Aggression and Anger-Related Traits Associated With a Polymorphism of the Tryptophan Hydroxylase Gene

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Background: *Central nervous system (CNS) serotonergic activity correlates inversely with human aggressive behavior, and individual differences in aggressive disposition are at least partially heritable. This study was conducted to evaluate the possible association between measures of antagonistic behavior and an intronic polymorphism of the gene coding for tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin biosynthesis.*

Methods: *Locally recruited men and women (*n = *251) were genotyped for the A218C polymorphism located in intron 7 of the TPH gene. All subjects were administered standard interview and questionnaire indices of aggression and anger-related traits of personality; in a portion of subjects, CNS serotonergic activity was assessed by neuropsychopharmacologic challenge (prolactin response to fenfluramine hydrochloride).*

Results: *Persons having any TPH U allele scored significantly higher on measures of aggression and tendency to experience unprovoked anger and were more likely to report expressing their anger outwardly than individuals homozygous for the alternate L allele. In men, but not women, peak prolactin response to fenfluramine was also attenuated among subjects having any U allele, relative to LL homozygotes.*

Conclusions: *Individual differences in aggressive disposition are associated with an intronic polymorphism of the TPH gene in a nonpatient sample of community-derived volunteers.* Biol Psychiatry 1999;45:603–614 © *1999 Society of Biological Psychiatry*

Key Words: Aggression, anger, genetics, serotonin, tryptophan hydroxylase, temperament

Introduction

Indices of central nervous system (CNS) serotonergic activity have been found to correlate inversely with measures of lifetime aggression, assaultiveness, irritability, and impulsivity in numerous clinical and forensic populations, including patients having personality and substance abuse disorders, men incarcerated for impulse-related crimes, and children with disruptive behavior disorders (eg., Brown et al 1979; Brown et al 1982; Coccaro et al 1989; Coccaro et al 1995; Coccaro et al 1997; Kruesi et al 1990; Limson et al 1991; Linnoila et al 1983; O'Keane et al 1992; Virkkunen et al 1994). Similar relations have been shown in healthy individuals as well (Roy et al 1988; Cleare and Bond 1997; Manuck et al 1998). For instance, we recently reported that interview and self-report measures of aggression and impulsivity correlated negatively in a nonpatient sample with subjects' prolactin responses to orally administered fenfluramine, a standard neuropsychopharmacologic challenge used to assess central serotonergic responsivity (Manuck et al 1998). As others have observed also (Roy et al 1988; Cleare and Bond 1997), this association was seen predominantly in men. Despite some inconsistencies among published studies (eg., Fishbein et al 1989; Stoff et al 1992; Wetzler et al 1991), the preponderance of current evidence indicates that, at least in males, low CNS serotonergic function is associated with heightened aggression, and perhaps especially, aggression of an irritable and impulsive nature.

Aggression and anger-related dispositions are partially heritable, as documented in twin studies of adult populations (Coccaro et al 1993; Rushton et al 1986; Tellegen et al 1988). Moreover, first-degree relatives of personality disordered (PD) patients who show blunted prolactin responses to fenfluramine are more likely to exhibit personality traits indicative of impulsive PD than relatives of probands showing a greater prolactin responsivity (Coccaro et al 1994b). Although there is yet little direct evidence for the heritability of central serotonergic activity in humans (Oxenstierna et al 1976; Meltzer and Arora 1988), heritable variation in cerebrospinal fluid (CSF) concentrations of the serotonin matabolite, 5-hydroxyin-

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doleacetic acid (5-HIAA), has been documented in rhesus monkeys *(Macaca mullata)* (Higley et al 1993). Notably, low CSF 5-HIAA in this species is also associated with aggressiveness, low social affiliation, high-risk behaviors (e.g., early emigration from natal groups, long leaps in the forest canopy), and premature mortality, often resulting from fight-inflicted wounds (Higley et al 1996; Higley et al 1992; Mehlman et al 1994).

To the extent that genetic factors account for some portion of interindividual variability in aggression-related traits and may do so through variations in CNS serotonergic activity, it follows that heritable influences might be expressed through differences in genes regulating serotonin synthesis, release and reuptake, metabolism, or receptor activation. Polymorphisms have been identified in several genes that code for elements of the serotonin system, including tryptophan hydroxylase (the rate-limiting enzyme in serotonin biosynthesis), the serotonin transporter, monoamine oxidase, and several of the serotonin receptors (Goldman 1996). Much attention has been focused on the tryptophan hydroxylase (TPH) gene, which is located on the short arm of chromosome 11 (11p14-p15.3) and contains an adenine→cytosine transversion at nucleotide 218 in intron 7 (Nielsen et al 1997). By convention, the two alleles of this polymorphism are designated U and L, with the less prevalent U allele having a frequency of about .40 in Caucasian populations. Because this polymorphism is not believed to influence serotonin biosynthesis directly, any significant association with behavior would most likely reflect linkage disequilibrium between the A218C polymorphism and functional variation in a coding or regulatory region of the TPH, or possibly a neighboring, gene.

Nielson et al (1994) reported that among arsonists and violent offenders, the A218C L allele was more common in persons who had attempted suicide than among those who had not. This finding was subsequently replicated both by association and linkage analysis in a separate sample involving subjects genotyped for a second adenine→cytosine transversion in intron 7 (A779C), which is in strong disequilibrium with the A218C polymorphism (Nielsen et al 1998). In other studies, however, suicidality was found unrelated to either the A218C polymorphism in patients with bipolar or unipolar affective disorder (Bellivier et al 1998; Furlong et al 1998) or a different, uncharacterized polymorphism of the TPH gene in persons hospitalized for suicide attempts, relative to normal control subjects (Abbar et al 1995). Recently, Mann et al (1997) reported that, among inpatients with major depression, the less common A218C U allele was more frequent in persons who had a history of attempted suicide; this association was independent of depression severity or comorbid borderline personality disorder. A further case-control study also found the U allele associated with attempted suicide, this effect strengthening somewhat when suicide attempts were violent in nature and occurred in patients with major depressive disorder (Buresi et al 1997). Thus, whether a reliable association exists between suicidal behavior and a polymorphism of the TPH gene remains unclear, as well as the extent to which discrepant findings may reflect population differences (e.g., in diagnostic classification or ethnicity) or other methododological limitations of association studies and studies on limited samples.

