Article

Serotonin Transporter Gene (SLC6A4) Promoter Polymorphisms and the Susceptibility to Posttraumatic Stress Disorder in the General Population

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Objective: There has been debate whether polymorphisms within the serotonin transporter-linked polymorphic region (5-HTTLPR) moderate susceptibility to posttraumatic stress disorder (PTSD). The authors investigated 5-HTTLPR genotypes and their interaction with the number of traumatic events in the prediction of PTSD in a general population sample.

Method: Analyses were based on data from 3,045 subjects who participated in the Study of Health in Pomerania. All participants were assessed with the PTSD module of the Structured Clinical Interview for DSM–IV. The short (S)/long (L) polymorphism of 5-HTTLPR (rs4795541) and the A-G polymorphism (rs25531) were genotyped.

Results: Among the participants, 1,663 had been exposed to at least one traumatic event, and 67 (4.0%) developed PTSD. Among those who had experienced less than three traumatic events, the lifetime prevalence of PTSD was 2.6%, 3.5%, and 4.3% for those with zero, one, and two L_A alleles, respectively, but the lifetime prevalence was 0%, 7.3%, and 19.6%, respectively, among those with three or more traumatic experiences. This finding suggests that there is an additive excess risk for frequent trauma in the L_A/L_A genotype, which was confirmed by the relative excess risk due to interaction (RERI). In allelic analysis, RERI was 3.3. Thus, the odds ratio for PTSD in L_A allele carriers exposed to three or more traumas was 3.3 times higher as a result of the interaction between PTSD and the L_A allele.

Conclusions: An additive gene-environment interaction with the high expression L_A allele of 5-HTTLPR and frequent trauma in PTSD was found. The attributable proportion indicated that more than 60% of all L_A allele carriers who were exposed to three or more traumas developed PTSD as a result of an interaction between genotype and exposure.

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Since the introduction of posttraumatic stress disorder (PTSD) as a diagnosis in DSM–III (1), PTSD has been recognized as a major issue in public health (2, 3). PTSD is characterized by distressing and/or impairing symptoms that occur after experiencing, witnessing, or being confronted with a traumatic event that includes an actual or perceived threat to the self or others (4). The disorder involves repeated and intrusive memories related to the trauma, avoidance of trauma-related stimuli, and hyperarousal (4).

Although the majority of individuals exposed to traumatic stress will experience some kind of transient distress, only a minority will develop PTSD (5, 6). Genetic factors have been shown to explain a relevant proportion of the variance of PTSD and are assumed to moderate the vulnerability to the adverse effects of traumatic stress (7). To date, very few studies have addressed the issue of genetic vulnerability to PTSD (8–10). There is evidence that the short (S) allele of the serotonin transporter-linked polymorphic region (5-HTTLPR) is associated with an increased sensitivity for anxiety (11) and depression (12–14) when a person is exposed to stressful life events. In addition, an association between the S allele and an increased reactivity of the amygdala was found in healthy subjects (15, 16). Thus, the 5-HTTLPR constitutes a genetic candidate region that may modulate emotional responses to traumatic events and thereby influence the risk for the development of PTSD. One Korean study (17) found an association of the 5-HTTLPR gene with PTSD in a clinical population. Recently, Kilpatrick et al. (18) investigated a posthurricane population in Florida (N=589), with 19 subjects (3.2%) suffering from posthurricane PTSD. Kilpatrick et al. reported an interaction among the following variables: high hurricane exposure, low social support, and the S/S genotype of the 5-HTTLPR. No direct gene effects on PTSD or two-way interactions were found. Given the small number of subjects with PTSD in the Kilpatrick et al. study and the fact that the genotype only became statisti-

TABLE 1. Baseline Characteristics of the Analytic Sample of Subjects Exposed to at Least One Traumatic Event

