ORIGINAL ARTICLE

Association Between Serotonin Transporter Gene Promoter Polymorphism (*5HTTLPR*) and Behavioral Responses to Tryptophan Depletion in Healthy Women With and Without Family History of Depression

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Background: Evidence suggests that serotonin transporter gene promoter polymorphism (*5HTTLPR*)–dependent low transcriptional activity of the human serotonin transporter gene may be a genetic susceptibility factor for depression. We studied the behavioral responses to tryptophan depletion (TD) in healthy women with and without a first-degree family history of depression and examined the relationship to *5HTTLPR* alleles.

Methods: Twenty-four healthy women with a negative family history of depression and 21 women with a positive family history of depression were genotyped for the polymorphism of the *5HTTLPR* and then entered a double-blind, placebo-controlled, randomized crossover TD study. The effects of these interventions were assessed with measures of depression and plasma tryptophan levels.

Results: The TD induced a robust decrease of plasma tryptophan levels in all women irrespective of family history of depression or *5HTTLPR* genotypes. The *s/s* genotype of the *5HTTLPR* was associated with an increased risk of developing depressive symptoms during TD irrespective of family history. In contrast, individuals with the *l/l* genotype did not develop depressive symptoms, irrespective of family history. Finally, *s/l* subjects without family history showed a mood response that was intermediate between the *s/s* and *l/l* subjects, while *s/l* subjects with a family history of depression showed the same depressiogenic effect of TD as seen in the *s/s* subjects.

Conclusions: The results of the present study suggest that the *s*-allele of the *5HTTLPR* and a positive family history of depression are additive risk factors for the development of depression during TD.

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THE INVOLVEMENT of serotonergic pathways in the pathogenesis of unipolar depression has been the subject of intensive research for many years. There is now substantial evidence suggesting that altered brain serotonergic transmission plays a key role in the development of depression.[1] Altered serotonin system indexes, including lower plasma tryptophan levels,[2,3] reduced cerebrospinal fluid 5-hydroxyindoleacetic acid levels,[4] decreased platelet serotonin uptake,[5] and blunted neuroendocrine responses in challenge studies of different serotonin receptors suggesting decreased brain serotonin responsiveness,[6-9] have been reported in depressed patients relative to healthy control subjects. Moreover, brain imaging studies suggest widespread impairment of serotonergic function in depression.[10,11]

The most widely reported serotonergic abnormality in major depression involves the serotonin transporter (5-HTT).[12] This is of particular interest because serotonin reuptake inhibitors, which are the mainstay of pharmacologic treatment of depression,[13] target the 5-HTT.[14] The human 5-HTT gene has been cloned and maps to chromosome 17q11.1-q12,[15,16] and 2 common polymorphisms have been described: a variable-number tandem repeat (VNTR) located in intron 2 (5-HTT-VNTR),[16] and a deletion-insertion in the transcriptional control region approximately 1 kilobase upstream of the transcription initiation site (*5HTTLPR*).[17] The promoter polymorphism has been shown to influence transcription activity and 5-HTT function. The short form of this variant, designated *s*, is associated with lower basal and induced transcriptional efficiency of the 5-HTT gene promoter, resulting in lower serotonin uptake activity, when compared with the long form, designated *l*.[17-21] The *l/l* genotype yielded

SUBJECTS AND METHODS

SUBJECTS

Forty-five white women, aged 19 to 53 years (mean±SD age, 26.3±4.9 years), were recruited for this study through advertisements between October 1997 and February 2000 (**Table 1**). All subjects were screened for present or past psychiatric Axis I diagnoses by means of the Structured Clinical Interview for *DSM-IV*, nonpatient version.[45] In addition, the Structured Clinical Interview for *DSM-IV* for Axis II Personality Disorders was administered.[46] Inclusion criteria for all subjects included willingness to participate in a TD study, good physical health, and the absence of any Axis I and II *DSM-IV* diagnoses. Physical examinations, including electrocardiogram and blood and urine tests, ensured that all participants were medically healthy. All subjects underwent urine pregnancy tests at the time of screening and on the morning of the day before initiation of the depletion procedures. Smokers were ineligible to participate.