The relation of TPH genotype to psychometric indices of impulsive aggression is examined in one published study of 40 patients with mixed PD diagnoses (New et al 1998). Although no association was observed in women, men homozygous for the A218C L allele scored higher on a measure of assaultiveness and irritability than subjects comprising the pooled UL and UU genotypes. These preliminary findings warrant cautious interpretation, however, due to sample restriction to PD patients, the small number of men studied ($n = 21$), and the presence of only two UU homozygous males.

The purpose of the current study was to further evaluate the possible association between TPH genotype and antagonistic behavior. Our sample involved 251 community-derived, men and women volunteers who were participants in a separate investigation concerning behavioral correlates of cardiovascular disease risk and risk factor modification. Behavioral measurements included both interview and questionnaire indices of aggression and anger-related traits of personality; in a portion of the sample, a fenfluramine challenge was administered to assess central serotonergic responsivity.

Methods and Materials

Subjects

Subjects were participants in the University of Pittsburgh Cholesterol and Risk Evaluation (CARE) project, a study of the neurobehavioral correlates of plasma lipid concentrations. Recruitment entailed media advertisements and locally distributed brochures and posters. Exclusion criteria included diagnoses of schizophrenia or delusional disorder, cancer, stroke, insulin-dependent diabetes, chronic kidney or liver disease; use of psychotropic, glucocorticoid, or hypolipidemic medication; and in women, pregnancy or lactation. Primary data collection involved the measurement of fasting lipids, blood pressure, and other cardiovascular risk factors; demographic characteristics; health-related quality of life and aspects of mood and personality (including traits related to aggression and anger); and in the majority of subjects, plasma prolactin responses to fenfluramine challenge. A diagnostic interview for DSM-III-R Axis I disorders (Structured Clinical Interview for DSM-III-R: Nonpatient edition [SCID-NP]) (Spitzer et al 1990) was also administered by a

trained interviewer and clinical psychologist, with psychiatric consultation and review. This protocol was approved by the University of Pittsburgh Biomedical IRB; after the study had been described to subjects, informed consent to participate was obtained.

DNA was extracted from lymphocytes or from squamous epithelium collected from buccal mucosa using cheek brushes (Puregene kit, Gentra Systems, Minneapolis, MN) in the fifth (final) year of the CARE project. In most instances, previous CARE participants were recontacted by phone, the purpose of the data collection described, and brushes sent to subjects for mucosal sampling; these were returned by mail and separate informed consent obtained at that time. Of 343 subjects on whom DNA was sought, 35 (10.2%) could not be located and 37 (10.8%) declined or did not respond. DNA amplification and TPH genotyping was successful on 262 subjects, 8 of whom had missing demographic or behavioral data. The remaining 254 subjects included 220 whites, 31 blacks, and 3 Asians. Because of their small number, Asian subjects were deleted for purposes the present analyses, leaving a total of 251 subjects (124 men, 127 women).

TPH Genotyping

The A218C polymorphism was genotyped as described by Nielsen et al (1994, 1997). Following amplification using unique sequence primers flanking np218 of exon 7, the 918 base pair (bp) product was digested with 1 unit of Nhel overnight at 37°C, and fragments resolved on 2% agarose gels. DNA fragments were visualized by ethidium bromide staining and UV transillumination. The less common (A, or U) allele yielded fragments of 860 and 58 bp, whereas the C (L) allele yielded fragments of 615, 245, and 58 bp (Nielsen et al 1997). Across all subjects, the proportion of U alleles was 41.4% and the distribution of U and L alleles conformed to Hardy-Weinberg equilibrium ($\chi^2_2 = 1.7$, n.s.). The proportion of U alleles was 42.1% among whites and did not differ from that of blacks (37.1%). The distribution of genotypes (UU, UL and LL) did not differ by ethnicity (all $\chi^2 <$ 1.0, n.s.).

Behavioral Assessments

Only a subset of behavioral measurements included in the parent project were selected for analysis to restrict experiment-wise error. Instruments were included based on their well-replicated correlation with indices of CNS serotonergic function (Brown et al 1979; Brown et al 1982; Coccaro et al 1989; Coccaro et al 1997; Manuck et al 1998; New et al 1998; Coccaro et al 1996), prior use in the one published association study linking aggression to the TPH polymorphism evaluated here (New et al 1998), or conceptual relevance to impulsive aggression.

The Life History of Aggression (LHA) interview was first reported by Brown et al (1979, 1982) and revised by Coccaro et al (1996, 1997) to assess aspects of aggressive behavior in three item clusters, or subscales: 1) aggression expressed toward others (verbal and physical assault) or toward inanimate objects (destruction of property), and temper tantrums; 2) antisocial behaviors involving disciplinary action (in school and workplace) and illicit acts, with and without police contact; and 3) injury to self. The 11-item LHA used here differed from Coccaro et al (1996) by deletion of an item on attempted suicide and inclusion of one additional question concerning conflict with authority. This semistructured instrument was administered by one of two experienced interviewers who recorded subjects' responses to each question and queried for corroborating detail by a common protocol. Items were scored by frequency of occurrence on a scale of 1 to 4 (never, rarely, occasionally, often) and referenced separately to subjects' experiences in childhood, adolescence, and adulthood. Excellent interrater reliability was observed between the two interviewers (kappa coefficients = 1.0, 0.92, and 0.86 for the childhood, adolescent, and adult codes, respectively). Scores on the "aggression" and "antisocial" factors described by Coccaro et al (1996) were calculated by summing the highest code assigned in the adolescent or adult age periods across applicable items. The subscale for self-injurious behavior was not computed due to an absence of reported acts in this category.

