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The Role of Memory-related Gene *WWC1* (*KIBRA*) in Lifetime Posttraumatic Stress Disorder: Evidence from Two Independent Samples from African Conflict Regions

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Background: Posttraumatic stress disorder (PTSD) results from the formation of a strong memory for the sensory-perceptual and affective representations of traumatic experiences, which is detached from the corresponding autobiographical context information. Because *WWC1*, the gene encoding protein KIBRA, is associated with long-term memory performance, we hypothesized that common *WWC1* alleles influence the risk for a lifetime diagnosis of PTSD.

Methods: Traumatic load and diagnosis of current and lifetime PTSD were assessed in two independent African samples of survivors from conflict zones who had faced severe trauma (*n* 392, Rwanda, and *n* 399, Northern Uganda, respectively). Array-based single nucleotide polymorphism (SNP) genotyping was performed. The influence of *WWC1* tagging SNPs and traumatic load on lifetime PTSD was estimated by means of logistic regression models with correction for multiple comparisons in the Rwandan sample. Replication analysis was performed in the independent Ugandan sample.

Results: An association of two neighboring SNPs in almost complete linkage disequilibrium, rs10038727 and rs4576167, with lifetime PTSD was discovered in the Rwandan sample. Although each traumatic event added to the probability of lifetime PTSD in a dose-dependent manner in both genotype groups, carriers of the minor allele of both SNPs displayed a diminished risk (*p* .007, odds ratio .29 [95% confidence interval .15–.54]). This effect was confirmed in the independent Ugandan sample.

Conclusions: This study reveals an association between two *WWC1* SNPs and the likelihood of PTSD development, indicating that this memory-related gene might be involved in processes that occur in response to traumatic stress and influence the strengthening of fear memories.

Key Words: Genetics, *KIBRA*, memory, posttraumatic stress disorder, risk, trauma, *WWC1*

Posttraumatic stress disorder (PTSD) affects the psychological health of a significant fraction of people victimized by war and conflict (1) and is associated with low functioning, high rates of suicidality (2), and adverse physical health outcomes (3). The number of traumatic stressors experienced (traumatic load) increases PTSD vulnerability in a dose-dependent manner (4,5). However, there exists a remarkable interindividual variability in PTSD susceptibility subsequent to trauma, of which some 30–40% can be explained by genetic factors (6,7).

The most prominent clinical feature of PTSD are intrusive recollections of the traumatic experiences that comprise reliving the trauma in forms of nightmares, thoughts, pictures, sensations, or flashbacks. Vivid intrusive memories are frequently accompanied by an inability to adequately remember the context and chronology of the traumatic events (8). Because these symptoms have been observed relatively invariant all over the globe, it was deduced that they share a common psychophysiological origin, that is, the structure of memories (8). Prominent theories of PTSD development distinguish between low-level, sensation-based representations, which can be easily triggered by sensory cues and abstract, context-bound representations of autobiographical experiences (9–14). The latter are essential for the allocation of events in time and space and are mainly mediated by the hippocampus and surrounding medial temporal lobe structures (10). Whereas these two forms of representation are generally well integrated, they are dissociated in PTSD. Likewise, a trauma reminder can activate the sensation-based representations of the trauma without activating the respective contextual information, resulting in the typical intrusive symptoms (9–14).

The consequent conceptualization of PTSD as a disorder of memory disturbance is supported by numerous studies reporting PTSD-related impairments in memory and cognition (15–17). Furthermore, cumulative evidence showed smaller hippocampal volume in patients with PTSD (18,19), which might represent either a consequence of traumatic stress or a preexisting vulnerability (20). Co-twin studies support that these observations can be partly explained by genetic factors, because smaller hippocampal volumes (21) and lower cognitive functioning (22) have been observed not only in Vietnam veterans with PTSD but also in their stay-at-home identical twins. Despite the importance of genetic factors as well as memory processes in PTSD etiology, few association studies explored genes involved in the molecular cascades of long-term memory formation.

The ubiquitously expressed protein KIBRA consists of 1113 amino acids and contains the following main structures (listed from N to C terminus): two WW-domains, responsible for the interaction with different proteins; a C2-like domain, implying

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that KIBRA is a calcium binding protein; and a glutaminergic stretch (23). The intronic single nucleotide polymorphism (SNP) rs17070145 located within *WWC1*, the gene encoding KIBRA, has been associated with episodic memory performance in a genome-wide scan (24). The reported association of rs17070145 minor (T-allele) and enhanced episodic memory performance was confirmed by the majority of replication studies (25–32), but nonreplications were also reported (33–37). A recent meta-analytic investigation summarized data from 17 samples and revealed a significant association of rs17070145 and episodic memory, which remained significant when including data from unpublished studies to counteract publication bias (38).

