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Cross-cultural gene–environment interactions in depression, post-traumatic stress disorder, and the cortisol awakening response: *FKBP5* polymorphisms and childhood trauma in South Asia

GxE interactions in South Asia

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Abstract

Despite increased attention to global mental health, psychiatric genetic research has been dominated by studies in high-income countries, especially with populations of European descent. The objective of this study was to assess single nucleotide polymorphisms (SNPs) in the *FKBP5* gene in a population living in South Asia. Among adults in Nepal, depression was assessed with the Beck Depression Inventory (BDI), post-traumatic stress disorder (PTSD) with the PTSD Checklist-Civilian Version (PCL-C), and childhood maltreatment with the Childhood Trauma Questionnaire (CTQ). *FKBP5* SNPs were genotyped for 682 participants. Cortisol awakening response (CAR) was assessed in a subsample of 118 participants over 3 days. The *FKBP5* tag-SNP rs9296158 showed a main effect on depressive symptoms ($p = 0.03$). Interaction of rs9296158 and childhood maltreatment predicted adult depressive symptoms ($p = 0.02$) but not PTSD. Childhood maltreatment associated with endocrine response in individuals homozygous for the A allele, demonstrated by a negative CAR and overall hypocortisolaemia in the rs9296158 AA genotype and childhood maltreatment group ($p < 0.001$). This study replicated findings related to *FKBP5* and depression but not PTSD. Gene–environment studies should take differences in prevalence and cultural significance of phenotypes and exposures into account when interpreting cross-cultural findings.

Background

Recent epidemiological and molecular-genetic studies support the role of gene–environment (GxE) interactions in elevating risk for mood and anxiety disorders. GxE interactions are defined as increased risk for or protection against a health condition including neuropsychiatric disorders within a specific environmental or experiential context such as exposure to environmental toxins or traumatic and stressful early life experiences. The growing literature on GxE interactions in mental illness suggests that genetic polymorphisms may associate with greater vulnerability or resilience in the context of stressful life events (Caspi et al., 2003; Kendler et al., 2005) and early childhood trauma (Binder et al., 2008; Bradley et al., 2008; Caspi et al., 2002; Weder et al., 2009). The vast majority of GxE interaction research has been conducted in high-income countries (HIC). One crucial need in the field is to examine whether GxE interactions can be identified across populations in different settings throughout the world. While the past decade can be characterized by rapid growth in global mental health research to develop an evidence base for treatment effectiveness in low-and-middle income countries (LMIC) (van Ginneken et al., 2013), including a number of

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ground-breaking randomized trials in South Asia (Chatterjee et al.; Patel et al., 2010; Rahman et al., 2008), there has been a dearth of research on GxE interactions in South Asia.

FKBP5 single nucleotide polymorphisms in depression and PTSD

The goal of the current study was to explore *FKBP5* single nucleotide polymorphisms (SNPs) related to depression and anxiety in a South Asian population. SNPs refer to differences in one nucleotide (e.g. A, C, T, G) in coding or non-coding regions of DNA. SNPs represent commonly occurring differences (i.e. having a prevalence $>1\%$) within a population (Cargill et al., 1999; Gray et al., 2000). SNPs are assumed to be relatively stable over time, thus groupings are less likely to be the result of random mutations within an individual. Some SNPs may have functional consequences, i.e. by changing the transcriptional activity of the gene of interest. However, most genetic association studies can only use proxies for these functional variants by genotyping linked marker SNPs, so called tag SNPs.

We selected SNPs associated with altered function of the hypothalamic–pituitary–adrenal (HPA) axis which have been studied previously in healthy individuals and persons with mental illness. Alterations in functioning of the HPA axis have been associated with neuropsychiatric disorders (Sapolsky & Plotsky, 1990), including in Asian populations (Hruschka et al., 2005; Kohrt et al., 2015). Cortisol, the end product of the HPA axis, is a steroid hormone produced by the adrenal glands based on neuroendocrine signalling through the hypothalamus and pituitary (Sapolsky et al., 2000). Typically, cortisol levels are highest in the morning and then begin to drop rapidly after waking; this change in cortisol levels is referred to as the cortisol awakening response (CAR) (Wust et al., 2000). Alterations in CAR and other dysregulation of the HPA axis have been reported in psychiatric disorders including depression and post-traumatic stress disorder (PTSD) (Heim et al., 1998; Holsboer, 2000; Shea et al., 2007; Yehuda, 1998). SNPs associated with alterations in functioning of the HPA axis are prime candidates for mediating GxE because they likely moderate acute reactivity, developmental sensitivity to stressful life events, and dysregulation among individuals with neuropsychiatric phenotypes. Depression and PTSD are predicted by interactions between early trauma and stressful life events with polymorphisms encoding regulatory receptors of several stress hormones, including corticotrophin-releasing hormone receptor 1 (CRHR1), glucocorticoid receptor (GR), and *FKBP5*, co-chaperone of GR (Bet et al., 2009; Binder et al., 2008; Bradley et al., 2008; Mahon et al., 2013).