The Buss-Durkee Hostility Inventory (BDHI) contains eight subscales assessing aspects of hostile behavior and ideation (Buss and Durkee 1957). Following New et al (1998), analyses were conducted on a composite of two Buss-Durkee subscales, Assault and Irritability, which have correlated consistently with central serotonergic activity in prior studies (Coccaro et al 1989; Coccaro et al 1995; New et al 1998).

The 35-item State-Trait Anger Expression Inventory (STAXI) assesses components of anger as an emotive state and disposition, including modes of anger expression (Spielberger 1991). Three of the instrument's seven subscales were selected for analysis. Angry Temperament reflects quick-temperedness, or a propensity to experience anger with minimal provocation; angry temperament is associated with impulsiveness and lack of anger control. In contrast, Angry Reaction denotes a tendency to express anger when demeaned by others, criticized, or treated unfairly. Anger-Out reflects the frequency with which anger is expressed as aggression toward persons or objects, either verbally or by physical assault. Reliabilities of the three anger subscales are all satisfactory, with alpha-coefficients of .70 to .89 across multiple samples (Spielberger 1991).

Fenfluramine Challenge

Both the D,L- and D-stereoisomers of fenfluramine cause presynaptic release of serotonin stores, inhibit reuptake, and by possible activation of postsynaptic serotonergic receptors, increase serotonin neurotransmission (Coccaro et al 1989; Borroni et al 1983; Quattrone et al 1983). Because stimulation of hypothalamic serotonergic receptors promotes pituitary release of prolactin, the change in plasma prolactin (PRL) concentration induced by fenfluramine provides an index of overall serotonergic responsivity in the hypothalamic–pituitary axis (Coccaro et al 1989; Coccaro et al 1994a).

In 167 subjects, D,L-fenfluramine hydrochloride was administered orally as a single dose and subjects' PRL levels were measured over 3.5 hours. Dosage between 30 and 60 mg was adjusted to a weight-relative administration of approximately .55

to .65 mg fenfluramine/kg of body weight. (Challenge data were not available on all subjects because weight-relative dosing was introduced after commencement of the CARE study.) Tests were conducted in the morning after 12-hour fast, at which time a 20-gauge, heparin-locked venous catheter was inserted. Following 30-min adaptation, a blood sample for determining baseline PRL concentration was obtained and the drug administered; subsequent blood samples were drawn 60, 120, 150, 180, and 210 min later. To index bioavailability, additional samples were obtained at 150 and 210 min for measuring plasma fenfluramine and norfenfluramine (active metabolite) concentrations. Samples were centrifuged immediately, separated, and stored at −70°C until analysis. Methods for determining plasma prolactin, fenfluramine, and norfenfluramine concentrations are described elsewhere (Manuck et al 1998).

This protocol is shorter than the standard 5-hour fenfluramine test due to practical constraints on participation and scheduling among nonpatient volunteers. In a validation sample of 42 individuals, peak PRL concentrations over 3.5 and 5 hours correlated .91, indicating high concordance between the abbreviated and standard sampling intervals. [Analyses of CARE participants without histories of DSM-III-R Axis I disorders, described in a separate report (Manuck et al 1998), showed measures of aggression and impulsivity to correlate inversely with peak PRL responses under the shortened challenge described here. Subjects included in the prior report who were also genotyped for the TPH polymorphism comprise 29% of the present sample.]

Statistical Analysis

Demographic characteristics of the sample are presented first, in addition to SCID-assessed Axis I diagnoses and, among subjects administered the fenfluramine challenge, mean body weight, fenfluramine dose, plasma fenfluramine and norfenfluramine concentrations during challenge, baseline PRL, and peak PRL response to fenfluramine. The latter variable was calculated as the arithmetic difference between the highest PRL value obtained following drug administration and PRL concentration at baseline: ΔPRL[fen] (Coccaro et al 1989; Manuck et al 1998). For descriptive purposes, men and women were also compared on each of the foregoing variables. These comparisons were made by *t* test or χ^2 analysis, as appropriate to the scale of measurement.

Because the frequency of UU homozygotes was relatively low within each gender, UU and UL genotypes were combined for statistical analysis (also replicating the analytic procedures of New et al 1998). Aggression and anger-related traits were then compared between subjects having any U allele and LL homozygotes. A single three-factor multivariate analysis of variance (MANOVA) was conducted, supplemented by univariate analyses (ANOVA, *t* test) in the event of a significant multivariate F-ratio. In addition to TPH genotype, MANOVA factors included gender of subject and the presence or absence of a SCID diagnosis of current or past substance abuse or dependence. The latter factor was included because alcohol and other substance abuse is associated with heightened aggression in both clinical and population studies (Swanson 1993) and was relatively common in the current sample.

Two secondary analyses were also conducted. First, on the possibility that current psychopathology might affect subject responding on interview and self-report instruments or otherwise modulate associations between genotype and behavior, we recomputed the three-factor MANOVA excluding 46 individuals having SCID-assessed DSM-III-R Axis I diagnoses at the time of testing. (With the exclusion of contemporaneous Axis I psychopathology, substance abuse in this analysis denotes a past, rather than "current or past," abuse disorder.) Second, even though allelic frequencies did not differ by ethnicity, in a third analysis we further restricted the MANOVA to data of whites alone. Effect sizes for all significant MANOVA outcomes were estimated by the eta-square (η^2) statistic, which is analogous to R^2 in multiple regression and reflects the proportion of explained variance in the dependent variables (Huberty and Smith 1982). Strength of association was also expressed by the correlation of TPH genotype (assigned values of 0 [LL], 1 [UL], and 2 [UU]) with each aggression and anger measurement, across all subjects, and in men and women separately.