The FH data were collected by means of the Structured Clinical Interview for *DSM-IV* for Axis I diagnoses. Interviews with first-degree relatives (n=150) were conducted by telephone, which has been shown to provide results comparable with those of interviews done face-to-face.[47,48] Moreover, relatives were asked to provide us with information regarding their treatment for depression and potential comorbid disorder. In case we were unable to obtain a personal interview with the relative (n=12; 7 men and 5 women) we used medical records and information provided by the study subject to determine his or her psychiatric diagnosis. Three relatives refused participation in the interview, 2 relatives had died of suicide, and 7 relatives had died of disorders primarily unrelated to their psychiatric disorder.

Inclusion criteria for positive FH of depression (FH+) were the presence of at least 1 first-degree relative with major depression according to *DSM-IV* criteria and the documentation of at least 2 episodes of depression that did require treatment, and/or an attempted or successful suicide during a depressive episode. Families who did not meet these criteria were considered as having a negative FH of depression (FH–).

All subjects were informed about the study design, which was approved by the Ethics Committee of the University of Vienna, Vienna, Austria, and written informed consent was obtained from all participants at the time of initial screening. The informed consent included information about the potential risk of transient mood alterations during the depletion sessions.

DESIGN OF THE STUDY

At the time of the initial screening, blood was drawn from all subjects for genotyping. After their relatives had been screened, subjects were assigned to either the FH+ or FH– group. According to their genotype (*s*/*s*, *s*/*l*, or *l*/*l*) and FH, subjects were enrolled into a double-blind, placebo-controlled crossover TD study and were randomly assigned to undergo either TD first and sham depletion second or sham depletion first and TD second. To avoid carryover effects, a period of at least 6 days between each depletion procedure was established. All women were studied during the follicular phase of their menstrual cycle.

We used a modified methodology for TD and sham depletion as described previously.[49] The TD was induced on day 1, at 8:30 AM, by administration of 50 white capsules containing an amino acid mixture consisting of L-isoleucine (4.2 g), L-leucine (6.6 g), L-lysine (4.8 g), L-methionine (1.5 g), L-phenylalanine (6.6 g), L-threonine (3.0 g), and L-valine (4.8 g). During sham depletion, subjects received on day 1, at 8:30 AM, 50 white capsules containing 31.5 g of lactose.

The effects of TD and sham depletion were evaluated with measures of depression and measures of plasma total and free tryptophan concentrations within each depletion procedure on day 1, at 8:30 AM, 2:00 PM, and 4:30 PM, and on day 2, at 8:30 AM. Patients did not eat on day 1 of the study until about 5:00 PM. Thereafter, patients returned to unrestricted food intake.

Four raters, blind to the FH and genotype of the subjects and to the depletion condition, used an 18-item version of the Hamilton Depression Rating Scale (HDRS) modified from a standard version to assess mood.[50] The items assessing sleep, diurnal variation, eating, and weight change were omitted. The intraclass correlation coefficient[51] among raters was 0.95.

All subjects were contacted by one of us (A.N.) about 1 month after completion of the study to obtain information about their mood. Subjects were encouraged to contact a clinician of the research team also in the future if persistent mood alterations occur.

BIOCHEMICAL ASSAYS

Genotyping

DNA was extracted from whole blood by means of a kit (QIAamp Blood Isolation Kit; QIAGEN GmbH, Hilden, Germany). Primers flanking the 5-HTT promoter polymorphic region (5HTTP-F 5′-TGA ATG CCA GCA CCT AAC CC-3′; 5-HTTP-R 5′-TTC TGG ′TGC CAC CTA GAC GC-3′) were used to generate a 406–base pair (deletion)/450–base pair (insertion) fragment.[32] Polymerase chain reaction was performed in a final volume of 25 µL consisting of 50-ng DNA, l µmol/L of each primer, 200-µM deoxynucleotide triphosphate, 100-µM 7-deaza–guanosine triphosphate, 5% dimethyl sulfoxide, 10-mM Tris hydrochloride (pH 8.3), 50-mM potassium chloride, 1.5-mM magnesium chloride, and 2.5 U of DNA polymerase (AmpliTaq Gold; PerkinElmer, Langen, Germany). Annealing was carried out at 61°C for 30 seconds, extension at 72°C for 1 minute, and denaturation at 95°C for 30 seconds for 40 cycles. Polymerase chain reaction products were separated on a 3% agarose gel (FMC NuSieve 3:1; Biozym Diagnostic GmbH, Oldendorf, Germany) and visualized by ethidium bromide staining.