Characteristic	Total Sample (N=3,045)				Subjects With ≥1 Traumatic Experience			
	Subjects Without Traumatic Experience (N=1,382)		Subjects With ≥1 Traumatic Experience (N=1,663)		No Lifetime PTSD (N=1,596)		Lifetime PTSD (N=67)	
	N	%	N	%	N	%	N	%
Gender (female)	741	53.6	830	49.9*	787	49.3	43	64.2*
Education (years)								
<10	457	33.1	774	46.5***	736	46.1	38	56.7
10 (reference)	739	53.5	653	39.3	626	39.2	27	40.3
>10	186	13.5	236	14.2**	234	14.7	2	3.0*
Depression (past 12 months)	67	4.8	162	9.7***	129	8.1	33	49.3***
Mini-Mental State Examination status (score ≤23)	35	2.5	67	4.0*	59	3.7	8	11.9**
Symptom criteria present[a]								
Subjects with B criterion symptoms	0	0	1,101	66.2	1,034	64.8	67	100
Subjects with C criterion symptoms	0	0	642	38.6	575	36.0	67	100
Subjects with D criterion symptoms	0	0	90	5.4	23	1.44	67	100
5-HTTLPR genotypes								
SS (reference)	202	14.6	270	16.2	264	16.5	6	9.0
S_LG	75	5.4	92	5.5	91	6.8	1	1.5
S_LA	599	43.3	702	42.2	675	42.3	27	40.3
L_GL_G	9	0.7	10	0.6	9	0.6	1	1.5
L_AL_G	96	7.0	114	6.9	109	6.8	5	7.5
L_AL_A	401	29.0	475	28.6	448	28.1	27	40.3*
5-HTTLPR "triallelic" genotypes								
SS or S_LG or L_GL_G (reference)	286	20.7	372	22.4	364	22.8	8	11.9
L_AL_G or S_LA	695	50.3	816	49.1	784	49.1	32	47.8
L_AL_A	401	29.0	475	28.6	448	28.1	27	40.3*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	50.0	13.3	57.6	15.6***	57.6	15.5	57.9	17.0
Number of traumatic events	0	0	1.6	1.0	1.6	0.9	2.2	1.3***

[a] ≥1 positive item.
*p<0.05. **p<0.01. ***p<0.001.

cally significant at the level of three-way interaction, these results should be regarded with caution.

Based on meta-analyses and power simulation on gene-by-environment interaction studies in depression, Munafo et al. (14) concluded that findings implicating the S allele of the 5-HTTLPR could have occurred by chance alone. Munafo et al. pointed to the need for more rigorous methodological approaches in gene-by-environment research.

In light of these limitations of the research thus far, the objective of our general population study of 3,045 community residents was twofold. We aimed to 1) investigate the direct effects of 5-HTTLPR on PTSD and 2) assess gene-by-environment effects, following an approach proposed by several experts (19–23) to examine possible biological interactions. We used the number of traumatic events as a quantitative environmental exposure variable because the number of traumatic events can be considered an approximation to the individual severity of exposure. Accordingly, higher numbers of traumatic events have been associated with an increased risk of subsequent PTSD (24).

Method

General Population Sample

In the present study, data from the Study of Health in Pomerania were used (13, 25, 26). The target population was made up of adult German residents (age range: 20 to 79 years old) residing in Northeastern Germany in three cities and 29 communities, with a total population of 212,157. The total number of eligible participants in the sample was 6,267. Of these, 4,310 Caucasian subjects participated in the Study of Health in Pomerania–0 (1997–2001). Follow-up examination (Study of Health in Pomerania–1) was conducted 5 years after baseline, and 3,300 subjects were assessed. All participants gave written informed consent. The study was approved by the local Institutional Review Board and conformed to the principles of the Declaration of Helsinki. Complete sociodemographic and psychometric data as well as the 5-HTTLPR genotypes were available from 3,045 subjects from the Study of Health in Pomerania–1.

Interview and Psychometric Assessments

The health-related interview of the Study of Health in Pomerania–1 involved the PTSD module of the Structured Clinical Interview for DSM-IV (SCID) (27), the Mini-Mental State Examination (28), and the Composite International Diagnostic–Screener (29).