Furthermore, KIBRA is expressed in memory-related brain areas (i.e., hippocampus and cortex) (39) and is supposed to be involved in neuroplastic processes through interaction with its binding partners (40,41). For example, KIBRA interacts with protein kinase Mζ, a brain-specific, constitutively active variant of protein kinase Cζ (42,43), which was found to be necessary for long-term potentiation maintenance (44). Further interactions of KIBRA involve dendrin (23), as well as synaptopodin (45), involved in the organization of the cytoskeleton and synaptic plasticity. Moreover, it was shown in vitro and in vivo that KIBRA is part of an α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptor (AMPAR) complex. Adult KIBRA knockout mice showed large impairments in hippocampal long-term potentiation and fear memory, indicating that KIBRA regulates memory processes by regulating AMPAR trafficking (46). In summary, the initial reports of a genetic association of the *WWC1* rs17070145 and human memory were extended by subsequent studies showing potential underlying cellular mechanisms (42,43,46).

We report the results of the first study investigating the association of common *WWC1* alleles on the risk for PTSD development in two independent samples from conflict zones. In contrast to the majority of previous association studies on PTSD, we investigated a large number of dense tag SNPs covering *WWC1* (±100 kbp). We hypothesized that common *WWC1* alleles by influencing memory strength are associated with the risk for PTSD development subsequent to traumatic stress. Because the samples were investigated several years after the end of the respective conflicts, and spontaneous remission was likely to occur in the interim, our main hypothesis was to find an association with lifetime PTSD.

Methods and Materials

Samples

Rwandan Sample. A total number of 466 survivors of the Rwandan genocide were interviewed in the refugee settlement Nakivale, Uganda, in 2006–2007 (47–50). However, after accounting for missing data (exclusion of *n* 20) and genotyping errors (exclusion of *n* 54), analyses were based on a sample of *n* 392 (194 women, mean age 34.62, SD 5.89). All procedures were approved by the Ethics Committee of the University of Konstanz, Germany, and the University of Mbarara, Uganda.

Ugandan Sample. A sample of 454 survivors of the conflict with the Lord's Resistance Army (LRA), including a large proportion of forcefully recruited (child) soldiers, were interviewed in the former Internally Displaced Persons Camp Anaka. After genotypic quality control (exclusion of *n* 49) and accounting for missing data (exclusion of *n* 16), *n* 399 (213 women, mean age 32.27, SD 11.83) study participants entered statistical analysis. All procedures were approved by the Ethics Committee of the German Psychological Society and the Ugandan National Council for Science and Technology.

In both samples, only one family member (defined as one member of a household) was allowed to participate in the study to avoid population substructure caused by kinship of participants. The trained interviewers approached the families in their homes, gave a detailed explanation of all study procedures, and asked for one family member (above the age of 18 years) who was significantly affected by the conflict to volunteer to participate. If more than one family member volunteered to participate, the most affected person was chosen. Before study participation, subjects gave written informed consent. All procedures followed the *Declaration of Helsinki*.

Behavioral Data

Similar diagnostic instruments were used in both samples. Current and lifetime PTSD cases were diagnosed in a structured interview on the basis of the Posttraumatic Diagnostic Scale (PDS) (51). Traumatic load was estimated by assessing the number of traumatic event types experienced (i.e., combat experiences, injuries by weapon, rape) by means of a 36-item event checklist, which has been used in previous studies (47–50). This event list, which was initially applied to the Rwandan sample, was extended by 26 items to include several atrocities specific to the LRA (e.g., forced to eat human flesh). Depressive symptoms were ascertained with the depression section of the Hopkins Symptom Checklist (HSCL-D) (52).

Trained local interviewers and expert psychologists in the field of trauma from the Universities of Ulm and Konstanz conducted the diagnostic interviews. Local interviewers attended 6-week training on the concepts of PTSD, depression, and quantitative data assessment before data collection. All diagnostic instruments were translated into the local languages, Kinyarwanda (Rwandan sample) and Luo (Ugandan sample), respectively, followed by blind back-translations and subsequent corrections by independent translators. The psychometric qualities of the translated instruments were ensured by assessing retest reliability and consistency with expert ratings (53,54).