The study investigates polymorphisms in *FKBP5*, the hsp90-associated co-chaperone of GR, which regulates GR sensitivity. When *FKBP5* is bound to the GR receptor complex, cortisol binds with lower affinity and nuclear translocation of the receptor is less efficient (Davies et al., 2005; Scammell et al., 2003; Westberry et al., 2006; Wochnik et al., 2005). *FKBP5* mRNA and protein expression are induced by GR activation via intronic hormone response elements, providing an ultra-short feedback loop for GR sensitivity (Binder et al., 2004; Vermeer et al., 2003).

SNPs in the gene encoding the co-chaperone have been shown to associate with differential up-regulation of *FKBP5* following GR activation and differences in GR sensitivity (Binder et al., 2004, 2008). GR activation and sensitivity are mediated by allele-dependent differences in the three-dimensional structure of the gene; some alleles enable contact of a glucocorticoid enhancer element in intron 2 of the gene with the transcription start site, whereas other alleles do not allow contact with the enhancer element, thus precluding transcription (Klengel & Binder, 2015; Klengel et al., 2013). SNPs rs9296158, rs3800373, rs1360780, and rs9470080 show strong linkage disequilibrium among populations of European descent (Binder et al., 2004) and tag the underlying functional variant.

Alleles associated with enhanced expression of *FKBP5* lead to increased GR resistance following GR activation and hence decreased efficiency of negative HPA feedback in healthy controls, resulting in prolonged HPA activation following exposure to stress (Buchmann et al., 2014; Ising et al., 2008; Menke et al., 2013). The dysregulated stress response might be a risk factor for stress-related psychiatric disorders.

To date, psychiatric GxE studies of *FKBP5* have been conducted in community populations of African Americans, European Americans, Germans, and New Zealanders of European descent, as well as a population of prisoners in Italy (Appel et al., 2011; Bevilacqua et al., 2012; Binder et al., 2008; Bortsov et al., 2013; Collip et al., 2013; Mahon et al., 2013; Xie et al., 2010; Zimmermann et al., 2011). Binder et al. (2008) initially reported GxE interactions with *FKBP5* SNPs. The study included a cohort of over 750 urban, low income African American non-psychiatric outpatients exposed to high levels of childhood and adult trauma. *FKBP5* polymorphisms robustly interacted with level of child abuse to predict severity of adult PTSD symptoms, and this relationship was only significant for child trauma and not for adult trauma exposure (Binder et al., 2008). Subsequent studies have been conducted in other European American and African American populations, with outcomes of interest including depression,

PTSD, and chronic pain (Bortsov et al., 2013; Mahon et al., 2013; Xie et al., 2010). Studies with German populations have demonstrated an association of *FKBP5* and stressors including child abuse on risk for depression (Appel et al., 2011; Zimmermann et al., 2011). A study of Italian prisoners demonstrated an association with *FKBP5* and aggression (Bevilacqua et al., 2012).

Recently, allele-specific *FKBP5* DNA demethylation has been identified as a mediator of genetic interactions with childhood trauma (Klengel & Binder, 2015; Klengel et al., 2013). The combined genetic and epigenetic effects likely lead to a long-term disinhibition of glucocorticoid-stimulated *FKBP5* which in turn alters the set-point of the HPA axis as well as to differential interaction with other signalling partners of *FKBP5* that may be implicated in psychiatric disorders, including the androgen receptor, calcineurin and tau-protein (Zannas & Binder, 2014).

The current study adds to the growing literature by exploring the relationship between this functional *FKBP5* locus, self-reported symptoms of mental illness, and HPA activity, assessing both main genetic effects and potential GxE interactions between these SNPs and child maltreatment. The unique contribution is the cross-cultural setting of a rural population in South Asia. This setting in Nepal is an area of high trauma exposure due to a history of political violence and it is characterized by a high prevalence of depression (Kohrt et al., 2012). We hypothesize that previously identified SNPs associated with increased risk of depression and PTSD in the context of child maltreatment will also demonstrate increased risk in this South Asian, low-income population, which has a high burden of trauma exposure.