Assessments of Plasma Total and Free Tryptophan Concentrations

Patients were asked to rest for 30 minutes before each blood draw. Collected blood was immediately centrifuged for 17 minutes at 4°C and 3000 rpm. Serum was frozen at –70°C until analyzed. Proteins were precipitated by adding 20 mL

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of 70% perchloric acid to 400 mL of serum. Centrifugation Centrifugation (Hettich Mikro 22 R centrifuge; Hettich Zentrifugen, Tuttlingen, Germany) for 30 minutes at 14 000 rpm (at 4°C) gave a colorless liquid above a yellowish white precipitate. Of this liquid, 100 mL was injected into the high-performance liquid chromatography, leaving another 100 mL for a second injection. Plasma total tryptophan concentrations were assessed by means of high-performance liquid chromatography with fluorometric detection. For detection of free tryptophan, samples were filtered through a 10-kd filter (Chemicon; Millipore Corporation, Bedford, Mass). Tryptophan in the ultrafiltrate was measured by high-performance liquid chromatography with fluorometric detection.[52]

DATA ANALYSIS

All behavioral and biochemical data were analyzed with a 4-way analysis of variance (ANOVA) with repeated measures using genotype (*l*/*l*, *s*/*l*, and *s*/*s*), and FH (FH+ vs FH–) as between-subject factors, and condition (TD vs sham depletion) and time as within-subject factors. Potential order effects were assessed with a 5-way ANOVA including order of depletion sessions. With all Huynh-Feldt correction coefficients equal to 1, sphericity of the repeated-measures design was assumed, and thus uncorrected *P* values are reported. Significant interactions found in the ANOVAs were further examined with paired *t* tests, comparing baseline scores with peak values during the depletion session. Between-group differences were assessed by means of unpaired *t* tests. Since all *t* tests were hypothesis driven, no multiple-comparison correction was made in the individual *t* test. Pearson correlation coefficients were calculated to evaluate the relationship between plasma tryptophan levels and behavioral changes. Results are reported as means±SDs. Differences were considered significant at $P<.05$ (2-tailed).

higher levels of 5-HTT function and expression than did the *s*/*l* and *s*/*s* genotypes, which did not differ significantly from each other. Altogether, both in vitro and in vivo studies showed that the *s*-allele leads to reduced transcription and expression.

In the initial study[22] linking *5HTTLPR* genotypes and behavioral variants, the authors report that individuals with either 1 or 2 copies of the *s*-promoter region variant exhibit significantly greater levels of neuroticism, defined as increased levels of anxiety, hostility, and depression, than subjects homozygous for the long genotype in the sample as a whole and also within sibships. Also, the subjects exhibited increased scores for Harm Avoidance on the Tridimensional Personality Questionnaire.[23] Subsequent attempts to replicate associations between *5HTTLPR* and personality traits agreed with these initial findings,[24,25] but others disagreed.[26-28] Ongoing research explores possible associations between the *5HTTLPR* variants and categorically defined neuropsychiatric disorders, including affective illness,[20,29,30] panic disorder,[31] autism,[32] obsessive-compulsive disorder,[33] schizophrenia,[34] alcoholism,[35] and Alzheimer disease.[36,37] Results of studies disagree about the potential role of *5HTTLPR* in the pathogenesis of depression.[29,38-40]

Tryptophan depletion (TD) is a widely used research paradigm to study the behavioral effects of transient reduced synthesis of brain serotonin[41,42] by depletion of its precursor tryptophan.[43,44] The literature on the behavioral effects of TD in healthy control subjects is somewhat controversial. Taken together, all studies report a high variability in the mood responses to TD, and depressed mood occurred in only a subset of the subjects.