In the sample of 392 Rwandan survivors, 172 (44.87%) fulfilled DSM IV-TR criteria of current PTSD, and 277 (70.66%) reported a lifetime history of PTSD. On average, study subjects reported almost 12 different traumatic event types (mean 11.98, SD 5.26, range 0–25), and only two subjects reported no trauma exposure at all. Besides, the mean HSCL-D score in the sample was 1.76 (SD .59), indicating a high prevalence of depressive symptoms.

Prevalence of PTSD symptoms was smaller in the Ugandan sample, in which *n* 40 (10%) fulfilled diagnosis of current and *n* 223 (55.8%) of lifetime PTSD. The mean traumatic load score was 24.09 (SD 8.48, range 3–59). This relatively high value, compared with the Rwandan sample, can be understood as a consequence of the aforementioned extension and precision of the event list. The mean HSCL-D-score was 1.58 (SD .56).

Genotyping

Saliva samples were collected with Oragene Self Collection Kits (DNA Genothek, Ottawa, Ontario, Canada), and DNA was isolated by use of standard protocols. Genotyping was performed as described in the *Genome-Wide Human SNP Nsp/Sty 6.0 User Guide* (Affymetrix Inc., Santa Clara, California). All tag SNPs investigated within *WWC1* were ascertained by use of the USCS human genome browser hg 19 release (55), and the analysis window was enlarged for 100 kbp flanking the 5′ and 3′ regions, respectively, to ensure inclusion of most regulating regions of

WWC1 (56). Genetic data quality control measures as implemented in GenABEL (57) (see Supplement 1 for details on genotyping and quality control) were applied before statistical analysis. Furthermore, genetic fingerprinting analysis was performed to ensure that no double probes were included. These quality control measures led to the aforementioned exclusion of *n* 56 individuals (Rwandan sample) and *n* 49 (Ugandan sample), respectively. This relatively high rate of genotyping quality impairments can be seen as a consequence of the field study conditions faced in African refugee settlements.

Statistics

The influence of the 115 tagged *WWC1* SNPs and traumatic load on current and lifetime PTSD was first analyzed by fitting logistic regression models in the Rwandan discovery sample. To obtain sufficient group sizes for each SNP analysis, heterozygous and homozygous carriers of minor alleles were assigned to one group. According to our main hypothesis, we initially fitted a model that investigated the main effects of traumatic load and *WWC1* genotype on the dependent variable lifetime PTSD. Hypothesis testing for the specific predictors of interest was performed by contrasting nested logistic models by means of likelihood ratio (LR) tests (58). More precisely, the significance of a predictor was estimated by comparing the likelihood of a larger, encompassing model, which includes the predictor, and a smaller, inner model that includes all variables of the encompassing model but the predictor to be tested. Because a total number of 115 SNPs was analyzed, a correction for multiple comparisons that is applicable to genetic data with high correlations caused by linkage disequilibrium (LD) was necessary. We used the approach of Gao (59), which takes the empirical correlation matrix between the SNPs investigated into account, to estimate the effective number of tests (M_{eff_G}). Subsequently, Holm's stepwise *p* value correction (60) was performed to correct for M_{eff_G}, which was 89 in this study.

After the discovery of an association of two SNPs with lifetime PTSD, which remained highly significant even after correction for multiple comparisons, we investigated whether the inclusion of a genotype × traumatic load interaction effect, or additional covariates that might influence PTSD, would improve model fit. Therefore, models including and excluding the interaction and covariates were evaluated by use of Akaike's Information Criterion (61) and explanatory power (coefficient of discrimination [D]), defined as the ability of the regression model to correctly predict the categorical outcome (62). The model including no interaction between genotype and traumatic load but including HSCL-D as a covariate yielded the lowest Akaike's Information Criterion and the highest D value (Table 1). Statistical inference is reported on the basis of this model.

We subsequently genotyped saliva samples from an independent validation sample from Northern Uganda to perform a replication analysis. In this sample, hypothesis-driven association testing was performed to investigate whether we can replicate the identified associations of traumatic load and genotype, by fitting the same logistic regression model defined as the final model in the discovery sample.

We further investigated whether we could identify an association of the identified *WWC1* polymorphisms with the different PTSD symptom dimensions, as measured by the current PDS subscores for intrusions, avoidance, and hyperarousal, fitting ordinary linear regressions. We did not investigate lifetime PDS subscores because symptom frequency cannot be reliably quantified retrospectively. Because residuals from linear models were not normally distributed, we used permutation tests to assess the significance of the genotype, while taking traumatic load and HSCL-D into account. The specific permutation test used 10,000 random permutations of the residuals of the variable of interest from a regression on the other variables (63,64). Statistical analyses were performed with the use of the software packages GenABEL 1.7-0 (57), glmperm 1.04 (65) and snp.plotter .4 (66) in R 2.14.1 (67).