The fenfluramine challenge was evaluated by ANOVA with two between-subjects factors, TPH genotype and the presence or absence of a substance abuse diagnosis. Due to estrogenic influences on PRL synthesis, storage, and release (including the PRL response to fenfluramine) in women (O'Keane et al 1991), this analysis was conducted separately in males and females. All multivariate and univariate tests were conducted using the Statistical Package for Social Sciences (SPSS), Version 8.0. Finally, all dependent measures were adjusted prior to analysis for covariation with age and socioeconomic status (as indexed by income and years of education).

Results

Subject Characteristics

As shown in Table 1, subjects' mean age was in the mid-40s (range = 25 to 60 years) and the sample as a whole was well-educated, averaging several years of post-secondary schooling. About two-thirds of subjects were married and 79% were employed full or part-time. Although men and women were similar in age and marital and employment status, representation of blacks was somewhat greater among women, and men reported more years of education and slightly higher income. Regarding Axis I diagnoses, substance abuse disorders were more common in men, whereas prior history of mood disorders predominated in women. χ^2 analyses showed no significant relationship between TPH genotype (UU+UL, LL) and any category of Axis I psychopathology, whether by past history or lifetime occurrence (i.e., current or past) in either men or women (all $\chi^2_1 < 1.8$, n.s.).

With respect to the fenfluramine challenge, women received less drug than men due to their lesser body weight, but plasma fenfluramine/norfenfluramine concen-

Table 1. Demographic characteristics and history of Axis 1 diagnoses among all subjects and in men and women separately, and fenfluramine challenge measurements in men and women.[a]

	All Subjects (n = 251)	Men (n = 124)	Women (n = 127)	Statistic	p
Age (years)	45.7 (.55)	45.3 (.79)	46.1 (.75)	$t_{249} = -.8$	n.s.
Marital Status (% married)	62.9	66.1	59.8	$\chi^2_1 = 1.0$	n.s.
Education (years)	15.3 (.18)	15.8 (.24)	14.8 (.27)	$t_{249} = 2.6$	<.009
Employment Status (% employed)	79.3	80.6	78.0	$\chi^2_1 = .3$	n.s.
Income (graded 1–7[b])	4.7 (.11)	4.9 (.15)	4.4 (.14)	$t_{249} = 2.2$	<.03
Race (% white)	87.6	91.9	83.5	$\chi^2_1 = 4.2$	<.05
Axis I					
Mood disorders [# (%)]					
Current	13 (5.2)	7 (5.6)	6 (4.7)	$\chi^2_1 = .1$	n.s.
Past	58 (23.1)	22 (17.7)	36 (28.3)	$\chi^2_1 = 4.0$	<.05
Anxiety disorders [# (%)]					
Current	16 (6.4)	5 (4.0)	11 (8.7)	$\chi^2_1 = 2.3$	n.s.
Past	24 (9.6)	8 (6.5)	16 (12.5)	$\chi^2_1 = 2.7$	n.s.
Substance abuse/dependence [# (%)]					
Current	15 (6.0)	14 (11.3)	1 (<1)	$\chi^2_1 = 12.3$	<.001
Past	82 (32.7)	55 (44.4)	27 (21.2)	$\chi^2_1 = 15.2$	<.001
Other disorders[c] [# (%)]					
Current	9 (3.6)	3 (2.4)	6 (4.7)	$\chi^2_1 = .9$	n.s.
Past	6 (2.4)	3 (2.4)	3 (2.4)	$\chi^2_1 = .6$	n.s.

Fenfluramine Challenge	Men (n = 84)	Women (n = 83)	Statistic	p
Weight (kg)	186.9 (3.10)	151.0 (2.75)	$t_{165} = 8.7$	<.0001
Fenfluramine dose (mg)	53.0 (.84)	43.1 (0.97)	$t_{165} = 7.7$	<.0001
Baseline PRL (ng/mL)	6.3 (.25)	6.7 (0.41)	$t_{165} = -.9$	n.s.
ΔPRL[fen] (ng/mL)	2.6 (.34)	5.5 (0.52)	$t_{165} = -4.5$	<.0001
Plasma measurements (ng/mL)				
Fenfluramine, 150 min	42.7 1.71	40.2 1.78	$t_{165} = .9$	n.s.
Norfenfluramine, 150 min	7.9 .36	7.5 0.46	$t_{165} = .8$	n.s.
Fenfluramine, 210 min	48.3 1.49	47.8 1.55	$t_{165} = .2$	n.s.
Norfenfluramine, 210 min	10.8 .41	10.4 0.49	$t_{165} = .6$	n.s.

[a]Unless indicated otherwise, values listed are group means (±SEM).
[b]Income grades: 1) < $10,000; 2) $10,000–14,999; 3) $15,000–24,999; 4) $25,000–34,999; 5) $35,000–49,999; 6) $50,000–74,999; 7) > $75,000.
[c]Other Axis I diagnoses: current; adjustment disorder (2 male, 1 female); hypochondriasis (1 m, 1 f), somatization pain disorder (1 m); bulimia nervosa or eating disorder NOS (4 f); past; pathological gambling (2 m); anorexia nervosa (1 m, 1 f), bulimia nervosa (1 f).

trations during the challenge did not vary by gender, indicating comparable drug exposure. No gender difference in baseline PRL was detected, but relative to men, peak PRL responses to fenfluramine (ΔPRL[fen]) were significantly greater among women.