The variability in the reported mood-lowering effects of TD in healthy subjects may result from a differing susceptibility to the behavioral effects of TD. The present study was designed to explore the relationship between family history (FH) of depression, *5HTTLPR* genotypes, and behavioral and biological responses to TD. We recruited healthy women with and without a positive first-degree FH of depression. We hypothesized that women with a positive first-degree FH of depression will experience a transient lowering of their mood during TD, in contrast to women without a positive first-degree FH of depression. On the basis of the evidence that the *s*-allele of the *5HTTLPR* is associated with reduced 5-HTT functions, we tested the hypothesis that women carrying the *s*/*s* and *s*/*l* genotypes are more vulnerable to the mood-lowering effects of TD and thus will show more pronounced behavioral responses.

RESULTS

BIOCHEMICAL EFFECTS

The administration of the tryptophan-deficient amino acid mixture resulted in a profound decline in plasma total and free tryptophan concentrations (**Table 2**), whereas only modest decreases were observed during sham depletion. The repeated-measures ANOVA of plasma tryptophan was significant for both total tryptophan (condition×time interaction: $F_{3,117}=149.42$, $P<.001$) and free tryptophan (condition×time interaction: $F_{3,117}=68.30$, $P<.001$). There was no effect of *5HTTLPR* genotype (genotype×condition×time interaction: $F_{6,117}=0.20$, $P=.98$) and FH of depression (FH×condition×time interaction: $F_{3,117}=1.20$, $P=.31$) for free tryptophan. The order of depletion sessions (tryptophan-deficient amino acid mixture or lactose first) did not affect the outcome.

BEHAVIORAL EFFECTS

The 4-way ANOVA assessing the effects of TD in the sample as a whole determined a significant genotype×FH×condition×time interaction ($F_{6,117}=3.13$, $P=.007$). The evaluation of to what extent FH influenced the behavioral responses to TD showed a significant FH×condition×time interaction ($F_{3,117}=2.99$, $P=.03$). This

Table 1. Demographic Characteristics of Healthy Female Subjects With (FH+) and Without (FH–) a Family History of Depression

	5HTTLPR Genotype*					
	FH+			FH–		
	s/s	*s/l*	*l/l*	*s/s*	*s/l*	*l/l*
No. of subjects	5	7	9	4	10	10
Age, mean ± SD, y	24.6 ± 1.8	26.1 ± 1.7	25.3 ± 3.1	27.5 ± 3.1	28.5 ± 8.9	27.2 ± 5.5
No. of relatives/subject, mean ± SD	4.0 ± 0.7	3.3 ± 0.8	3.6 ± 0.9	3.8 ± 0.5	3.0 ± 0.8	3.3 ± 0.9
No. of affected relatives/subject, mean ± SD	1.6 ± 0.5	1.4 ± 0.8	1.6 ± 0.7	0	0	0

*5HTTLPR indicates serotonin transporter gene promoter polymorphism.

Table 2. Changes in Plasma Total and Free Tryptophan Concentrations During Tryptophan Depletion and Sham Depletion*

	5HTTLPR Genotype					
	Tryptophan Depletion			Sham Depletion		
	s/s	*s/l*	*l/l*	*s/s*	*s/l*	*l/l*
Positive Family History of Depression						
Total plasma tryptophan						
Baseline, mg/dL†	11.1 ± 1.1	9.6 ± 1.4	10.3 ± 1.5	9.1 ± 1.4	9.1 ± 1.3	9.7 ± 1.1
Posttreatment (+5 h), mg/dL†	2.6 ± 1.2	2.3 ± 1.2	2.0 ± 1.1	7.9 ± 1.4	7.5 ± 0.9	7.5 ± 1.7
% Change	–77	–76	–81	–13	–17	–22
Free plasma tryptophan						
Baseline, mg/dL†	1.6 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.3 ± 0.5	1.1 ± 0.3	1.1 ± 0.1
Posttreatment (+5 h), mg/dL†	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	1.1 ± 0.5	0.8 ± 0.3	0.9 ± 0.5
% Change	–75	–83	–82	–15	–27	–18
Negative Family History of Depression						
Total plasma tryptophan						
Baseline, mg/dL†	9.1 ± 0.6	10.4 ± 0.7	10.5 ± 0.9	9.3 ± 0.7	10.5 ± 1.3	10.6 ± 1.3
Posttreatment (+5 h), mg/dL†	1.5 ± 0.4	1.8 ± 0.9	2.8 ± 1.0	7.9 ± 1.4	9.2 ± 2.5	8.3 ± 0.9
% Change	–84	–83	–73	–15	–12	–22
Free plasma tryptophan						
Baseline, mg/dL†	0.9 ± 0.3	1.1 ± 0.4	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.2 ± 0.3
Posttreatment (+5 h), mg/dL†	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	0.9 ± 0.3
% Change	–77	–73	–75	–20	–10	–25