Results

Rwandan Sample

Two neighboring SNPs within *WWC1*, rs10038727 and rs4576167, were significantly associated with a diminished risk of lifetime PTSD development (Figure 1, upper panel) with and without inclusion of the covariate's age, sex, or comorbid depressive symptoms (HSCL-D). A main-effect model of traumatic load and genotype with HSCL-D as a covariate was chosen on the basis of goodness-of-fit parameters. The two SNPs were in almost complete LD (r^2 .99; Figure 1, lower panel) and showed similar genotype distributions (*n* 86 minor allele carriers, *n* 306 noncarriers), leading to identical statistical outcomes for both variants (Table S1 in Supplement 1).

Logistic regression analysis revealed strong main effects of both traumatic load and genotype: Whereas each traumatic event added 23% to the odds of lifetime PTSD in a dose-dependent manner (Figure 2, main effect traumatic load LR_1 51.29, $p < .001$, odds ratio [OR] 1.23 [95% confidence interval (CI) 1.15–1.31]), minor allele carriers of both SNPs exhibited less than one third of the odds to develop PTSD (Figure 2, main effect genetics LR_1 15.69, $p_{uncorrected}$ .000075, $p_{Gao\ Holm}$ .007, OR .29 [95% CI .15–.54]). Nominal association *p* values of all *WWC1* SNPs and lifetime PTSD can be found in Table S2 (Supplement 1).

After correcting for multiple comparisons, we found no *WWC1* genotype-dependent effects on current PTSD in the Rwandan discovery sample. However, a hypothesis-driven analysis involving only rs10038727 and rs4576167 would have revealed a nominally significant effect of these SNPs on current

Table 1. Goodness of Fit Statistics for the Different Models Estimated

Models Estimated	D	AIC
Null Model (No Parameters Estimated)	0	476.43
Main Effect Model: Traumatic Load + Genotype	.23	386.76
Interaction Model: Traumatic Load × Genotype	.23	387.91
Main Effect Model: Traumatic Load + Genotype with Covariate Age	.23	387.40
Main Effect Model: Traumatic Load + Genotype with Covariate Sex	.23	388.74
Main Effect Model: Traumatic Load + Genotype with Covariate Depressive Symptoms	.33	342.55

Higher values of D and lower values of the AIC indicate improvement of model fit.
AIC, Akaike Information Criterion; D, coefficient of discrimination.