Aggression and Anger

The MANOVA for aggression and anger-related traits revealed significant omnibus effects of each of the three between-subjects factors: TPH genotype ($F_{6,241} = 2.6$; $p < .02$; $\eta^2 = .062$), gender ($F_{6,241} = 9.9$; $p < .0001$; $\eta^2 = .204$), and substance abuse history ($F_{6,241} = 7.0$; $p < .0001$; $\eta^2 = .146$). There were no significant multivariate interactions. Results of follow-up univariate ANOVAs are summarized in Table 2. People with any U allele scored significantly higher than LL homozygotes on four of the six dependent measures: LHA-assessed Aggression and Antisocial behavior, Angry Temperament, and Anger Out. On each of these measures, the mean scores of TPH heterozygotes also fell intermediate between subjects homozygous for the U and L alleles. In addition, supplemental analyses comparing the latter two groups (i.e., UU versus LL homozygotes, collapsed across gender and substance abuse history) revealed significant group differences for LHA-Aggression ($t_{117} = 2.8$, $p < .007$), Angry Temperament ($t_{117} = 2.8$, $p < .007$), and Anger Out ($t_{117} = 2.4$, $p < .02$); however, these groups did not differ significantly on the LHA-Antisocial factor ($t_{117} = 1.7$, $p = .097$), Angry Reaction ($t_{117} < 1$, n.s.), or "assault + irritability" scores ($t_{117} < 1$, n.s.).

Independent of genotype, men (relative to women) and people with current or past substance abuse diagnoses scored higher on the LHA Aggression and Antisocial

Table 2. Mean scores (± SEM) for aggression and anger-related personality traits as a function of TPH genotype, gender, and lifetime occurrence of substance abuse/dependence

		Life History of Aggression		Buss-Durkee Hostility Inventory	State-Trait Anger Expression Inventory		
	n	Aggression	Antisocial	Assault + Irritability	Angry Temperament	Angry Reaction	Anger-Out
Genotype							
LL	81	8.6 (.32)	7.8 (.30)	6.7 (.41)	5.1 (.14)	8.2 (.29)	13.4 (.30)
UL	132	9.2 (.24)	8.3 (.23)	6.8 (.33)	5.7 (.16)	7.9 (.18)	14.0 (.24)
UU	38	10.2 (.45)	8.7 (.55)	7.2 (.71)	6.1 (.41)	8.4 (.41)	14.8 (.55)
[UU + UL]	170	9.4 (.22)	8.4 (.22)	6.9 (.30)	5.8 (.15)	8.0 (.17)	14.2 (.22)
	$F_{1,243}$[a]	5.4	4.8	<1.0	7.3	<1.0	4.3
	p	<.03	<.03	n.s.	=.007	n.s.	=.04
Gender							
Male	124	9.9 (.27)	9.7 (.25)	7.4 (.34)	5.7 (.17)	7.8 (.19)	14.1 (.27)
Female	127	8.5 (.22)	6.8 (.18)	6.3 (.34)	5.4 (.16)	8.4 (.22)	13.8 (.24)
	$F_{1,243}$	5.2	44.6	1.7	<1.0	6.1	<1.0
	p	<.03	<.0001	n.s.	n.s.	<.02	n.s.
Substance Abuse							
SA[a]	160	8.5 (.19)	7.2 (0.18)	6.3 (0.30)	5.4 (.14)	8.0 (0.18)	13.5 (.21)
SA+	91	10.3 (.33)	9.9 (0.30)	7.8 (0.44)	5.8 (.21)	8.3 (.26)	14.6 (.33)
	$F_{1,243}$	14.1	36.5	5.1	1.3	2.2	5.7
	p	<.001	<.0001	=.025	n.s.	n.s.	<.02

[a] *F* test for mean difference between UU + UL and LL genotypes.
[b] SA = substance abuse; + = positive; − = negative.

factors. Anger Out and Buss-Durkee "assault + irritability" scores were also higher among people with a substance abuse history, while Angry Reaction scores were significantly greater in women than among men.

Correlations between the several dependent measures are listed in Table 3. The six aggression and anger indices correlated significantly in almost all instances, reflecting modest-to-moderate covariation among scales and correlations of similar magnitude in men and women. We next correlated TPH genotype (i.e., where LL = 0, UL = 1, UU = 2) with each dependent measure, as shown in Table 4. Consistent with results of the univariate ANOVAs, genotype correlated significantly (though modestly) with LHA-assessed Aggression and Antisocial behavior, Angry

Table 3. Intercorrelation of aggression and anger-related personality traits in men ($n = 124$; above diagonal) and women ($n = 127$; below diagonal)

	Life History of Aggression		Buss-Durkee Hostility Inventory	State-Trait Anger Expression Inventory		
	Aggression	Antisocial	Assault + Irritability	Angry Temperament	Angry Reaction	Anger-Out
Life History of Aggression						
Aggression	—	$r = .51$ $p < .0001$	.34 <.0001	.34 <.0001	.15 n.s.	.32 <.0002
Antisocial	.30 <.0005	—	.19 <.04	.22 <.02	n.s.	.33 <.0002
Buss-Durkee Hostility Inventory						
Assault + Irritability	.34 <.0001	.18 <.04	—	.55 <.0001	.45 <.0001	.49 <.0001
State-Trait Anger Expression Inventory						
Angry Temperament	.41 <.0001	.08 n.s.	.63 <.0001	—	.36 <.0001	.60 <.0001
Angry Reaction	.17 n.s.	.16 n.s.	.43 <.0001		—	.37 <.0001
Anger-Out	.36 <.0001	.24 <.006	.64 <.0001	.60 <.0001	.34 <.0001	—

Table 4. Correlation of TPH genotype with aggression and anger-related personality traits among all subjects and in subjects without current Axis I psychopathy

	All Subjects			Subjects Without Current Axis I Disorders		
	Total (n = 251)	Men (n = 124)	Women (n = 127)	Total (n = 205)	Men (n = 99)	Women (n = 106)
Life History of Aggression						
Aggression	r = .20	.25	.10	.24	.27	.18
	p < .002	= .004	n.s.	< .001	= .006	= .072
Antisocial	.16	.25	.03	.24	.32	.07
	< .01	< .005	n.s.	< .001	< .002	n.s.
Buss-Durkee Hostility Inventory						
Assault + Irritability	.06	.07	.02	.02	−.03	.05
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Spielberger Anger Expression Inventory						
Angry Temperament	.19	.26	.10	.21	.25	.16
	< .003	< .003	n.s.	< .003	< .02	= .095
Angry Reaction	.01	.11	.02	.00	.05	−.02
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Anger-Out	.17	.23	.10	.23	.28	.16
	< .008	< .01	n.s.	< .002	< .006	= .095

Temperament, and Anger Out. Although neither the MANOVA nor univariate analyses revealed a significant interaction of genotype and gender, gender-specific correlations (Table 4) show the covariation of TPH genotype with aggression and anger measures to be statistically robust in men, but weak and nonsignificant among women.

