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Association Study of Trauma Load and *SLC6A4* Promoter Polymorphism in Posttraumatic Stress Disorder: Evidence From Survivors of the Rwandan Genocide

Iris-Tatjana Kolassa, PhD; Verena Ertl, MS; Cindy Eckart, MS; Franka Glöckner, MS; Stephan Kolassa, PhD; Andreas Papassotiropoulos, MD; Dominique J.-F. de Quervain, MD; and Thomas Elbert, PhD

Objective: As exposure to different types of traumatic stressors increases, the occurrence of posttraumatic stress disorder (PTSD) increases. However, because some people exhibit either surprising resilience or high vulnerability, further influencing factors have been conjectured, such as gene-environment interactions. The *SLC6A4* gene, which encodes serotonin transporter, has been identified as predisposing toward differential emotional processing between genotypes of its promoter polymorphism.

Method: We investigated 408 refugees from the Rwandan genocide and assessed lifetime exposure to traumatic events, PTSD (according to *DSM-IV*) status, and genotype of the *SLC6A4* promoter polymorphism. The study was conducted from March 2006 to February 2007.

Results: The prevalence of PTSD approached 100% when traumatic exposure reached extreme levels. However, persons homozygous for the short allele of the *SLC6A4* promoter polymorphism showed no dose-response relationship but were at high risk for developing PTSD after very few traumatic events. This genotype influence vanished with increasing exposure to traumatic stressors.

Conclusion: We find evidence for a gene-environment interplay for PTSD and show that genetic influences lose importance when environmental factors cause an extremely high trauma burden to an individual. In the future, it may be important to determine whether the effectiveness of therapeutic interventions in PTSD is also modulated by the *SLC6A4* genotype.

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Corresponding author: *Iris-Tatjana Kolassa, PhD, Clinical Psychology and Neuropsychology and Zukunftskolleg, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany (Iris.Kolassa@uni-konstanz.de).*

Who develops posttraumatic stress disorder (PTSD) after experiencing a traumatic event, and who does not? Traumatic stressors add up and render a person more vulnerable to develop PTSD[1,2]: the greater the number of various types of traumatic stressors experienced by an individual (eg, types of natural disasters, combat exposure, types of torture, abuse/rape, forcible female circumcision), the more likely the individual is to develop PTSD and the more pronounced the symptoms will be—a dose-response effect. However, some people seem to develop PTSD after experiencing a single traumatic event; others are more resilient and do not show PTSD symptoms even after multiple traumatic experiences. It has been suggested that genetic factors might play a role in the risk of developing PTSD[3,4] in the form of a gene-environment interaction.[5] Of several candidate gene loci for PTSD,[6–8] one with mounting evidence is the serotonin transporter (5-hydroxytryptamine transporter, 5-HTT) gene (*SLC6A4*)[6,9] through a polymorphism in its promoter region.

5-Hydroxytryptamine transporter is an integral membrane protein that transports the neurotransmitter serotonin from synaptic spaces back into presynaptic neurons. This transport terminates the action of serotonin in the synaptic cleft. The *solute carrier family 6 neurotransmitter transporter, serotonin, member 4* (SLC6A4) gene, which encodes 5-HTT, is located on human chromosome 17q11.1-q12.[10] The promoter region of the *SLC6A4* gene contains a polymorphism with rarer "short" *s* and more frequent "long" *l* alleles in the *serotonin transporter linked polymorphic region* (5-HTTLPR).[11] The *s* allele is associated with lower 5-HTT expression and function,[12,13] thus potentially with longer-acting serotonin effects.

In a clinical context, gene-environment interactions occur when the effect of exposure to an environmental pathogen on a person's health is dependent on his or her genotype[5]—for example, if the susceptibility to develop PTSD after traumatization depends not only on the cumulative exposure to traumatic events but also on genetic predisposition. Gene-environment interactions have been reported for various disorders.[5] The *SLC6A4* promoter polymorphism has been extensively studied in depression research: the *s* carrier status seems to lead to increased acquisition of conditioned

Table 1. Genotype Groups by Number of Traumatic Event Types and Time Elapsed Since Worst Events in 408 Rwandan Genocide Refugees

	Genotype							
	Short/Short		*Short/Long*		*(Ultra)Long/Long*		Statistics[a]	
Variable	Mean	SD	Mean	SD	Mean	SD	*F*	*P*
Number of traumatic event types during lifetime	12.69	4.39	12.85	5.03	12.55	5.00	0.14	.87
Time elapsed since worst event, y	10.88	3.88	11.60	3.30	11.64	3.51	0.37	.69