The PTSD module of the SCID (27) was used to assess trauma exposure and PTSD. The following traumatic events were assessed: combat or war zone experience, physical assault, rape, childhood sexual abuse, natural disaster, serious or nearly fatal accident, imprisonment and/or torture, life-threatening illness, sudden and unexpected death of a loved one, and witnessing or learning about traumas experienced by others. If a participant answered "no" to each of the trauma questions, the module was terminated. In the event of trauma exposure, the interview was continued, assessing DSM-IV PTSD symptoms, including criterion A2 (experiencing high distress during/after the event), criterion B (five re-experiencing symptoms), criterion C (seven avoidance symptoms), and criterion D (five arousal symptoms). For the diagnosis of PTSD to be determined, we required at least one symptom of re-experiencing, three avoidance symptoms, and two symptoms of hyperarousal. If participants did not pass the re-

TABLE 2. Association Between the PTSD Diagnosis and SLC6A4 Genotypes Among Subjects Exposed to at Least One Traumatic Event (N=1,663)

	Subjects With PTSD	Subjects Without PTSD		Analysis (logistic regression)					
				Unadjusted			Age and Gender Adjusted		
Genotype	N	N	Absolute PTSD Risk (%)	Odds Ratio	95% CI	p	Odds Ratio	95% CI	p
SS or SL_G or L_GL_G (reference)	8	364	2.2	1			1		
L_AL_G or SL_A	32	784	4.1	1.9	0.8–4.1		1.8	0.8–4.1	
L_AL_A	27	448	6.0	2.7*	1.2–6.1		2.7*	1.2–6.0	
Genotypes						0.04			0.05
Number of L_A alleles						0.01			0.01

[a] Fully adjusted for age (10-year age groups [20s, 30s, 40s, etc.]), gender, age and gender, number of traumatic events, low (<23) Mini-Mental State Examination scores (yes/no), and depression within the last 12 months (yes/no).
*p<0.05.

TABLE 3. Allelic Interaction Adjusted for 1,663 Subject Clusters Between Lifetime Diagnosis of PTSD and the Number of Traumatic Experiences

		Subjects With PTSD	Subjects Without PTSD		Analysis (logistic regression)					
					Unadjusted		Age and Gender Adjusted		Fully Adjusted[a]	
Exposed to Three or More Traumatic Events	Risk Allele	N	N	Absolute PTSD Risk (%)	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
No	S or L_G (reference)	39	1,266	3.1	1		1		1	
No	L_A	57	1,454	3.9	1.3	0.8–1.9	1.3	0.8–1.9	1.2	0.8–1.9
Yes	S or L_G	9	246	3.7	1.2	0.5–2.6	1.5	0.6–3.4	1.1	0.5–2.8
Yes	L_A	29	226	12.8	4.2*	2.2–7.8	5.3*	2.6–10.8	4.7*	2.1–10.1

[a] Fully adjusted for age (10-year age groups [20s, 30s, 40s, etc.]), gender, age and gender, low (<23) Mini-Mental State Examination scores (yes/no), and depression within the last 12 months (yes/no).
*p<0.05.

quired diagnostic threshold (e.g., at least one re-experiencing symptom), the interview was terminated.

Depression that occurred during the 12 months prior to the examination was assessed by face-to-face interview using the Composite International Diagnostic–Screener (29). The screening questions for depressive disorders, according to DSM-IV, were as follows: "feelings of sadness or depressed mood for a period of at least 2 weeks" and "lack of interest, tiredness, or loss of energy for a period of at least 2 weeks." If both items were endorsed, we assigned the label "depression at the syndrome level." Based on the Mini-Mental State Examination score (28), subjects were classified as low (Mini-Mental State Examination score <23) or high (Mini-Mental State Examination score ≥23) scoring. This variable was used to adjust for cognitive decline in some older participants.

Genotyping of the 5-HTTLPR

The SLC6A4 gene harbors a variable number tandem repeat polymorphism in the transcription control region of the gene that is located approximately 1 kb upstream from the transcription initiation site. This area has been associated with differential expression of the transporter (rs4795541) (30). Both variants (S and long [L]) differ by a 43 base pair insertion/deletion. Within the inserted fragment, an additional common single nucleotide polymorphism (SNP) occurs (rs25531) and has been reported to further affect the transcriptional activity of the SLC6A4 promoter by the genotype-dependent generation of an AP2 transcription factor binding site in the rs25531 G allele (31). Together, this leads to the thought that 5-HTTLPR is triallelic with the following alleles: S, L_A, and L_G.