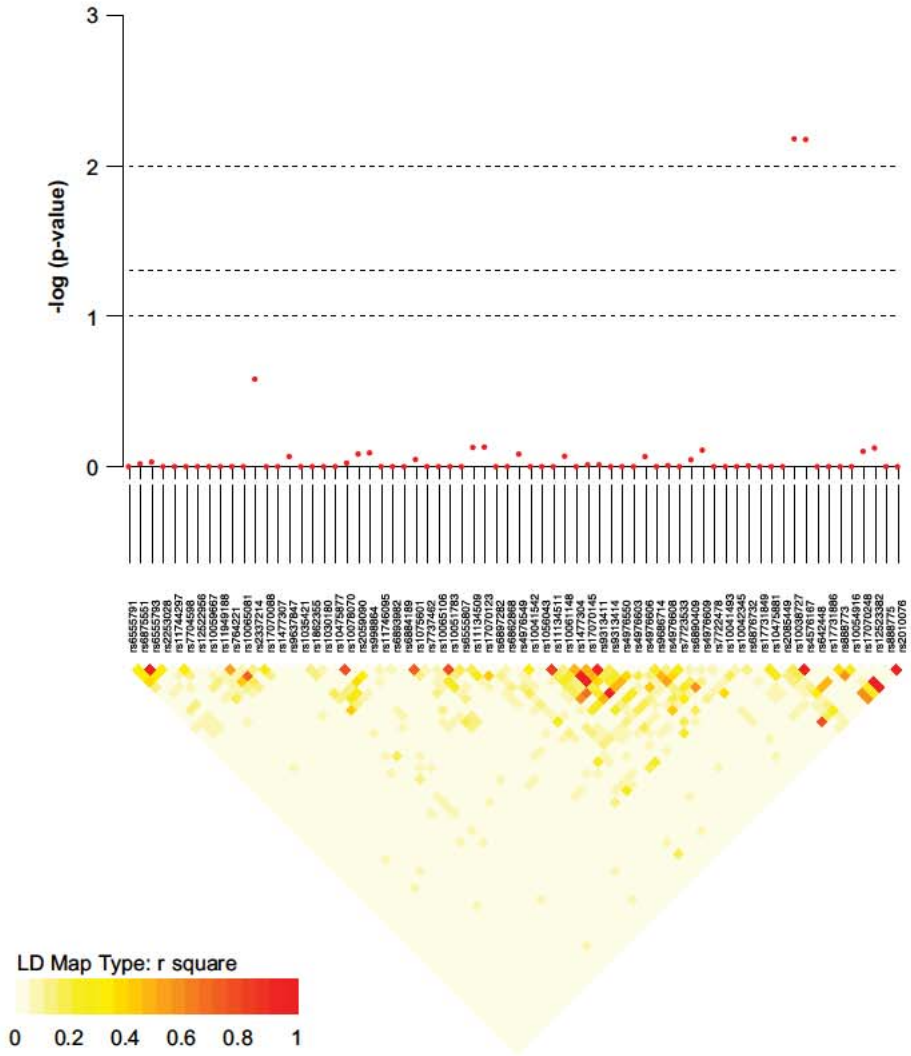

Figure 1. Rwandan sample. (Upper panel) Single nucleotide polymorphisms (SNPs) rs10038727 and rs4576167 significantly predict the risk of lifetime posttraumatic stress disorder in a sample of survivors from the Rwandan genocide. Red circles represent $\log_{10} p$ values of SNPs assessed within *WWC1* plotted against chromosome position (after correction for multiple comparisons). Horizontal lines show significant thresholds ($p < .1$, $p < .05$, $p < .01$, respectively). Empirical p values were derived from a main effect model with genotype and traumatic load as predictors and inclusion of comorbid depression as a covariate. (Lower panel) Linkage disequilibrium (LD) map created in snp.plotter 0.4. (66). To obtain a clear illustration, only intragenic *WWC1* SNPs are displayed.

PTSD (LR_1 5.32, $p_{\text{uncorrected}}$ .02, OR .48 [95% CI .26–.89], Figure S1 in Supplement 1).

Ugandan Sample

A replication analysis was performed in an independent sample of war survivors in Northern Uganda, using the same statistical model (main-effect model of traumatic load and *WWC1* genotype with HSCL-D as covariate). In the Ugandan population, rs10038727 and rs4576167 displayed a similarly high level of LD (r^2 .99); however, a slightly different frequency of minor allele carriers accounts for small differences in statistical outcomes (Table S3 in Supplement 1). Associations of *WWC1* genotype were significant with and without inclusion of age, sex, or HSCL-D as covariates; however, model-fit parameters strongly favored the inclusion of HSCL-D (Table S4 in Supplement 1). As previously observed in the Rwandan sample, minor allele carriers of rs10038727 and rs4576167 displayed a reduced risk toward lifetime PTSD development (rs10038727: LR_1 4.84, p .03, OR .58 [95% CI .35–.94]; rs4576167: LR_1 4.35, p .04, OR .59 [95% CI .36–.97], Figure S2 in Supplement 1). Furthermore, an equally strong association of the two *WWC1* SNPs with current PTSD was observed (Figure 3; rs10038727: LR_1 4.58, p .03, OR .31 [95% CI .10–.99]; rs4576167: LR_1 4.64, p .03, OR .30 [95% CI .10–.99]).

Analyses of Current PTSD Symptomatology

Our explorative analysis on the effect of *WWC1* rs10038727 and rs4576167 on the PDS symptom subscores revealed a significant association with the PDS intrusion score (LR 3.92, $p_{\text{perm}} < .05$) and the avoidance score (LR 5.29, p_{perm} .02) in the Rwandan sample. In the Ugandan sample, by contrast, we failed to find an association with any of the PDS subscores. However, inspection of the descriptive data (Table 2) depicted reduced symptoms on all three symptom dimensions in minor