When the 18% of subjects having current Axis I disorders were excluded from analysis, the multivariate F-ratio for TPH genotype grew somewhat stronger ($F_{6,192} = 4.4$; $p < .001$; $\eta^2 = .12$) and the effects of gender ($F_{6,192} = 7.2$; $p < .001$; $\eta^2 = .18$) and substance abuse history ($F_{6,192} = 5.6$; $p < .001$; $\eta^2 = .15$) remained significant. As in the previous analysis, there were no significant multivariate interactions. For TPH, univariate testing again showed significant main effects of genotype on four of the six dependent measures: the LHA-Aggression and Antisocial factors, Angry Temperament, and Anger Out. As shown in Table 5, individuals having any U allele scored significantly higher on each of these measures than persons homozygous for the L allele, and again, subjects of UL genotype tended to score intermediate to UU and LL homozygotes. Separate comparison of subjects homozygous for the U and L alleles also showed significant group differences for the LHA-Aggression ($t_{88} = 2.9$, $p < .004$) and Antisocial ($t_{88} = 2.2$, $p < .04$) factors, Angry Temperament ($t_{88} = 2.8$, $p < .007$), and Anger Out ($t_{88} = 2.8$, $p < .006$). Similarly, correlations with TPH genotype remained significant for the same four aggression and anger measure-

Table 5. Mean scores (± SEM) for life history of aggression, angry temperament, and anger-out in subjects without current Axis I psychopathy, as a function of TPH genotype

				Life History of Aggression						State-Trait Anger Expression Inventory					
	Sample (n)			Aggression			Antisocial			Angry Temperament			Anger-Out		
Genotype	Total	White	Black	Total	White	Black	Total	White	Black	Total	White	Black	Total	White	Black
LL	60	52	8	8.0	8.2	6.9	7.4	7.8	4.9	4.9	5.0	4.1	13.0	13.1	12.4
				(.36)	(.41)	(.33)	(.35)	(.37)	(.32)	(.16)	(.18)	(.53)	(.37)	(.40)	(.87)
UL	115	104	11	9.1	9.2	8.2	8.3	8.4	7.5	5.7	5.7	5.1	13.9	14.1	12.8
				(.27)	(.28)	(.76)	(.25)	(.26)	(.84)	(.17)	(.18)	(.44)	(.25)	(.25)	(.99)
UU	30	26	4	9.9	10.2	8.3	8.8	9.0	7.9	6.0	5.8	7.4	14.9	14.9	15.1
				(.51)	(.54)	(1.28)	(.62)	(.66)	(2.07)	(.46)	(.49)	(1.39)	(.62)	(.65)	(2.11)
[UU + UL]	145	130	15	9.3	9.4	8.2	8.4	8.5	7.6	5.8	5.7	5.7	14.1	14.3	13.4
				(.24)	(.25)	(.63)	(.24)	(.25)	(.79)	(.17)	(.18)	(.53)	(.23)	(.24)	(.92)
	$F_{1,197}$[a]	$F_{1,174}$	—	F 9.1	6.6	—	6.1	2.8	—	7.1	3.9	—	5.1	3.9	—
				p = .003	= .011		< .02	= .098		= .008	< .05		< .03	< .05	

[a] F test for mean difference between UU + UL and LL genotypes.

ments, as indicated in Table 4. Within-gender coefficients continued to be significant among men, while on three measures (LHA-Aggression, Angry Temperament, and Anger Out) correlations approached, but did not achieve, significance in women ($p < .10$).

MANOVA outcomes did not change when analysis was further restricted to whites only (without current Axis I diagnoses); significant omnibus effects still obtained for TPH genotype ($F_{6,169} = 4.0$; $p = .001$; $\eta^2 = .12$), gender ($F_{6,169} = 5.9$; $p < .001$; $\eta^2 = .17$), and substance abuse history ($F_{6,169} = 4.4$; $p < .001$; $\eta^2 = .13$), and no multivariate interactions were observed. However, univariate testing here showed significant main effects of genotype on LHA-Aggression, Angry Temperament, and Anger Out, but not the LHA-Antisocial factor (or, as in previous analyses, Angry Reaction or Buss-Durkee "assault + irritability" scores). The pattern of mean differences (also shown in Table 5) was similar to that observed in analyses of the more inclusive samples. The comparison of UU and LL homozygotes again proved significant for LHA-Aggression ($t_{76} = 2.8$, $p < .007$) and Anger Out ($t_{76} = 2.4$, $p < .02$), but not for the LHA-Antisocial factor ($t_{76} = 1.7$, $p = .093$), Angry Temperament ($t_{76} = 1.8$, $p = .079$), Angry Reaction ($t_{76} < 1$, *n.s.*), or "assault + irritability" scores ($t_{76} < 1$, n.s.). On the other hand, TPH genotype continued to correlate significantly, across all subjects and in men alone, with LHA-Aggression ($r_{180} = .24$ and, in men, $r_{91} = .27$, $p < .005$, respectively), LHA-Antisocial behavior ($r = .21$ and .32, $p < .005$), Angry Temperament ($r = .16$ and .22, $p < .05$), and Anger Out ($r = .22$ and .28, $p < .003$).