We developed a restriction fragment length polymorphism method that allows for determination of both variants (S/L; rs25531) within one assay. The 5-HTTLPR region was polymerase chain reaction amplified using the oligonucleotide primers SLC6A4_SE (5′-CTCCTAGGATCGCTCCTGCATC-3′) and SLC6A4_AS (5′-GGACCGCAAGGTGG-GCGGGAGGCTTGGAG-3′), resulting in amplicons of 294 base pairs for the S variant and 337 base pairs for the L variant. The restriction enzyme *BcnI* (Fermentas) digested the rs25531 variant differentially, in addition to two constitutive restriction sites in the amplicon. This resulted in the following fragments: 200, 61, and 33 base pairs for the S allele; 243, 61, and 33 base pairs for the L_A allele; and 70, 173, 61, and 33 base pairs for the L_G allele. The detection of fragments of 173, 200, and 243 base pairs in 4% agarose gels allowed for allocation to the respective alleles. Representative samples of different genotypes were further verified by sequencing of the amplicons. Based on previous reports on gene expression, we classified the genotypes into the following three functional "triallelic" genotypes: L_A/L_A; L_G/L_A or S/L_A; and L_G/L_G or L_G/S or S/S (18, 31).

The main analyses were repeated using the classical "biallelic" classification without separating L_G from L_A (see the data supplement accompanying the online version of this article).

Statistical Analyses

Comparisons between groups were performed using Mann-Whitney U tests (continuous data) and unadjusted logistic regression (nominal data). The Cochran-Armitage Trend Test was used to determine an additive mode of inheritance. Criteria B, C, and D were dichotomized into present (≥1 positive item) or absent (0 items). The analysis of direct gene effects between the triallelic genotypes and the diagnosis of PTSD was performed using logistic regression.

To assess gene-environment interaction using the crude prevalence of lifetime PTSD, we used tables with three genotype categories crossed with two levels of trauma frequency (19, 20). The number of traumatic events was dichotomized (<2/≥2 events, <3/≥3 events, and <4/≥4 events) and analyzed in separate regression models. Estimated absolute risks, odds ratios, and 95% confidence intervals (CIs) were calculated for the resulting six-by-two

Age, Gender, and Number of Traumatic Events Adjusted			Fully Adjusted[a]		
Odds Ratio	95% CI	p	Odds Ratio	95% CI	p
1			1		
2.0	0.9–4.4		1.8	0.8–4.1	
2.8*	1.3–6.4		2.8*	1.2–6.5	
		0.04			0.04
		0.01			0.01

tables. In several cases, conventional maximum likelihood estimations for the dichotomized outcome were potentially biased as a result of an expected cell count <5. We examined this situation in three ways. First, based on the bootstrap approach, robust estimates were calculated as described in the present article. In any instance in which there was a genotype-trauma combination with no cases of PTSD, median-unbiased estimates were used. Second, odds ratios and CIs were alternatively estimated using a semi-Bayes approach (32). The posterior approximations were derived from an ordinary logistic regression (32). Third, the number of symptoms for criterion D was regressed in a count model (negative binomial regression).

Genotype differences in susceptibility to trauma were estimated by biological interaction, which is measured by departure from an additive model (22). For the quantification of the magnitude of an interaction effect, the relative excess risk due to interaction (RERI) was calculated. To clarify this, we computed the crude absolute PTSD prevalence for each category and then the absolute excess risk due to additive interaction as well as the corresponding crude RERI. It is not straightforward to compute the adjusted absolute excess risk in logistic regression. However, the adjusted RERI is easily estimated using the following basic formula for RERI (22) (OR=odds ratio):

$$RERI=OR_{\text{risk allele and high trauma}}-OR_{\text{risk allele and low trauma}}-OR_{\text{no risk allele and high trauma}}+1$$

Additionally, the proportion of disease among subjects with both exposures (traumatic events ≥3 and at least one L_A allele) that was attributable to their interaction (attributable proportion) was calculated for two dichotomous determinants as follows (22) (AP=attributable proportion):

$$AP=RERI/OR_{\text{risk allele and high trauma}}$$

As recommended (22), the coding was chosen in such a manner that in the presence of additive interaction, both RERI and attributable proportion were greater than zero; whereas in the absence of interaction, both RERI and attributable proportion were equal to zero. If the 95% CI excluded zero, then the p value for the interaction was significant ($p<0.05$). A bias-corrected and accelerated bootstrap approach (10,000 bootstrap samples) was applied in order to yield the CI of RERI and attributable proportion (19, 21). RERI and attributable proportion were calculated for an allelic model (L_A versus L_G/S), a dichotomized model (zero or one versus two L_A alleles), and a continuous genetic model (zero, one, or two L_A alleles).