[a]Describes *F* and *P* values derived from permutation tests with 10,000 permutations.

fear responses[14] and elevates the risk for depression in the context of environmental adversity.[15–18] Furthermore, homozygous *s* carriers have been reported to be at increased risk for posthurricane PTSD and major depression, but only under the combined environmental conditions of high hurricane exposure and low social support.[3]

METHOD

We investigated 408 refugees (218 male, 190 female; mean age = 34.68 years, SD = 5.87, age range, 17–68 years) from the Rwandan Civil War who were living in the Nakivale refugee camp in southwestern Uganda from March 2006 to February 2007. All subjects had experienced multiple highly aversive traumatic situations and were examined by trained experts using a structured interview based on the Posttraumatic Diagnostic Scale[19–21] with the help of trained interpreters. The Posttraumatic Diagnostic Scale is widely used in industrialized countries as a screening instrument for the diagnosis and severity of PTSD based on *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM-IV*) criteria. Its internal consistency (Cronbach $\alpha = 0.92$), test-retest reliability ($\kappa = .74$); and—as measured against the Structured Clinical Interview for *DSM-III-R* (SCID)[22]—validity ($\kappa = 0.65$, with 82% agreement), sensitivity of 0.89, and specificity of 0.75 are high.[19] Traumatic events were assessed with a checklist of 36 war- and non–war-related traumatic event types, eg, bombing or shelling, injury by weapon, rape, accidents.[1,7] The procedures were approved by the ethics committee of the University of Konstanz, Germany, and the Mbarara University of Science and Technology, Mbarara, Uganda. After complete description of the study to the subjects, written informed consent was obtained.

The Posttraumatic Diagnostic Scale and event list were completed in form of a standardized interview. Interviewers were first trained in a 6-week course on principles of quantitative data collection and interviewing techniques. Instruments were translated into Kinyarwanda using several steps of translations, blind back-translations, and subsequent corrections by independent groups of translators.[23] Following the translations, the psychometric properties of the translated scales were investigated in a validation study including a retest spanning a 2-week period and a cross-validation with expert rating.[24]

Of the sample, 81.1% fulfilled criteria for lifetime PTSD according to *DSM-IV*.[25] All but 1 subject had experienced at least 1 event fulfilling the A1 and A2 criteria according to *DSM-IV* for a traumatic event. On average, participants had experienced 12.6 different traumatic event types (SD = 4.98; range, 0–25).

Genotyping

Saliva samples were obtained from each person using an Oragene DNA Self-Collection Kit (DNA Genotek, Ottawa, Ontario, Canada). Deoxyribonucleic acid was extracted from saliva using standard protocols. Subjects were genotyped[11] with the triallelic classification: short/short (n = 16), short/long (n = 109), long/long (n = 275), and ultralong/long (n = 8). Because of the low frequency of the ultralong allele, participants were reclassified into 3 groups: short/short (*ss)*, short/long (*sl*), and (ultra)long/long (*[u]ll*, n = 283). Results reported below did not change if *ull* carriers were considered a separate group.

The genotype distributions did not deviate from Hardy-Weinberg equilibrium ($\chi^2_3 = 3.30$, $P = .35$). There were more carriers of the *l* allele than in an African American population studied previously[11] ($\chi^2_2 = 19.18$, $P < .0001$). On the other hand, the genotype distribution did not differ from a different African American population studied previously[26] ($\chi^2_2 = 1.97$, $P = .37$).

Genotype groups did not differ in gender (*ss,* 10 male, 6 female; *sl*, 54 male, 55 female; *ll,* 154 male, 129 female; $\chi^2 = 1.30$, $P = .52$). There were no significant differences in number of traumatic event types experienced or time elapsed since most stressful event between the genotype groups (Table 1), and only 1 participant had not experienced any traumatic events. Similarly, there were no differences between types of traumatic events experienced as measured by χ^2 tests: after correcting for the multiple tests (1 per event type) using Holm's stepwise procedure,[27] all *P* values exceeded .34.