Methods

Sample

The study took place in a rural mountainous district of north-western Nepal. A cross-sectional community design using an n^{th} random-sampling frame of households was employed to recruit one adult per household in four village development committees (VDCs) with sampling criteria adjusted per village population and demographics (Kohrt et al., 2012). Total adult population of the sampled area was approximately 9,000 at the time of the study (HMG-CBS, 2003). The sample comprised two groups: participants who entered the study in 2000 with follow-up participation in 2007–2008 (Kohrt et al., 2012), and those who first entered in 2007–2008. We employed the same sampling frame techniques for both periods. All saliva collections and phenotype measurements reported here were collected in 2007–2008. All interviews were conducted in participants' homes, with only the interviewer and participant present. Interviews lasted 60–90 minutes.

The sample included four main ethnic/caste groups: 'high' Hindu caste Indo-Aryan groups (Brahman and Chhetri), a 'low' Hindu caste Indo-Aryan group (Dalit, locally also referred to as Nepali), and Tibeto-Burmese language speaking groups of Mongolian-descent who were predominantly Buddhist (Janajati). Caste/ethnicity was recorded through self-report using locally defined and salient categories (Kohrt et al., 2009): individuals were asked to self-identify according to one of these four categories. These are social groupings based on historical caste and ethnic distinctions in Nepal (Höfer, 2004; Whelpton, 2005). They are not racial categories. Inter-caste and inter-ethnic marriages and child-bearing have occurred throughout history and are increasingly common in Nepal (Niraula, 1994; Subedi, 2011).

Ethical approval

All individuals provided verbal consent. The protocol was approved by the Institutional Review Board of Emory University, Atlanta, Georgia, and the Nepal Health Research Council, with modifications approved by Tribhuvan University Teaching Hospital/Institute of Medicine. Participants with high levels of psychological distress were evaluated by the study's principal investigator and referred for mental health support.

Mental health and exposures

The 21-item Beck Depression Inventory (BDI-Ia) was used to assess depression symptoms over the prior 2 weeks (Beck et al., 1961). Items are scored 0–3 with an instrument range of 0 to 62. The scale has been translated into Nepali and validated for use in Nepal (Kohrt et al., 2002) with a cut-off score of 20 or greater for moderate depression; sensitivity = 0.73, specificity = 0.91, test–retest reliability = 0.84 (Kohrt et al., 2012). The 17-item PTSD Checklist-Civilian Version (PCL-C) is a rating scale for assessing PTSD symptoms in contexts where administration of a structured interview schedule is not feasible (Weathers et al., 1993). The PCL-C assesses symptom severity within the past week. The English language measure has good psychometric properties in American populations (Weathers et al., 1994), and the validated Nepali version performs similarly in Nepal (Tol et al., 2007) with a cut-off score of 50 or above indicating need for intervention (Thapa & Hauff, 2005). For the sample in the study, internal reliability (Cronbach's alpha) was 0.83. Test–retest reliability (Spearman-Brown coefficient) was 0.82.

The brief version of the Childhood Trauma Questionnaire (CTQ) was used to assess child maltreatment (Bernstein & Fink, 1998; Bernstein et al., 2003). The Traumatic Events Inventory (TEI) was used to assess lifetime history of adult trauma (Schwartz et al., 2005, 2006). We excluded items of childhood trauma on the TEI from our analyses because they were assessed through the CTQ. The 64-item Stressful Life Events Rating Scale for Cross Cultural Study (SLERS) records exposure to daily stressors over the prior 12 months (Zheng & Lin, 1994) and has shown significant association with depression, anxiety, and locally defined psychosomatic complaints in Nepal (Kohrt et al., 2005, 2009). All of the exposure measures went through transcultural translation procedures (Van Ommeren et al., 1999).

DNA collection and genotyping

Individuals first rinsed their mouths with water. Within 3–5 min, they chewed Parafilm to facilitate saliva production. Saliva was collected with Oragene kits (DNA Genotek, Ottawa, ON, Canada) and stored in a cool, dry place before being shipped first to Kathmandu and then to Emory University, Atlanta, USA. DNA was extracted using the Purelink 96 Genomic DNA kit (Invitrogen). Four *FKBP5* SNPs (rs1360780, rs3800373, rs9296158, and rs9470080) were genotyped using the previously described protocol (Binder et al., 2008) for TaqMan assays on an ABI7900 lightcycler. Saliva for genotyping was collected from 705 individuals. For 23 samples (3.2%), insufficient human DNA could be extracted from the saliva samples, so the final genotyping was performed on 682 DNA samples.