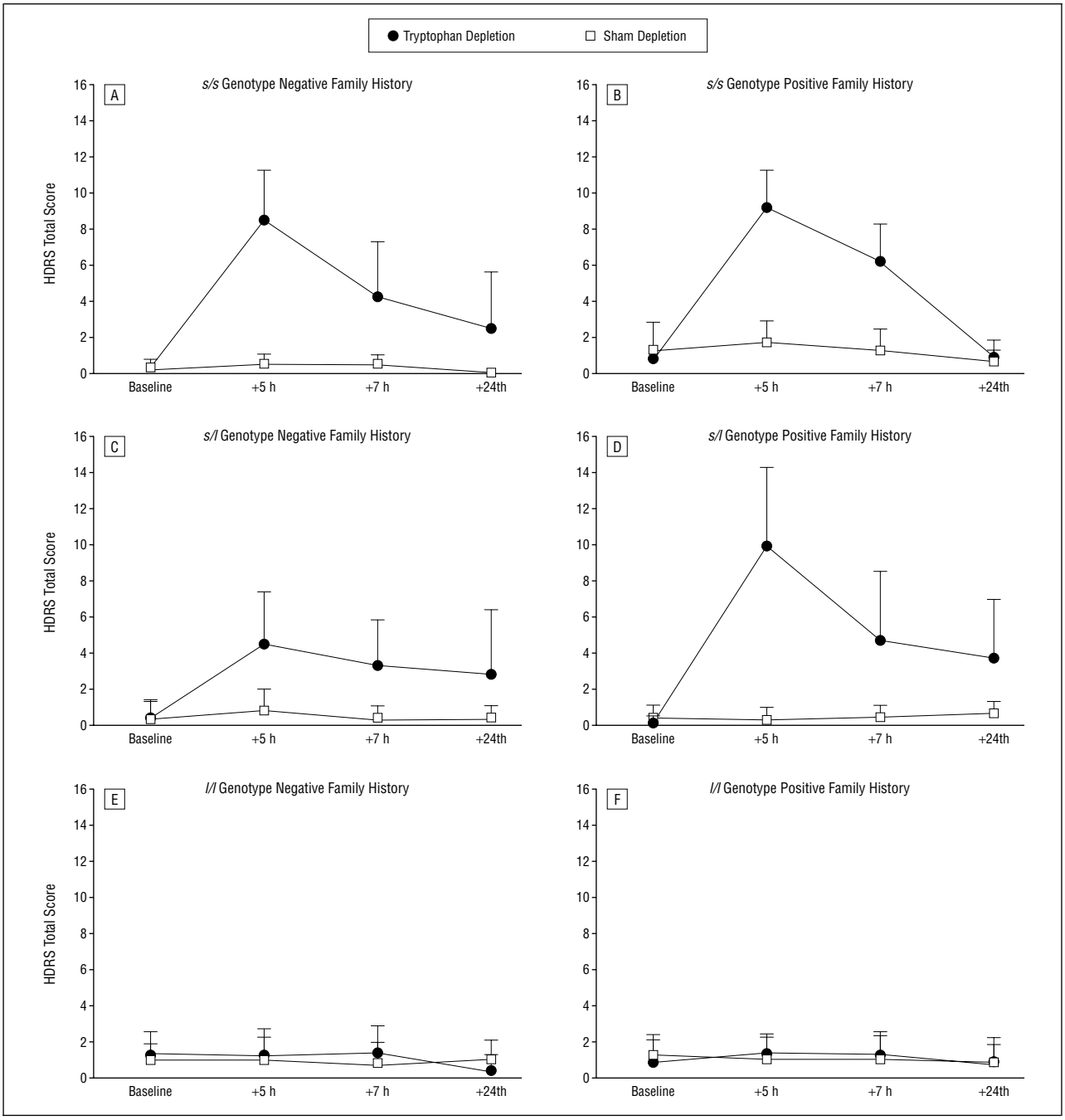
*Data are given as mean ± SD, unless otherwise indicated. *5HTTLPR* indicates serotonin transporter gene promoter polymorphism.
†To convert to micromoles per liter, multiply by 48.97.