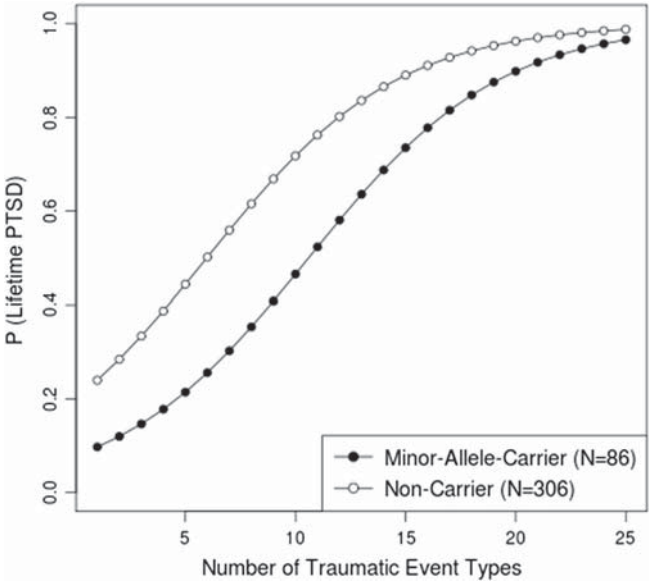

Figure 2. Rwandan sample. Fitted values of probability for lifetime posttraumatic stress disorder (PTSD) are plotted against the number of traumatic event types for minor allele versus non minor allele carriers of rs10038727. Carriers of the minor allele are at lower risk than noncarriers for development of PTSD after traumatic experiences. Identical results were obtained for rs4576167.

allele carriers in both samples. Furthermore, the nonsignificant findings in the Ugandan sample appeared to be related to the presence of one outlier in the minor allele carrier group, who presented with an intrusion score of 14, more than 7 SD above the group mean. Exclusion of this outlier would result in significant associations with the intrusion (rs10038727: *LR* 5.07, p_{perm} .02; rs4576167: *LR* 5.35, p_{perm} .02) and hyperarousal score (rs10038727: *LR* 5.24, p_{perm} .02; rs4576167: *LR* 5.52, p_{perm} .02).

Importantly, genotype groups did not differ in traumatic load in both samples (Rwandan sample: $t_{141.1}$ .44, *p* .65; Ugandan sample: rs10038727: $t_{210.4}$ .18, *p* .86; rs4576167: $t_{214.4}$ .2, *p* .84). The influence of the two SNPs was specific for PTSD because no association with depressive symptoms was found in the Rwandan sample (LR_1 .02, *p* .87) or in the Ugandan sample (rs10038727: LR_1 .82, *p* .36; rs4576167: LR_1 .79, *p* .38). Demographic characteristics of both samples by genotype groups are summarized in Table 2.

In both samples, the two SNPs displayed no deviation from Hardy-Weinberg Equilibrium and were called in every study participant. Manual inspection of signal intensity cluster plots revealed good performance of calling algorithms (Figure S3 in Supplement 1). Both SNPs map between the 19th and 20th exon of *WWC1,* at the carboxyterminal part of KIBRA (55). SNP rs10038727 is a G → A polymorphism and rs4576167 is a G → C polymorphism.

Discussion

We found a strong association of two SNPs within *WWC1*, rs10038727 and rs4576167, with a reduced risk to lifetime PTSD development in two independent samples. This novel association points toward a potential clinical relevance of this gene in disorders that involve the formation of pathological fear memories.

We replicated the typical dose-dependent effect of traumatic load on the risk of lifetime PTSD in both genotype groups. However, minor allele carriers of the two SNPs identified exhibited reduced risk for lifetime PTSD development in both samples, confirming our main hypothesis. The effect of the two *WWC1* SNPs on current PTSD did not survive multiple comparison correction in the Rwandan discovery sample. Nevertheless, a small but nominal significant association with current PTSD would have been present without applying correction. Yet, compared with the large effect on lifetime PTSD in the discovery sample, this effect was relatively small. By contrast, in the Ugandan sample, the strengths of the associations of *WWC1* genotype with current and lifetime PTSD were comparable. One potential rationale for the different strengths of the associations with lifetime and current PTSD observed in the two independent samples might consist in the time course of traumatic experiences: Whereas 60% of the Rwandan participants experienced their worst traumatic event in 1994, the year of the genocide, the prevalence of the worst event was distributed over two decades of war in the Ugandan sample, with many atrocities still occurring recently (Figure S4 in Supplement 1). A further explanation might be that *WWC1* genotype by influencing memory processes also affects the likelihood of spontaneous remission from PTSD. This can be investigated in the present study if we define remission as the diagnosis of a lifetime but no current PTSD. However, in both samples, no genotype-dependent effects on spontaneous remission were found.