Table 6. Mean prolactin response to fenfluramine (±SEM) in men and women as a function of TPH genotype and lifetime occurrence of substance abuse/dependence

	Men		Women	
	n	ΔPRL[fen][a]	*n*	ΔPRL[fen][a]
Genotype				
LL	26	3.3 (.60)	30	5.5 (.88)
UL	42	2.3 (.44)	43	5.7 (.64)
UU	16	2.3 (.75)	10	4.4 (1.68)
[UU+UL]	58	2.3 (.38)	53	5.3 (.61)
	$F_{1,80}$[b]	4.0	$F_{1,79}$[b]	<1.0
	p	<.05	*p*	n.s.
Substance Abuse				
SA−[c]	47	3.0 (.47)	65	5.6 (0.57)
SA+	37	2.1 (.42)	18	5.0 (1.03)
	$F_{1,80}$	3.6	$F_{1,79}$	<1.0
	p	<.07	*p*	n.s.

[a] ng/mL.
[b] *F* test for mean difference between UU + UL and LL genotypes.
[c] SA = substance abuse; + = positive; − = negative.

The availability of limited data on black subjects in this sample (<13%) afforded insufficient statistical power to permit their analysis separately. In Table 5, however, mean values among blacks are listed by genotype beside those of whites and of all subjects without current Axis I diagnoses, for LHA-assessed Aggression and Antisocial behavior, Angry Temperament, and Anger Out (i.e., the four measures associated with TPH genotype in univariate analyses of all subjects combined and, with the exception of the Antisocial scale, in whites alone). Despite the small number of blacks with each genotype, means listed in Table 5 are consistent with those observed in whites and in the sample as a whole. On average, blacks having any U allele scored higher than LL homozygotes on each measure, and blacks of UL genotype scored intermediate to subjects homozygous for the U and L alleles. Any trend that might be inferred from the pattern of mean differences among genotypes in blacks, therefore, is fundamentally the same as that seen in whites and on analysis of all subjects together.

Fenfluramine Challenge

As noted previously, fenfluramine challenge data were analyzed separately in men and women due to gender-specific endocrine (ovarian) influences on fenfluramine-induced PRL responsivity (O'Keane et al 1991). Preliminary analyses showed neither genotype nor substance abuse history to be associated with baseline PRL levels, fenfluramine dose, or plasma fenfluramine/norfenfluramine concentrations during challenge, in either men or women. As indicated in Table 6, mean ΔPRL[fen] scores were significantly lower in men having any U allele than among LL homozygotes, with the UU and UL genotypes showing equivalent PRL responses.

Though only approaching significance, men with histories of substance abuse (current or past) also tended to show blunted PRL responsivity, independent of genotype. The effects of the TPH polymorphism on ΔPRL[fen] scores did not remain significant, however, when reanalyzed on the reduced sample involving subjects without current Axis I disorders ($F_{1,64} = 1.6$; $p = .22$) or when further restricted to whites alone ($F_{1,59} = 1.1$; n.s.). In no analysis was the interaction of TPH genotype and substance abuse history significant. Among all men administered the fenfluramine challenge, ΔPRL[fen] values correlated negatively with LHA-Aggression scores ($r_{82} = -.25$, $p < .025$), but did not covary significantly with other behavioral measurements. In women, neither TPH genotype nor substance abuse history was associated with peak PRL responses to fenfluramine (Table 6); aggression and anger measurements also were unrelated to ΔPRL[fen] scores in women.

Discussion

In this study, persons possessing one of the two alleles of an intronic polymorphism in the TPH gene (the A218C U allele) scored significantly higher on measures of aggression and propensity to unprovoked anger, and were more likely to report expressing their anger outwardly, than individuals homozygous for the alternate L allele. TPH heterozygotes also tended to score intermediate to subjects homozygous for the U and L alleles. These associations were independent of variability in age and social class, did not vary by history of substance abuse, and strengthened on removal of subjects with current Axis I psychopathology. Additionally, TPH genotype covaried with aggression and anger-related personality traits in correlational analyses, but did so significantly only in men and among men and women combined.

Interestingly, the interview-derived index of aggression history used in this study (the LHA) has also been found to correlate inversely with numerous measures of central serotonergic function in both clinical and nonpatient populations (Brown et al 1979; Brown et al 1982; Coccaro et al 1989; Coccaro et al 1995; Coccaro et al 1997; Manuck et al 1998; Coccaro et al 1996), and has been validated against an observational measure of aggressive responding in laboratory testing (Cherek et al 1997). In a preliminary study of regional cerebral metabolic rates of glucose using positron emission tomography among PD patients, moreover, higher scores on the LHA were associated with reduced glucose uptake in orbital prefrontal cortex, an area of dense serotonergic innervation often thought to be implicated in the regulation of impulsive behavior, including aggression (Goyer et al 1994). Regarding anger-related measurements, the UU and UL genotypes were related to tendencies to express angry feelings openly and against others, either physically or verbally (Anger Out), and to experience such feelings on minimal provocation (Angry Temperament), but not to anger arousal induced by real or perceived ill-treatment or personal affront (Angry Reaction). This pattern of association is also consistent with evidence that low CNS serotonergic activity predisposes to aggression of a predominantly irritable and impulsive nature (Coccaro et al 1989).

To our knowledge, these data are the first to show possible association of a genetic polymorphism with variability in aggressive disposition, as seen in an unselected sample of community volunteers. Because subjects were evaluated only for Axis I disorders, the sample cannot be characterized with respect to all forms of psychopathology. The lifetime occurrence of mood and substance abuse disorders was also about 10 to 20% greater than United States population estimates based on DSM-III-R criteria (Kessler et al 1994). Not surprisingly, substance abuse was itself positively associated with subjects' Anger Out and "assault + irritability" scores and with LHA-assessed aggression and antisocial behavior. However, these relations were independent of the effects of genotype on LHA and Anger Out scores, and substance abuse (as well as other Axis I disorders) were unrelated to the distribution of TPH alleles in this sample. In addition, men having any U allele scored higher on Angry Temperament than LL homozygotes, but unlike Anger Out and LHA measurements, Angry Temperament did not vary by substance abuse history.