indicates that FH+ subjects had more prominent responses to the depressiogenic effects of TD than did FH– subjects.

The evaluation of whether the mood-lowering effects of TD may be explained by *5HTTLPR* genotypes exhibited profound effects. We found a significant main effect of genotype ($F_{2,39}$=9.43, P<.001) and a highly significant genotype × condition × time interaction ($F_{6,117}$=12.54, P<.001). Post hoc comparisons showed significant increases of HDRS total scores from baseline in *s/s* carriers irrespective of their FH of depression (**Figure**, A and B). Most prominent effects of TD were found 5 hours after ingestion of the tryptophan-deficient amino acid mixture (FH+: 0.8±0.5 vs 9.2±2.1, t_4=–10.3, P<.001; FH–: 0.3±0.5 vs 8.5±2.9, t_3=–6.6, P=.007). Similarly, *s/l* carriers showed significant increases of HDRS total scores 5 hours after ingestion of the tryptophan-depleting amino acid mixture (FH+: 0.1±0.4 vs 10.0±4.5, t_6=–5.7, P<.001; FH–: 0.5±0.9 vs 4.6±2.9, t_9=–4.22, P=.002) in both the FH+ and FH– groups (Figure, C and D). Notably, the mood-lowering effects of TD were significantly more pronounced in the FH+ group than in the FH– group (unpaired t test: t_{15}=–3.00, P=.009). In contrast, no significant behavioral changes were found in *l/l* carriers in both the FH+ (t_8=–2.0, P=.08) and FH– (t_9=0.0, P=1.0) groups (Figure, E and F).

Six subjects developed HDRS total scores greater than 10 during TD. Two subjects had HDRS total scores of 16 and 15. However, in none of our subjects did the development of depressive symptoms, reflected by the increase of HDRS scores, require treatment, and the effects were transient. In the morning after TD, all subjects were fully recovered, and most symptoms had completely disappeared.

The analysis of the mood item of the HDRS agreed with the results from the HDRS total scores and indicated that in some individuals one of the core symptoms of depression was affected. We found a trend toward significance in the FH × condition × time interaction ($F_{3,117}$=2.5, P=.06) and a highly significant genotype × condition × time interaction ($F_{6,117}$=4.4, P<.001).



Scores (means±SDs) on a modified version of the Hamilton Depression Rating Scale (HDRS, 21-item version) of healthy female subjects (n=45) with positive and negative first-degree family histories of depression and differing serotonin transporter gene promoter polymorphism *(5HTTLPR)* genotypes.

COMMENT

In the present study, we examined the relationship between neurochemistry, behavior, and genetics. This study is, to our knowledge, the first to assess behavioral responses to TD in healthy female control subjects in relation to their FH of depression and their individual *5HTTLPR* polymorphisms. The *s/s* genotype was associated with an increased risk of developing depressive symptoms during TD, irrespective of the FH for depression. In contrast, women with the *l/l* genotype did not develop depressive symptoms to TD, irrespective of the FH for depression, implying that this genotype exerted a protective effect on mood in the TD paradigm. Finally, healthy female FH– subjects with the *s/l* genotype showed a mood response to TD that was intermediate between those of the *s/s* and *l/l* subjects, while *s/l* FH+ subjects showed the same depressiogenic effects of TD seen in the *s/s* subjects. Conclusively, the *s*-allele of the *5HTTLPR* and a positive FH of depression appear to be additive risk factors for the development of depression during TD.