Statistics

Dependent variables were analyzed by logistic linear regressions using the software package R.[28–30] The number of lifetime traumatic event types was modeled via a restricted cubic spline[31] to include possible nonlinearities. Three knots, set at the 10%, 50%, and 90% quantiles of the covariate distribution (Table 2.3 in Harrell[31]), minimized Akaike's Information Criterion (AIC).[32] Nested models were compared using likelihood ratio (LR) tests[31]; statistical significance was assessed by nonparametric permutation tests,

Figure 1. Proportion of Participants With Lifetime Posttraumatic Stress Disorder (PTSD) and Fitted Values Against Lifetime Number of Event Types

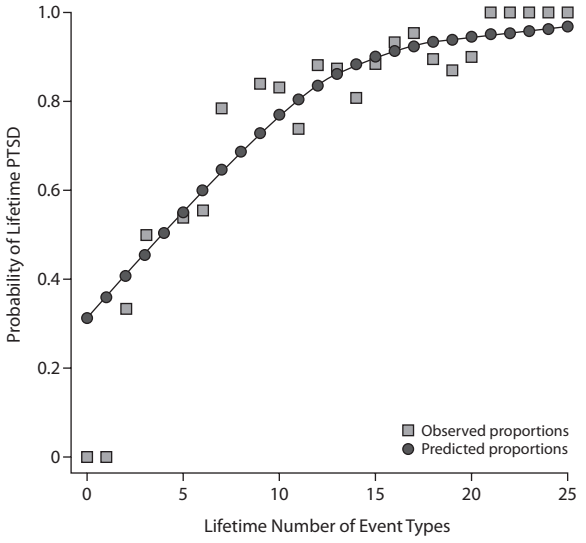
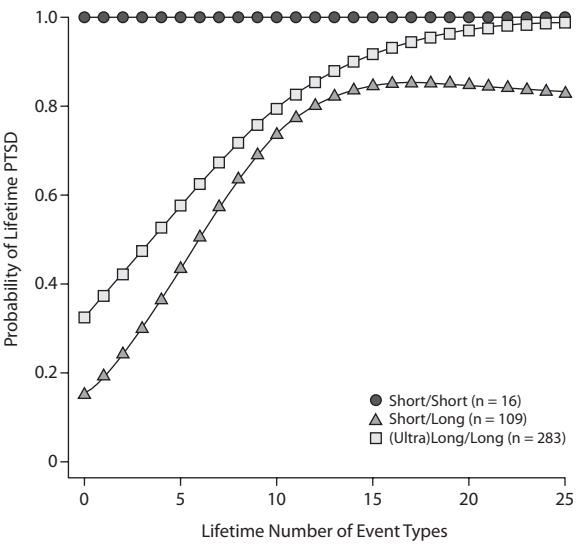


Figure 2. Fitted Values of Probability for Lifetime Posttraumatic Stress Disorder (PTSD) Against Lifetime Number of Event Types for the Different Genotypes



using 10,000 random permutations.[33] All reported *P* values for LR tests are 1-tailed; all others are 2-tailed.

RESULTS

Higher numbers of different lifetime traumatic event types led to a higher prevalence of lifetime PTSD in a dose-response relationship (LR = 43.86, $P < .0001$) (Figure 1). Lifetime PTSD was nonlinearly related to the number of different lifetime traumatic event types (coefficient of nonlinear spline basis function $\beta = -.029$, $t = -3.02$, $P = .003$). The probability of lifetime PTSD increased for small to medium numbers of lifetime event types but asymptotically approached 1 for large numbers of event types (beyond ~ 15 event types).

The probability of developing lifetime PTSD was found to depend on genotype (main effect of genotype, LR = 6.92, $P = .008$). Figure 2 shows that the fitted probability for lifetime PTSD was 100% for participants homozygous for the *s* allele, independent of the number of traumatic event types (LR = 4.71, $P = .68$). Other participants followed an S-shaped dose-response relationship as seen in Figure 1, with an effect of number of event types in *sl* carriers (LR = 13.89, $P = .001$) and in *ll* carriers (LR = 34.02, $P < .0001$). There was little difference between genotypes beyond about 15 traumatic event types. There was a trend toward an interaction between genotype groups and number of traumatic events experienced (LR = 7.85, $P = .11$).

The probability of suffering from current PTSD as well as the probability of remission from lifetime PTSD exhibited neither genotype group effects nor interactions with the number of traumatic event types experienced.

DISCUSSION

5-Hydroxytryptamine transporter is a key component of the neurochemical regulation of amygdala function, with lower 5-HTT availability leading to higher amygdala activation in emotion recognition.[34] Correspondingly, there is consistent evidence for a link between the 5-HTTLPR *s* allele and relatively heightened amygdala activation to emotional relative to neutral stimuli[35] and to fearful stimuli in particular.[36] Thus, homozygosity for the *s* allele may be linked to increased PTSD risk after trauma through increased amygdala reactivity and altered processing of emotional information.