Saliva cortisol measures

From the 705 individuals, a subset of 120 individuals was selected randomly for participation in diurnal saliva collection for cortisol analysis. Of these, 119 completed the collection protocol by providing samples at waking, 30 min after waking, and at 7 pm for 3 days, totalling nine samples; 118 participants had samples sufficient for analyses. Participants were instructed neither to eat nor drink anything prior to collection, nor to brush their teeth during the interim period between the two collections. Three trained research assistants conducted all collections, arriving at individuals' houses prior to waking to assure fidelity of collection times and procedures.

Collection tubes were treated with sodium azide to prevent breakdown of cortisol during the period of storage in rural Nepal and shipment to the USA, which required between 3 to 6 weeks from time of collection until freezing in the Laboratory for Comparative Human Biology. Analyses were performed with a Salimetrics (State College, Pennsylvania) ELISA kit, including a modification for ether extraction of cortisol to prevent sodium azide interference with the ELISA; inter-assay 7.812% coefficient of variation (CV), intra-assay 3.315% CV. Cortisol awakening response (CAR) was assessed by comparing levels at waking and 30 min after waking.

Statistical analyses

The four main goals of the statistical analyses were to determine whether (1) *FKBP5* tag-SNP genotypes have main effects on depression or PTSD symptoms, (2) *FKBP5* SNP genotypes interact with exposure to child maltreatment to influence adult depression or PTSD, (3) *FKBP5* SNP genotypes have main effects on CAR, and (4) *FKBP5* SNP genotypes interact with exposure to child maltreatment to influence CAR. Statistical analyses were performed with SPSS version16.0 (SPSS, 2007).

Haploview version 3.3.2 (Barrett et al., 2005) was used to investigate the linkage disequilibrium pattern among the four *FKBP5* SNPs, performed separately for each caste. The tagger version implemented within the software was used for tag-SNP selection with an r-squared cut-off of 0.75.

The outcomes of interest for the first two goals were continuous total scores for the BDI and PCL-C. To determine main effects, we employed general linear models (GLM) that included the *FKBP5* genotypes in a genotypic model: genotypes entered as dummy variables. In the models, we controlled for covariates of interest: sex, age, caste/ethnicity, childhood maltreatment (CTQ), adult lifetime trauma (TEI), and stressful life events in the past year (SLERS). CTQ scores were coded as quartiles (0–31, 32–35, 36–40, and greater than 41). TEI scores were coded as quartiles (0, 1, 2–3, or 4 or more events across the lifetime). SLERS scores were coded as tertiles (0–14, 15–22, or 23 and more events).

Caste/ethnicity captures important experiences contributing to risk and psychopathology stratification (Kohrt et al., 2009), therefore caste/ethnicity was included as a dummy variable. To reduce the likelihood of reporting false positive association due to population stratification, we performed separate analyses for Chhetri caste alone, which comprised the majority of the total sample. To assess interaction effects, we included the childhood trauma by SNP term in GLM with the full model of genotypes and covariates. Child maltreatment by genotype was the only interaction tested because of the existing literature on *FKBP5* and childhood trauma.

We then examined the association of *FKBP5* genotype and childhood maltreatment on CAR. For

each of the 3 days of collection, CAR was calculated using the difference of $\log_{10}$ waking cortisol and $\log_{10}$ 30 min after waking cortisol. Log values were used because of the non-normal distribution of cortisol levels. Because of variance lost using averages, we included all 3 days in a linear mixed model (LMM) that allows repeated measures (Hruschka et al., 2005). To assess main effects of *FKBP5* genotype on CAR, we created a LMM that included fixed effects for *FKBP5* genotype (homozygous risk group 'AA' versus the combined category of the other homozygous group and the heterozygous group, herein referred to as 'G-carrier') and childhood maltreatment (CTQ, median split). Caste/ethnicity was included as a fixed-effect dummy variable to control for population stratification. Other fixed effects included variables with known associations with cortisol levels: sex, age (tertiles), menstrual status (pre/menstrual versus non-menstruating women and men), and vegetarianism (self-identification as 'veg' versus 'non-veg').

Random effects were: subjects, day of collection, duration of prior night's sleep (hours), clock time at first collection, and time difference between waking and first collection (minutes) in cases where participants awoke before research assistants' arrival for collection. To test for interaction effects, we added *FKBP5* genotype by CTQ median split as a fixed effect to the model described above.

Results

Demographics

Sample demographics are provided in Table 1. The study was sex balanced (49.1% female). Mean age of the sample was 37 years (SD 13 years). Chhetri was the most common self-reported caste (63.2%). Similar numbers of participants reported Brahman (16.1%) and Dalit/Nepali (17.4%) castes. Ethnic minority Janajati groups comprised 3.2% of participants.