All analyses were performed as unadjusted (crude), as adjusted for age (10-year age groups [20s, 30s, 40s, etc.]) and gender, and as "fully adjusted" models, including low (<23) Mini-Mental State Examination scores (yes/no) and depression within the last 12 months (yes/no). Analyses were performed with STATA/SE software, version 10.1 (StataCorp LP, College Station, Tex.). Power analyses were performed using the program POWER (33).

Results

The description of clinical and demographic characteristics of the sample is provided in Table 1. There was no departure from the Hardy-Weinberg equilibrium (total sample [triallelic]: $\chi^2=2.53$, df=2, p=0.28; exposed sample [triallelic]: $\chi^2=1.18$, df=2, p=0.55). All genetic analyses were based on 1,663 subjects who reported exposure to at least one traumatic event. Sixty-seven subjects (4.03%) met the diagnostic criteria for lifetime diagnosis of PTSD. There were no associations between the number of traumatic events and the triallelic genotypes ($F=0.115$, df=2, 1660, p=0.89).

Gene Main Effects

Main effects of the three genotypes with the diagnosis of PTSD (L_A/L_A; L_G/L_A or S/L_A; and L_G/L_G or L_G/S or S/S) were analyzed by logistic regression analysis (Table 2). All associations between the L_A/L_A genotype and PTSD (both crude and adjusted) were statistically significant. There was clear evidence for a dose-response relationship, since among subjects with zero, one, and two L_A alleles the crude proportion of PTSD increased from 2% to 4% and 6%, respectively. The Cochran-Armitage test indicated a clear additive mode for L_A alleles ($\chi^2=6.78$, df=1, p=0.009), with no departure from linearity ($\chi^2=0.00$, df=1, p=0.99). The allelic analyses revealed an effect for the L_A allele (odds ratio=1.6; 95% CI=1.1–2.4) but not for the more rare L_G allele (odds ratio=0.7; 95% CI=0.3–1.6) on PTSD in the fully adjusted model (see the data supplement accompanying the online version of this article). No associations were established between the 5-HTTLPR genotypes and criteria B, C, or D ($p\geq0.05$).

Gene-Environment Interaction

In allelic analyses, the estimated absolute risk among subjects who were not exposed to three or more traumatic events was 3.1% for those without the L_A allele and 3.9% for those with the L_A allele; whereas the risk among subjects who were exposed to three or more traumatic events was 3.7% for those without the L_A allele but 12.8% for those with the L_A allele (Table 3). Thus, based on the same difference in absolute risks for the L_A allele in trauma-exposed and nonexposed subjects, the absolute additive excess risk for trauma-exposed subjects with the L_A allele was as follows: 12.8%–3.7%–3.9%+3.1%=8.3%. The unadjusted RERI was then 8.3%/3.1%=2.7, which is in terms of the odds ratio equivalent to 2.7=4.2–1.2–1.3+1 (Table 3). Thus, the unadjusted odds ratio of having the lifetime diagnosis of PTSD among subjects with ≥3 traumas and the L_A allele relative to those without the L_A allele was 2.7 times higher than the condition in which no interaction between traumas and the L_A allele was present (e.g., absolute risk of 4.5% instead of 12.8% or, equivalently, odds ratio=4.5/3.1=1.5 instead of odds ratio=4.2). For the final model, RERI was 3.3 (95% CI=1.2–8.2 [Table 4]). Importantly, RERI–1>0 holds for the 95% CI. The unadjusted at-

TABLE 4. Interaction Between the Number of Traumatic Events and L_A Alleles for Lifetime Diagnosis of PTSD (outcome)

Exposed to Three or More Traumatic Events	L_A Allele	Subjects With PTSD	Subjects Without PTSD		Analysis (logistic regression)					
					Unadjusted		Age and Gender Adjusted		Fully Adjusted[a]	
		N	N	Absolute PTSD Risk (%)	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
No (reference)	0 (reference)	8	303	2.6	1		1		1	
No	1	23	660	3.5	1.3	0.6–3.0	1.3	0.6–3.0	1.1	0.5–2.5
No	2	17	397	4.3	1.6	0.7–3.8	1.6	0.7–3.8	1.5	0.6–3.6
Yes	0	0	61	0.0	0.5[b]	0–3.0	0.6[b]	0–5.3	0.7[b]	0–7.2
Yes	1	9	124	7.3	2.7	1.0–7.3	3.6	1.3–9.9	2.7	0.9–7.8
Yes	2	10	51	19.6	7.4[c]	2.8–19.7	9.7[c]	3.4–27.4	8.0[c]	2.6–24.1