The human serotonin transporter is the initial site of action for several antidepressants, eg, selective serotonin reuptake inhibitors (SSRIs), which are used to treat mood but also anxiety disorders such as PTSD. In affective disorders, homozygosity for the *s* allele has been associated with poorer therapeutic responses to SSRI treatment.[37,38] Thus, not only are subjects homozygous for the *s* allele at higher risk to develop PTSD after traumatic events, but they may also benefit less from a neuropharmacologic therapeutic approach.

In studies of gene-environment interactions, it is frequently unclear whether the gene under consideration moderates not only individuals' response to the environment but also—by influencing their behavior—their exposure to environmental risk factors. For example, a gene may increase an individual's probability of encountering a traumatic event, eg, by influencing the decision to become a policeman or firefighter. This would lead to higher prevalence of PTSD through a different route than enhanced

vulnerability after stressors.[6,39] However, in the present population, this problem is much attenuated, as the genocide affected the whole Rwandan population—only 1 participant had not experienced a traumatic event, and there were no differences in the number of traumatic event types experienced between the genotype groups.

A common drawback in genetic association studies specific to PTSD is that it is unclear how many individuals in a "healthy" (non-PTSD) group might have developed PTSD if they had experienced a traumatic event or "enough" different traumatic event types,[6,9] which increases the danger of false-negative results.[40] The sample investigated in this study consisted of a highly traumatized population, so it was possible to compare persons who had developed PTSD after experiencing traumatic events to severely trauma-exposed controls without PTSD.

Subjects homozygous for the *s* allele of the *SLC6A4* promoter polymorphism were found not to be subject to the cumulative effect of PTSD development, wherein multiple traumata accumulate to increase the probability of developing PTSD, but to be likely to develop PTSD even after very few traumatic events. This effect is not due to a selection effect of subjects homozygous for the *s* allele to have experienced more traumatic events than other participants; therefore, it does not appear to be mediated by a higher propensity to experience traumatic situations. Still, it should be kept in mind that the relationship between homozygosity for the *s* allele and PTSD is not deterministic but one of increased susceptibility only.

Although the present analysis found both a dose-response relationship between the number of traumatic event types experienced and the probability to develop lifetime PTSD and a main effect of genotype, with participants homozygous for the *s* allele having a fitted probability of 1 to develop trauma after traumatization, the interaction between genotype and traumatic load narrowly failed significance. This outcome may be due to low power, as the 16 participants with the *ss* genotype—of which 15 suffered from lifetime PTSD—were "spread out" over a large span of observed traumatic load. However, a clear main effect of genotype with high vulnerability of homozygous carriers of the *s* allele has been found in populations with low traumatic load,[3] while other work has shown that prevalence of PTSD across genotypes reaches very high levels with increasing trauma load.[1,2] Together, these known effects make us confident that a statistically significant interaction between genotype and number of traumatic event types experienced can be found with sufficiently powered studies. In light of the low prevalence of the *ss* genotype, future studies on the interplay of *SLC6A4* genotype and traumatic load may thus require even larger sample sizes.

Additionally, no effects of genotype and no interaction with traumatic load were found in the analysis of current PTSD and remission from lifetime PTSD. In the analysis of remission from PTSD, this may be a consequence of lower sample size, as only participants with lifetime PTSD could be considered for the remission analysis. On the other hand, any gene-environment interaction on current PTSD may be masked by the effects of remission over the 13 years after the Rwandan genocide, with a corresponding increase in statistical noise. Alternatively, it is possible that remission may be subject to influences of genes other than the one considered here.

Further research could focus on whether the effectiveness of various types of therapeutic interventions in PTSD[24] is modulated by *SLC6A4* genotype. As homozygous carriers of the *s* allele of *SLC6A4* have been found to react less well to SSRIs, their therapeutic outlook may be worse than that of heterozygous or homozygous *l* allele carriers.

Author affiliations: Clinical Psychology and Neuropsychology (Drs I.–T. Kolassa and Elbert and Mss Ertl, Eckart, and Glöckner) and Zukunftskolleg (Dr I.-T. Kolassa), University of Konstanz, Konstanz, Germany; Research and Innovation, SAF Simulation, Analysis and Forecasting AG, Tägerwilen (Dr S. Kolassa); Division of Molecular Psychology, and Life Sciences Training Facility, Biozentrum, University of Basel (Dr Papassotiropoulos); and Division of Psychiatry Research, University of Zürich (Dr de Quervain), Switzerland.
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