Genotype frequency and linkage disequilibrium

Genotype frequencies in the total sample and by caste are presented in Table 2. Linkage disequilibrium (LD) of the SNPs for each of the four castes is presented in Fig. 1. SNPs were highly correlated in Chhetri, Brahman and Janajati, but LD was less extended in the Dalit/Nepali subsample. rs9296158 was selected as common tag-SNP, tagging genotyped *FKBP5* variants with an $r^2 \geq 0.75$ in all four castes. This SNP has shown the most significant interaction for child abuse and risk of PTSD (Binder et al., 2008).

Table 1. Sample demographics.

Demographics	No. (%) of participants
Sex	
Male	347 (50.9)
Female	335 (49.1)
Ethnicity/caste	
Brahman	110 (16.1)
Chhetri	431 (63.2)
Dalit/Nepali	119 (17.4)
Janajati	22 (3.2)
Education	
None	306 (44.9)
Grade 1–5	101 (14.8)
Grade 6–10	146 (21.4)
School leaving certificate and above	134 (18.9)
Marital status	
Unmarried	64 (9.4)
Married	571 (83.7)
Widow/widower	44 (6.5)
Divorced	3 (0.4)
Occupation	
Unemployed	18 (2.6)
Manual labour	11 (1.6)
Farmer	474 (69.5)
Office holder	179 (26.2)
Household monthly income	
None	172 (25.2)
NPR 1–2000	243 (35.6)
NPR 2001–4000	100 (14.7)
NPR 4000+	167 (22.5)
Age (years) Mean (SD)	37.0 (13.0)

N = 682; SD, standard deviation; NPR, Nepalese rupee (1000 NPR ≈ 10 USD).

Mental health phenotype and exposures

Mean BDI scores showed moderate depression severity (mean = 18.44, SD = 7.62). The mean PCL-C score for PTSD symptoms was 40 (SD = 8). Mean depression and PTSD scores did not differ by caste/ethnicity (see Table 3): BDI score ($F = 2.27$, $p = 0.08$), PCL-C score ($F = 0.46$, $p = 0.71$). Exposure levels for childhood maltreatment and SLEs did differ by caste/ethnicity while lifetime adult trauma did not (see Table 4): CTQ score ($\chi^2 = 67.42$, $p < 0.001$), SLERS score ($\chi^2 = 64.31$, $p < 0.001$), and TEI score ($\chi^2 = 14.01$, $p = 0.12$).

Main and interaction effects for mental health outcomes

We identified a main effect ($p = 0.030$) for rs9296158 and total BDI score when controlling for sex, age, caste/ethnicity, childhood maltreatment, adult trauma, and stressful life events. There was no significant main effect for PTSD symptoms ($p = 0.875$). The rs9296158 AA genotype is associated with overall greater BDI scores compared with to the other two genotypes (see Table 5, $\beta = 1.67$, 95%CI, 0.00–0.34, $p = 0.03$).

Table 2. *FKBP5* SNP frequency by caste/ethnicity.

			No. (%)				
FKBP5 SNP	Position	Genotype	Total sample	Dalit	Chhetri	Brahman	Janajati
rs3800373	35650460						
		CC	66 (9.7)	11 (9.2)	42 (9.8)	12 (10.9)	1 (4.5)
		AC	289 (42.6)	43 (36.1)	183 (42.8)	50 (45.5)	13 (59.1)
		AA	324 (47.7)	65 (54.6)	203 (47.4)	48 (43.6)	8 (36.4)
rs9296158	35675060						
		AA	68 (10.0)	12 (10.1)	43 (10.0)	12 (10.9)	1 (4.5)
		AG	303 (44.4)	52 (43.7)	188 (43.6)	51 (46.4)	12 (54.5)
		GG	311 (45.6)	55 (46.2)	200 (46.4)	47 (42.7)	9 (40.9)
rs1360780	35715550						
		TT	61 (9.0)	11 (9.2)	36 (8.4)	13 (11.8)	1 (4.5)
		CT	304 (44.6)	51 (42.9)	191 (44.4)	50 (45.5)	12 (54.5)
		CC	316 (46.4)	57 (47.9)	203 (47.2)	47 (42.7)	9 (40.9)
rs9470080	35754410						
		TT	65 (10.0)	10 (9.0)	42 (10.1)	12 (11.3)	1 (4.5)
		CT	266 (40.7)	43 (38.7)	163 (39.4)	48 (45.3)	12 (54.5)
		CC	322 (49.3)	58 (52.3)	209 (50.5)	46 (43.4)	9 (40.9)

N = 682; SNP, single nucleotide polymorphism.

When controlling for ethnicity and other covariates, rs9296158 also showed a significant interaction effect with child maltreatment on total BDI score ($p = 0.022$) (see Fig. 2), but not for PTSD (p = 0.552).