[a] Fully adjusted for age (10-year age groups [20s, 30s, 40s, etc.]), gender, age and gender, low (<23) Mini-Mental State Examination scores (yes/no), and depression within the last 12 months (yes/no).
[b] Data show median unbiased estimates based on 372 observations (reference and current line: 8+303+0+61).
[c] Data analysis for robust estimators are as follows: odds ratio=7.4 (95% CI=2.4–23.3), odds ratio=9.7 (95% CI=2.7–35.3), and odds ratio=8.0 (95% CI=2.0–32.4) for crude adjusted, age and gender adjusted, and fully adjusted models, respectively.

tributable proportion was 2.7/4.2=0.64 (64%), and the fully adjusted attributable proportion was 71% (95% CI=41–88), meaning that approximately 71% of PTSD diagnoses among subjects with ≥3 traumas and the L_A allele was attributable to the interaction between the two.

To avoid a sparse data problem, referring to a genotype-trauma combination with no cases of PTSD (Table 4), subjects with zero or one L_A allele were grouped into one category versus subjects with two L_A alleles. The absolute risks were 31/963=3.2% and 4.3% among nonexposed subjects without and with two L_A alleles, respectively, and 9/185=4.9% and 19.6% among exposed subjects without and with two L_A alleles, respectively (Table 4). This type of RERI was crude (13.6%/3.2%=4.25) and fully adjusted (5.5) (95% CI=0.8–18.3, p<0.05). The unadjusted attributable proportion was 4.25/(10×963/[31×51])=4.25/6.1=0.70 (70%), and the fully adjusted attributable proportion was 74% (95% CI=18–92).

Since the allelic analyses supported that subjects with only one L_A allele possibly had a higher risk for PTSD when trauma exposed compared with those subjects carrying no L_A alleles, we calculated RERI for the number of L_A alleles as a continuous variable. This type of RERI directly estimates the relative excess risk for one L_A allele compared with no L_A alleles. In the present study, RERI was 1.3 for the unadjusted model and 1.5 (95% CI=0.5–4.3, p<0.05) for the fully adjusted model (Table 4). This means that among subjects with ≥3 traumas the odds ratio of having PTSD was 1.5 times higher with one L_A allele compared with the absence of interaction between the L_A alleles and traumas. We confirmed these results using a Bayesian approach to address the sparse data problem (see the data supplement accompanying the online version of this article). Prior limits similar to the related CIs of the fully adjusted model for the number of symptoms from the D criterion were utilized (see the data supplement accompanying the online version of this article).

When four instead of three was used as a cutoff point for the number of traumas, RERI for the dichotomized genotype (zero or one L_A allele versus two L_A alleles) increased to 8.8 (95% CI=1.6–31.7, p<0.05) and attributable proportion increased to 86% (95% CI=33–97). Given the lower number of subjects with ≥4 traumatic events, the continuous models were less robust. When two was used as a cutoff point for the number of traumas, no statistically significant interactions emerged.

For the number of symptoms from the D criterion as the outcome (see the data supplement accompanying the online version of this article), no genotype-trauma combination with a zero count occurred. RERI for the continuous genetic model (zero, one, or two L_A alleles) was 1.2 (95% CI=0.5–2.8, p<0.05) and attributable proportion for the continuous genetic model was 72% (95% CI=23–126). No statistically significant interactions emerged when performing the analyses for B and C criteria irrespective of the trauma threshold.

