TPQ Harm Avoidance estimated from the NEO data in a subsample of 225 Caucasian individuals. One factor complicating interpretation of those results is that associations between serum cholesterol levels and personality traits including aspects of NEO Neuroticism and hostility have been reported [e.g., Suarez, 1999; Suarez et al., 1998]. Since half of the sample studied by Flory et al. [1999] had LDL-cholesterol levels  $\geq 160$  mg dl<sup>-1</sup> by design, it is possible that influences of serum cholesterol may have obscured the relatively small 5-HTTLPR personality trait association. In addition, these authors themselves note that the sample size required to have statistical power of 0.8 would be 350, considerably larger than the population they studied. We agree with these authors [Flory et al., 1999] that there is a "need for a replication attempt in a large normative sample stratified by age and sex," and, we would add, using a within family design, as in the present investigation.

## CONCLUSIONS

In summary, the present results replicated the majority of our previous findings, most notably a dominant effect of the 5-HTTLPR s allele on increased NEO Neuroticism and other measures of negative emotionality and on decreased agreeableness and related traits likely to affect social cooperation. The present and prior studies found this association in three populations with significantly different demographic characteristics, and also within families. The modest effect sizes in both studies are consistent with observations that variations in individual genes may have relatively small but replicable effects on a variety of quantitative traits in different species [brief review: Lesch et al., 1996]. The findings are intriguing in light of recent speculation that the recent appearance of the 5-HTTLPR genetic variation may have helped permit more sophisticated modulation of social behaviors during the evolution of higher order (postprosimian primates) [Lesch et al., 1997]. In this regard, an advantage of the five-factor NEO Personality Inventory, and the lexical tradition of personality theory on which it is based, is that this model of personality is consistent with evolutionary perspectives on personality. That is, the trait terms in natural language may best reflect individual behavioral differences important to group survival and reproductive success [Buss, 1995].

Our results illustrate how progress in neuropsychiatric genetics might be hastened by closer integration of neuroscience and genetic approaches and a dimensional, semiquantitative approach to behavioral phenotypes arising out of a large body of psychometric research. That the 5-HTTLPR personality trait associations were most strongly replicated with the NEO personality inventory suggests that the five-factor model of personality is a good starting point for this research effort. Further studies of the genetics of human behavioral traits using association techniques, linkage strategies, and newer methods in development

such as SNP analysis may be especially useful in refining conceptions of the heritable components of personality. Finally, investigating possible genetic differences associated with variation along behavioral dimensions within neuropsychiatric diagnoses may be a useful complement to the traditional strategy of looking for genetic differences between categorically defined neuropsychiatric diagnostic groups.

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