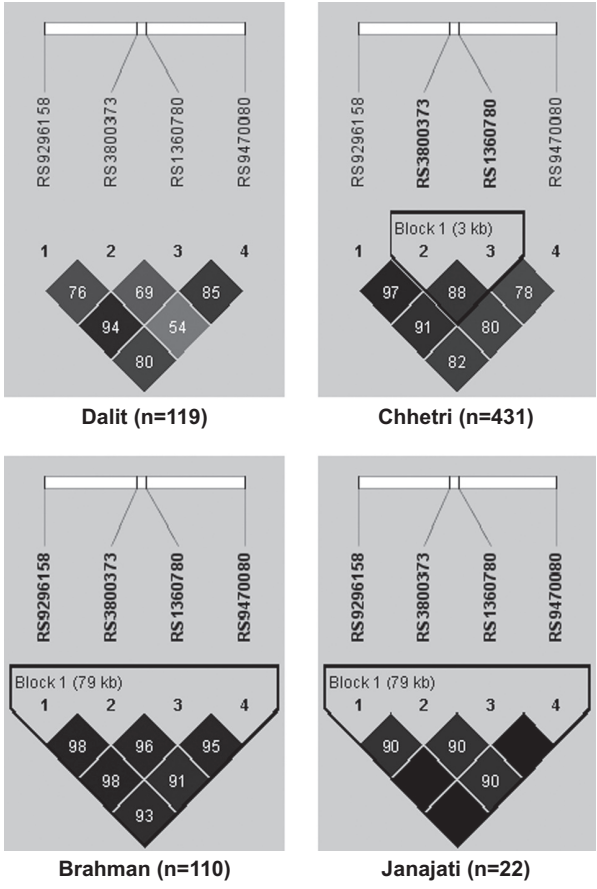

Fig. 1. Linkage disequilibrium R^2 plots for *FKBP5* by ethnicity/caste.

Analysis in the Chhetri-only group showed similar results, with a non-significant trend for the main effect ($p = 0.098$) and significant interaction effect ($p = 0.012$) for total BDI, but not for PCL-C (main: $p = 0.0431$; interaction: $p = 0.117$).

Main and interaction effects for cortisol awakening response

CAR analyses employed the waking sample and a second sample taken 30 min later, collected for three consecutive days, totalling 353 samples for collection time 1 and 346 samples for collection time 2 (see Table 6). Mean cortisol was 0.16 μg/dL (SD = 0.13) for waking collections, and 0.16 μg/dL (SD = 0.14) for the collections 30 min later.

Comparison among the four interaction groups (low CTQ and G-carrier, high CTQ and G-carrier, low CTQ and AA, high CTQ and AA) revealed significant group differences for time 1 collection ($F = 5.52$, $p = 0.02$) and for time 2 collection ($F = 4.59$, p = 0.03, see Table 6). There were no differences among the four interaction groups on time between waking and first collection ($F = 0.57$, $p = 0.45$).

Using LMM with fixed and random effects, we found no significant fixed main effects for CAR (delta of log time 1 and log time 2; see Table 7). However, addition of the interaction term for rs9296158 genotype (AA versus G-carrier) and childhood maltreatment (CTQ median split) yielded a significant interaction effect, as well as a main effect for vegetarianism. In the interaction, the AA genotype/high CTQ subgroup showed a decrease in cortisol from time 1 to time 2, whereas AA genotype/low CTQ showed an increase during that same

Table 3. Mental health outcomes by caste/ethnicity.

	Mean (SD)				
	Total sample	Dalit	Chhetri	Brahman	Janajati
Depression (BDI total score)	18.44 (7.62)	19.86 (6.62)	18.33 (7.62)	17.76 (8.34)	16.32 (8.04)
PTSD (PCL-C total score)	40.00 (8.00)	40.09 (6.59)	39.79 (8.48)	39.50 (8.77)	37.91 (10.24)

N = 682; BDI, Beck Depression Inventory; PCL-C, PTSD Checklist-Civilian Version; SD, standard deviation.

period (see Fig. 3). The 'G-carrier' groups showed no significant positive or negative CAR.

Total between-subject variation explained by the *FKBP5*-by-maltreatment interaction was 14%. Vegetarianism accounted for 1.2% of between-subject variation, with vegetarians having greater positive CAR. Amount of sleep accounted for 9.5% of within-individual variation whereby CAR increased with sleep duration.