As Figures 2 and 3 indicate, the relationships between *WWC1* genotype, traumatic load, and the outcome variables lifetime and current PTSD, respectively, appear somewhat different. Whereas the effect of the minor allele carrier status on lifetime PTSD is visible across all levels of traumatic load (Figure 2), the effect on current PTSD is more pronounced at higher levels of traumatic

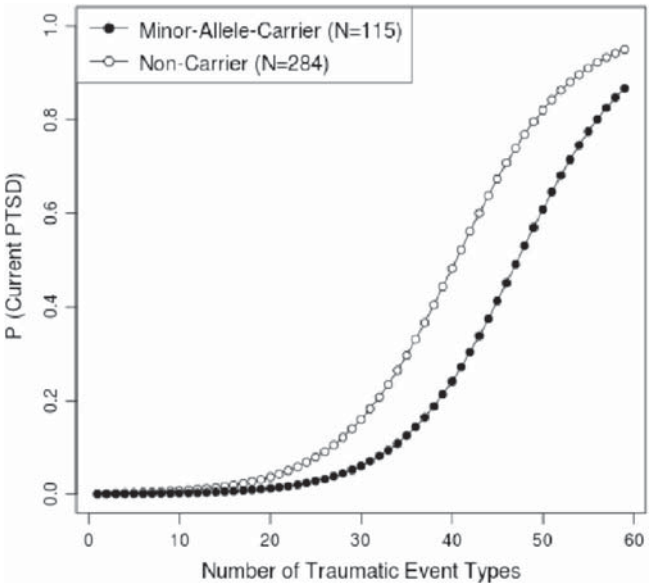

Figure 3. Ugandan sample. Fitted values of probability for current posttraumatic stress disorder (PTSD) are plotted against the number of traumatic event types for minor allele versus non minor allele carriers of rs10038727. Carriers of the minor allele are at lower risk than noncarriers for development of PTSD after traumatic experiences.

Table 2. Demographic Information of the Rwandan and Ugandan Sample by Genotype Group.

Rwandan Sample	Minor Allele Carriers (n = 86)	Noncarriers (n = 306)
Mean Age (SD)	34.88 (6.89)	34.59 (5.60)
Sex (% Women)	47.71%	55.81%
Mean Traumatic Load (SD)	12.14 (5.24)	11.86 (5.28)
Lifetime PTSD (%)	58.14%	74.18%
Current PTSD (%)	36.05%	46.08%
Mean PDS Sumscore Intrusions (SD)	4.02 (3.87)	4.66 (3.89)
Mean PDS Sumscore Avoidance (SD)	4.49 (4.76)	5.30 (4.86)
Mean PDS Sumscore Hyperarousal (SD)	3.83 (3.53)	4.00 (3.26)
Mean HSCL D (SD)	1.77 (.59)	1.75 (.59)
Ugandan Sample, rs10038727	**Minor Allele Carriers (n = 115)**	**Noncarriers (n = 284)**
Mean Age (SD)	33.10 (11.51)	31.94 (11.97)
Sex (% Women)	54.22%	51.30%
Mean Traumatic Load (SD)	23.97 (8.51)	24.14 (8.49)
Lifetime PTSD (%)	46.96%	59.51%
Current PTSD (%)	5.22%	11.97%
Mean PDS Sumscore Intrusions (SD)	.62 (1.67)	.90 (1.60)
Mean PDS Sumscore Avoidance (SD)	.70 (1.87)	1.00 (1.93)
Mean PDS Sumscore Hyperarousal (SD)	.66 (1.65)	1.06 (1.95)
Mean HSCL D (SD)	1.53 (.57)	1.60 (.61)
Ugandan Sample, rs4576167	**Minor Allele Carriers (n = 116)**	**Noncarriers (n = 283)**
Mean Age (SD)	33.01 (11.50)	31.97 (11.98)
Sex (% Women)	54.61%	50.86%
Mean Traumatic Load (SD)	23.96 (8.48)	24.14 (8.50)
Lifetime PTSD (%)	47.41%	59.36%
Current PTSD (%)	5.20%	12.00%
Mean PDS Sumscore Intrusions (SD)	.61 (1.67)	.90 (1.60)
Mean PDS Sumscore Avoidance (SD)	.69 (1.87)	1.01 (1.92)
Mean PDS Sumscore Hyperarousal (SD)	.66 (1.64)	1.06 (1.95)
Mean HSCL D (SD)	1.54 (.57)	1.59 (.61)

HSCL D, Hopkins Symptom Checklist; PDS, Posttraumatic Diagnostic Scale; PTSD, posttraumatic stress disorder.

Because the ratio of minor allele carriers to noncarriers was not identical for rs10038727 and rs4576167 in the Ugandan sample, results are reported separately for both single nucleotide polymorphisms.

load (Figure 3). This can be rationalized by the fact that the probability of spontaneous remission from PTSD also depends on traumatic load (4), that is, the chances to have PTSD several years after the conflict are considerably low if only few events have been experienced.