Previous TD studies in healthy control subjects have shown highly variable mood responses to TD. This may be explained by differing susceptibilities to the mood-

lowering effects of TD. Subjects with no personal history of depression but with a positive FH of affective disorders have been shown to be at risk to develop depressive symptoms during TD,[53-55] although one study disagrees.[56] Although the reasons for this discrepancy are unknown, the reported elevated dropout rate in that study may explain the differing results. Eleven (34%) of 32 subjects did not complete the study because of increased fatigue, loss of interest to complete the study, and having started an antidepressant treatment during the study. It can be speculated that, at least in some of these subjects, depressive symptoms may have occurred. Studies assessing mood responses to TD in healthy subjects without an FH of affective disorders suggest a greater risk for women to develop depressive symptoms during TD.[54,57] However, these findings remain controversial and could not be replicated by others.[58-60] Altogether, these studies and the present findings suggest that responses to lowered tryptophan availability may reveal a genetic vulnerability to depression in some individuals.

The present study has a number of potential limitations that warrant discussion. First, the sample size is large relative to previous TD studies, but subsamples were relatively small. Replication of our findings is needed. Second, we have to acknowledge that we have collected information about the FH of psychiatric illnesses only among first-degree relatives of our study subjects. This may be relevant, since evidence in the literature suggests that families with single cases of affective illnesses may differ from families with multiple affected individuals in different generations.[61,62] However, a strength of the present study is the personal interviews of the first-degree relatives, providing us with detailed information about psychiatric and medical illness and treatment of each first-degree family member.

Moreover, we studied only women in the present study, and thus our findings cannot be generalized to men. In view of the evidence on gender-related differences in serotonin system functioning in animals[63-65] and in humans,[41,66] we decided to include only women in the present study. The importance of differential modulation of serotonergic transmission between males and females is supported by their differing responsivity and tolerance to selective serotonin reuptake inhibitors and tricyclic antidepressants in the treatment of depression.[67] Previous studies showed that TD induces lowering of mood in FH+ males,[53] but not in FH– males.[53,68] However, *5HTTLPR* genotypes have not been assessed in the noted studies. Thus, it is of interest to extend our findings to a sample of healthy men with different *5HTTLPR* genotypes.

Another issue that raised our attention was the relatively young age of the subjects in the present study. However, we do not believe that the age of our subjects influenced the results of this study. Previous studies have shown that most patients with recurrent unipolar depression with at least 1 affected family member had experienced a depressive episode before 25 years of age.[69] Furthermore, the modal age at onset of depression is slightly lower in women than in men[70] and is even earlier in the offspring of depressed parents.[71-73]

We acknowledge that the use of a modified procedure to deplete tryptophan makes it difficult to compare results with those of other TD studies. This study used a smaller amino acid load (32 g) than previous TD studies (100 g). Nevertheless, we found decreases in plasma total and free tryptophan levels of 73% to 84% and 73% to 83%, respectively. This is comparable with other TD studies using the original method of Young and colleagues.[74] It must be considered that brain tryptophan concentrations depend not only on plasma tryptophan concentrations, but also on the concentrations of the other large neutral amino acids, competing with tryptophan for uptake at the blood-brain barrier.[75-77] Thus, one cannot conclude that the behavioral effects result from TD per se, since behavioral changes resulting from higher levels of the other amino acids cannot be excluded.

The question arises whether *5HTTLPR* genotypes may serve as genetic markers linked to different risks for developing depression in healthy subjects. This would be of particular interest and relevance because the proportions of *5HTTLPR* genotypes in the general population are as follows: *s/s*, 0.16; *s/l*, 0.48; and *l/l*, 0.36.[22,25] Thus, a substantial proportion of the general population are carriers of the *s*-allele. Preliminary evidence supports such an assumption. Neonates carrying the *s*-allele of the *5HTTLPR* show lowered alertness and visual and auditory behavior, perhaps reflecting reduced adult novelty-seeking behavior.[78] Follow-up studies of this sample showed most negative emotionality and most distress to daily situations in infants with the *s/s 5HTTLPR* genotype.[79] However, to answer the question of whether the *s*-allele is associated with an increased risk of developing depression, carefully designed, prospective genetic epidemiologic studies are needed.

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