Discussion

Our results demonstrate a clear gene main effect, with an additive relationship between the number of L_A alleles and the diagnosis of PTSD in community residents exposed to at least one traumatic event. The specificity of the "gain-of-function" L_A allele compared with the L_G allele on PTSD could be shown in the allelic analyses, since the effect of the L_G allele was not different from that of the S allele (see the data supplement accompanying the online version of this article). Further, significant gene-by-environment interactions with moderate CIs between the L_A alleles and the number of traumatic events on the manifestation of PTSD were established for ≥3 traumatic events. These interaction effects could already be shown in carriers of one L_A allele (versus zero L_A alleles) but were much stronger in carriers of two L_A alleles. The proportion of subjects with PTSD among those with both exposures (traumatic events ≥3 and at least one L_A allele) that was attributable to the interaction between traumatic events (<3 events/≥3 events) and the L_A alleles ranged from 61% to 74%, depending on the model. Since RERI–1>0 holds for the allelic analysis, a stronger condition than RERI>0 was met and a causal synergistic action may be assumed (34).

The 5-HTTLPR was not associated directly or through interaction with re-experiencing (criterion B) or avoidance behavior (criterion C). However, for the D criterion (hyperarousal), gene-by-environment effects were found. Since most subjects with the D criterion symptom also fulfilled the diagnosis of PTSD, we are unsure whether the association between the L_A alleles and hyperarousal was specific or emerged from the overlap with PTSD.

Our results of the increased susceptibility for PTSD in carriers of the L_A allele contradict other clinical and neurobiological findings that found the S allele to be associated with a higher susceptibility to anxiety and depression (12, 13, 18). However, many findings on the neurobiological function of the 5-HTTLPR were derived from cell cultures and animal models and have not been validated in humans (35). Some functional magnetic resonance imaging (fMRI) studies found an increased amygdala reactivity to fearful stimuli in healthy subjects carrying the S allele (15, 16). In contrast, a recent fMRI study in adolescents suffering from anxiety and depression found the L_A/L_A genotype to be associated with increased amygdala reactivity, whereas healthy adolescents showed an increased amygdala response when carrying the S(L_G) allele (36). This points to a putative differential neurobiological effect of the L_A versus the S(L_G) allele, depending on the psychobiological background of the probands.

Interestingly, obsessive-compulsive disorder (OCD) has recently been associated with "gain-of-function" polymorphisms of the L allele that increase the transcription of the serotonin transporter (31, 37). OCD shares psychopathological features with PTSD, such as conditioned fear, intrusive thoughts, avoidance behavior, and increased emotional and physiological arousal (38). A reduced synaptic availability of serotonin as a result of an increased reuptake might represent a risk factor for decreased prefrontal control over disinhibited subcortical circuits in PTSD and OCD (39, 40).

Our findings contradict the results of Kilpatrick et al. (18), which emphasized the problem of statistical power. Modeling the 80% power ($p<0.05$, two-sided) of a direct gene effect for our exposed sample, the detection of an odds ratio as low as 1.65 per L_A allele was feasible with 80% power in our sample. In contrast, in the study conducted by Kilpatrick et al. (18) the power was <50% for an effect size as low as 1.65 per S allele. Moreover, power simulations for the additive gene-by-environment effects in our sample demonstrated 76% power for a RERI=4 for the dichotomized model (L_A/L_A versus all other genotypes) (see the data supplement accompanying the online version of this article). In fact, we even found a RERI=5.5 in our analyses. Therefore, it is rather unlikely that the direct and gene-by-environment effects in our study occurred by chance.

Lee et al. (17) reported an association of the S allele with PTSD in patients in Korea. However, PTSD in clinical samples may be confounded by a high degree of comorbid psychopathology, and selection bias may occur through treatment seeking behavior. Moreover, the allele distribution of the S/L polymorphism in Asians is reversed relative to Caucasians. Further, Lee et al. (17) did not analyze the gain-of-function polymorphism rs25531. Thus, the validity of their results is limited, especially in Caucasian samples.

Limitations of the Study

In the present study, we cannot exclude the possibility that subjects with a high symptom load or PTSD had recall biases (i.e., subjects who reported more traumatic events in the past than those without PTSD or who reported fewer events because they only recalled the most severe traumatic events in the past). This misclassification is differential, and the direction of bias is unknown. However, we used a validated questionnaire in order to reduce the recall bias (27). Although the number of subjects with PTSD (N=67) was three times larger than that of the Kilpatrick et al. study and our power analyses demonstrated a robust statistical power, we still have to acknowledge that the sample size in our study was limited.

In addition to the number of traumatic events, the type of trauma may influence the risk for PTSD (24). Therefore, in replication studies, qualitative trauma-related variables should be investigated as well.

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