These findings may reflect a genetic correlate of normative variability in aggression and anger-related traits of personality. A dimensional interpretation must be considered speculative, though, without diagnostic information regarding Axis II disorders—particularly borderline and antisocial PD, which are both characterized by impulsive aggression and low CNS serotonergic activity (Coccaro et al 1989; O'Keane et al 1992; New et al 1998; Coccaro et al 1996). Although the low population prevalence of these disorders would tend to discount their influence in this study (Kessler et al 1994; Lenzenweger et al 1997; Lyons 1995; Zimmerman and Coryell 1989), a more definitive interpretation will require independent replication in samples rigorously screened for concomitant psychiatric morbidity. It should be noted, too, that association studies of unrelated individuals, such as that reported here, warrant cautious interpretation, as unknown sources of population stratification ("admixture") may potentially yield spurious relations between genetic markers and a behavioral phenotype (Berrettini 1997; Cloninger 1991; Greenberg et al 1998; Nimgaonkar 1997). For this reason, it is also important that future research extend the present findings by use of family-based association designs, in which intrafamilial comparisons mitigate possible confounding due to population substructuring.

In the one prior study of behavioral traits associated with this polymorphism, New et al (1998) reported that "assault + irritability" scores on the Buss-Durkee Hostility Inventory were higher in LL homozygotes than in subjects of UU and UL genotype, among 21 men of mixed PD diagnoses. We did not replicate this finding, and more generally, our results are directionally opposite those of New et al (1998). It may be noted, however, that the New et al study included only two UU homozygous men and that the mean "assault + irritability" score of these subjects did not differ from that of LL homozygotes [UU = 14.5; UL = 8.8 (n = 9); LL = 14.5 (n = 10)]. In contrast, TPH-associated measures of aggression and anger-related traits differed maximally in the present study between subjects homozygous for the U and L alleles, with heterozygotes scoring in the intermediate range. Mean differences between UU and

LL homozygotes alone were also statistically significant in most comparisons.

Interestingly, studies of suicidality exhibit some inconsistency in this same polymorphism and with respect to corresponding alleles of the related A779C polymorphism. Among men incarcerated for arson and violence in studies of Nielsen et al (1994, 1998), suicide attempts were more frequent in persons having the L allele, whereas the U allele predicted attempted suicide in two other investigations (Mann et al 1997; Buresi et al 1997). Somewhat consistent with the latter findings, though, are two additional observations reported by Nielsen et al (1998). First, suicidality was associated with the A779C U allele in one of two subsamples composed of "nonimpulsive" violent offenders (persons committing crimes of premeditation), and second, in the absence of suicidality, the U allele was more common in criminal offenders than among nonoffender controls. Nonetheless, there remain discrepancies in the literature on suicidality, as well as failures of replication (Bellivier et al 1998; Furlong et al 1998). Although it is tempting to speculate that genetic influences might vary within different ranges of the distribution of variability in a behavioral phenotype (Greenberg et al 1998) or among groups of disparate clinical characteristics, it is also possible that apparent inconsistencies reflect spurious association due to genetic admixture or sampling error in one or more studies. In this context, resolution of conflicting results among these few and, in many ways, dissimilar studies of selected clinical populations awaits further investigation involving well-characterized patient and normal samples.

That peak PRL responses to fenfluramine were attenuated here among subjects having any U allele, relative to LL homozygotes, suggests that the TPH polymorphism may also be related to variability in CNS serotonergic responsivity. This finding was relatively weak and did not retain significance when excluding current Axis I psychopathology and subjects of minority ethnicity, but is at least consistent with TPH-behavior associations in the full sample. Although Nielson et al (1994) previously reported finding the L allele associated with low CSF 5-HIAA concentrations in analyses restricted to impulsive, alcoholic offenders, this finding did not relicate on subsequent investigation among similarly defined offenders and control subjects. In contrast, Jonsson et al (1997) reported that among healthy volunteers, LL homozygotes for the A779C polymorphism had significantly higher CSF 5-HIAA concentrations than subjects of either UL or UU genotype, but only among men. This finding is directionally consistent with our own. Similarly, we did not find fenfluramine-induced PRL responses related to TPH genotype in women. As noted elsewhere (Manuck et al 1998; O'Keane et al 1991), the PRL response to fenfluramine is influenced by ovarian function and may vary as much as threefold over the menstrual cycle. We did not control for menstrual phase at the time of testing, thereby introducing additional measurement variability that may have obscured any genetic association with PRL responsivity in women.

As noted before, the A218C polymorphism is not thought to be functional for serotonin biosynthesis due to its intronic location (Nielsen et al 1997; Nielsen et al 1994; Mann et al 1997). To the extent allelic differences can be shown to predict indices of behavior and central serotonergic function, however, it is reasonable to suspect linkage disequilibrium with respect to a mutation in the coding region of the TPH (or a proximal) gene or in a regulatory sequence; less likely is the possibility that the polymorphism is itself functional in an intronic regulatory region of the TPH gene. Should the current findings replicate in other samples (particularly, as noted above, in family-based association studies) and with other, nearby markers, it will become important to identify any associated, functionally relevant mutation and to verify its effects on protein structure or gene expression. Whatever the outcome of such research though, it must be cautioned that single genetic polymorphisms, even those of known functional significance, can be expected to account for only a small portion of the heritable variation (and therefore even less of the total phenotypic variability) in any complex behavioral characteristic. Investigating influences of individual serotonin-associated genes will therefore advance our understanding of some of the mechanisms underlying antagonistic behavior, but implies no necessary constraint on the modification of such behavior, whether by social and environmental, psychotherapeutic, or pharmacologic intervention.

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