Discussion

This is the first study to assess the association of polymorphisms in *FKBP5* with mental health outcomes and childhood maltreatment in a South Asian population. Although the study with Nepalis did not replicate the previously reported interaction of *FKBP5* polymorphisms and early trauma on PTSD symptoms (Binder et al., 2008; Xie et al., 2010), we did find an interaction with depression, another measure of stress-related psychopathology which has also been observed in HIC settings (Appel et al., 2011; Mahon et al., 2013; Zimmermann et al., 2011). In the Nepali population the interaction predicted current depressive symptoms. The genotype previously associated with highest risk to develop PTSD in the presence of child abuse – AA at rs9296158 (Binder et al., 2008) – was associated with the most severe depressive symptoms. The A allele at rs9296518 has also recently been shown to associate with psychosis symptoms and cortisol level differences in interaction with childhood trauma (Collip et al., 2013).

These results meet the minimum requirement for statistical significance, and larger samples will be needed to demonstrate replication of these findings. Our study demonstrated significance at the $p = 0.02$ and $p = 0.03$ level which is comparable to some prior studies with similar sample sizes: $p = 0.02$ (Appel et al., 2011), $p = 0.01$–0.03 (Bevilacqua et al., 2012); however, other prior studies have demonstrated stronger significance of $p < 0.002$ (Binder et al., 2008), $p < 0.001$ (Zimmermann et al., 2011), $p = 0.004$–0.09 (Xie et al., 2010).

Functional neuroendocrine differences in the cortisol awakening response (CAR) also supported the GxE association. Opposite CARs were observed for low versus high childhood maltreatment within the AA genotype group, suggesting that early life trauma in combination with genetic *FKBP5* risk variants can lead to long-term changes in HPA regulation. The rs9296158 AA genotype group with childhood maltreatment exhibited a decrease in cortisol upon waking and overall hypocortisolism, which may

Table 4. Exposures by caste/ethnicity.

	No. (%)				
	Total sample	Dalit	Chhetri	Brahman	Janajati
Childhood maltreatment (CTQ score)					
0–31	206 (30.2)	12 (10.1)	136 (31.6)	46 (41.8)	12 (54.5)
32–35	164 (24.0)	22 (18.5)	107 (24.8)	29 (26.4)	6 (27.3)
36–40	169 (24.8)	37 (31.1)	101 (23.4)	29 (26.4)	2 (9.1)
41+	143 (21.0)	48 (40.3)	87 (20.2)	6 (5.5)	2 (9.1)
Adult lifetime trauma (TEI score)					
0 events	149 (21.9)	17 (14.3)	106 (24.7)	19 (17.3)	7 (31.8)
1 events	216 (31.8)	39 (32.8)	138 (32.2)	32 (29.1)	7 (31.8)
2–3 events	191 (28.1)	35 (29.4)	118 (27.5)	32 (29.1)	6 (27.3)
4 + events	124 (18.2)	28 (23.5)	67 (24.5)	27 (24.5)	2 (9.1)
Stressful life events in past year (SLERS score)					
0–14 events	248 (36.4)	14 (11.8)	178 (41.3)	42 (38.2)	14 (63.6)
14–22 events	232 (34.0)	38 (31.9)	150 (34.8)	40 (36.4)	4 (18.2)
23 + events	202 (29.6)	67 (56.3)	103 (23.9)	28 (25.5)	4 (18.2)

N = 682; CTQ, Childhood Trauma Questionnaire; TEI, Traumatic Events Inventory; SLERS, Cross-Cultural Stressful Life Events Rating Scale.

Table 5. General linear models (GLM) for *FKBP5* SNP rs9296158 for depression (BDI score) and PTSD (PCL-C score) with genotype main effect and gene–environment (childhood maltreatment) interaction.