Our explorative analysis of the symptom dimensions of PTSD further revealed a trend of reduced PTSD symptoms in minor allele carriers on all dimensions, with a significant association of the identified *WWC1* SNPs with intrusive and avoidance symptoms in the Rwandan sample, and a significant association with intrusive and hyperarousal symptoms only after the removal of one outlier in the Ugandan sample. From our memory-centered perspective, one would expect the strongest association with intrusive symptoms. Indeed, only for intrusive symptoms, there was suggestive evidence of a *WWC1* effect in both samples. However, there are limitations to the analyses of symptom dimensions in the current study. First, because it is impossible to reliably assess lifetime symptom subscores, our analyses were confined to current symptom dimensions. Second, the PDS only assesses the frequency of the reported symptoms but not their severity, which might account for the rather small effects observed.

Whether the observed associations can be generalized to other populations has yet to be investigated in replication samples of different ancestry. Data from the latest HapMap release (68) (Table S5 in Supplement 1) show that genotype distribution of rs10038727 and rs4576167 vary between populations. Still, the fact that the reduced PTSD risk in minor allele carriers was present at all levels of traumatic load (i.e., that we did not observe gene × environment interaction effects) increases the possibility that the effect could be generalized to other populations that faced less trauma than our severely traumatized samples.

Whereas the benchmark study of Papassotiropoulos *et al.* (24) reported an association of rs17070145 and memory performance, we found two additional SNPs located within *WWC1* associated with reduced PTSD risk. The previous findings of an involvement of rs17070145 in long-term memory justified hypothesis-driven association testing, but even without multiple comparisons correction, no association of rs17070145 and PTSD was found. Furthermore, the reported associations of rs10038727 and rs4576167 are independent of rs17070145, as indicated by low levels of LD (Rwandan sample: r^2 .002, Ugandan sample: r^2 .01). However, the genotype distribution of rs17070145 in the investigated African samples differed strongly from the distribution observed in samples of European origin (Table S6 in Supplement 1). Nevertheless, the results of our study underscore the importance of KIBRA in memory-related traits. Because KIBRA is known to be a versatile player in the brain that holds binding sides for various interaction partners (40,41), it is plausible that KIBRA influences mnemonic processes by various mechanisms influenced by different ge

Because we identified two novel intronic SNPs, the exact molecular mechanisms underlying the observed association with PTSD risk are unknown. However, data from the ENCODE project, initiated to functionally annotate the human genome, have shown that the vast majority of discovered disease-associated SNPs map at noncoding but functional regions (69). Indeed, searching data from ENCODE (70) and RegulomeDB (71) gave putative evidence for a regulatory function (RegulomeDB score of 5), because rs10038727 maps at a DNase hypersensitive site, indicating enhanced transcription factor binding affinity.

In summary, our results show that minor allele carriers of the two SNPs identified are at considerably lower risk for the development of PTSD, suggesting that memory-related protein KIBRA might be involved in molecular processes that occur as a consequence of traumatic experiences and influence the development and strengthening of pathological fear memories. It was previously shown by our work group that genetic variations, which account for normal variation in emotional memory performance in healthy subjects (i.e., ADRA2B and PRKCA polymorphisms), can become clinically relevant in the case of severe environmental stress and influence the strength of traumatic memories [(49,50); for an overview, see (14)]. A similar mechanism might be true for the two *WWC1* SNPs identified in this study.

Limitations of this study include the retrospective assessment of lifetime traumatic load. It is impossible to disentangle in a diagnostic interview if all reported traumatic experiences occurred before the first onset of a PTSD diagnosis. This is of little concern for the Rwandan sample because the majority of traumatic experiences occurred in 1994 (Figure S4 in Supplement 1). By contrast, Northern Uganda faced almost two decades of terror by the LRA. This higher variability in the timing of trauma exposure might also be one explanation for the smaller effect of *WWC1* genotype on lifetime PTSD observed in this sample.

Furthermore, our study does not allow any inference on the memory processes that might mediate the protecting effects of the identified variants. Different memory aspects have been discussed to promote reduced risk of PTSD development [i.e., diminished fear conditioning and hence memorization of emotional memories (72), enhanced encoding of contextual autobiographical memories (12), enhanced extinction learning and fear inhibition (73), or better extinction memory recall (74)]. Future studies should investigate *WWC1* alleles with respect to these different processes to gain further insight into the role of memory-related protein KIBRA in PTSD etiology.

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