		Depression (total BDI score)				PTSD (total PCL-C score)			
		Main effects only model[a]		Main effects and CTQ-*FKBP5* interaction model[b]		Main effects only model[c]		Main effects and CTQ-*FKBP5* interaction model[d]	
	No.	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value
Sex									
Male	347	Ref.[e]	< 0.001	Ref.	< 0.001	Ref.	0.02	Ref.	0.03
Female	335	5.47 (4.44–6.49)		5.37 (4.35–6.39)		1.44 (0.20–2.69)		1.39 (0.14–2.65)	
Age									
18–33 years	297	Ref.	< 0.001	Ref.	< 0.001	Ref.	0.34	Ref.	0.29
34–43 years	187	1.92 (0.73–3.11)		2.02 (0.83–3.20)		0.79 (− 0.66–2.24)		0.84 (− 0.62–2.29)	
44 + years	198	4.85 (3.68–6.03)		5.01 (3.84–6.18)		0.99 (− 0.44–2.42)		1.08 (− 0.36–2.52)	
Caste/ethnicity									
Chhetri	431	Ref.	0.05	Ref.	0.10	Ref.	0.09	Ref.	0.13
Dalit	119	− 1.44 (− 1.36–0.23)		− 1.09 (− 2.43–0.25)		− 0.96 (− 2.60–0.68)		− 0.90 (− 2.55–0.74)	
Brahman	110	− 1.36 (− 2.72–− 0.01)		− 1.26 (− 2.61–0.10)		− 2.05 (− 3.70–− 0.39)		− 1.89 (− 3.56–− 0.22)	
Janajati	22	− 2.53 (− 5.28–0.22)		− 1.98 (− 4.75–0.79)		− 1.23 (− 4.59–2.15)		− 1.15 (− 4.56–2.25)	
Childhood maltreatment (CTQ score)									
0–31	206	Ref.	0.001	Ref.	0.002	Ref.	0.66	Ref.	0.30
32–35	164	0.36 (− 0.98–1.69)		− 2.03 (− 4.05–0.00)		− 0.03 (− 1.66–1.60)		− 0.67 (− 3.15–1.82)	
36–40	169	1.56 (0.19–2.93)		0.73 (− 1.20–2.66)		0.46 (− 1.21–2.14)		0.30 (− 2.07–2.66)	
41+	143	2.88 (1.41–4.36)		1.52 (− 0.49–3.52)		0.99 (− 0.80–2.79)		0.69 (− 1.78–3.15)	
Adult lifetime trauma (TEI score)									
0 events	149	Ref.	< 0.001	Ref.	< 0.001	Ref.	< 0.001	Ref.	< 0.001
1 event	216	2.63 (1.27–3.99)		2.52 (1.16–3.88)		2.01 (0.34–3.67)		1.99 (0.32–3.65)	
2–3 events	193	2.88 (1.50–4.26)		2.80 (1.42–4.18)		4.30 (2.62–5.99)		4.29 (2.59–5.99)	
4 + events	124	3.60 (1.99–5.20)		3.51 (1.91–5.10)		6.67 (4.71–8.62)		6.70 (4.73–8.66)	
Stressful life events in past year (SLERS score)									
0–14 events	248	Ref.	< 0.001	Ref.	< 0.001	Ref.	< 0.001	Ref.	< 0.001
14–22 events	232	2.98 (1.78–4.17)		3.06 (1.87–4.25)		2.39 (0.92–3.85)		2.41 (0.95–3.88)	
23 + events	202	4.86 (3.51–6.21)		4.85 (3.51–6.19)		4.56 (2.92–6.21)		4.56 (2.91–6.22)	

(*Continued*)

Table 5. (*Continued*)

		Depression (total BDI score)				PTSD (total PCL-C score)			
		Main effects only model[a]		Main effects and CTQ-*FKBP5* interaction model[b]		Main effects only model[c]		Main effects and CTQ-*FKBP5* interaction model[d]	
	No.	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value	Effect size β (95%CI)	p-value
FKBP5 rs9296158									
AA	68	1.67 (0.00–3.43)	0.03	1.39 (−1.88–4.66)	0.02	−0.47 (−2.51–1.58)	0.83	0.17 (−3.84–4.19)	0.87
AG	303	−0.59 (−1.59–0.41)		−2.74 (−4.55–−0.93)		−0.34 (−1.56–0.86)		−0.92 (−3.15–1.30)	
GG	311	Ref.		Ref		Ref		Ref.	
Interaction: CTQ by Genotype									
		N/A[e]		See Fig. 2[d]	0.02		N/A[e]	Not reported[d]	0.58

N = 682; SNP, single nucleotide polymorphism; BDI, Beck Depression Inventory; PCL-C; PTSD Checklist-Civilian Version; CTQ, Childhood Trauma Questionnaire; TEI, Traumatic Events Inventory; SLERS, Cross-Cultural Stressful Life Events Rating Scale; CI, confidence interval.

[a]Main effect models include all variables shown: sex, age, CTQ quartiles, TEI quartiles, political violence quartiles, and SNP genotype.

[b]Interaction models include all main effects from model A (sex, age, caste/ethnicity, CTQ, TEI, SLERS, and *FKBP5*) and the interaction of *FKBP5* genotype and CTQ quartiles.

[c]Interaction not included in main effect model A.

[d]Regression coefficients not reported for interaction cells, see Fig. 3 for BDI means by genotype and severity of child maltreatment. Non-significant regression coefficients and confidence intervals not reported for PCL-C.

[e]Ref. (reference) indicates comparison group for estimation of effect size.

Fig. 2. Depression mean scores (error bars represent standard error of the mean) by *FKBP5* rs9296158 SNP genotypes and level of child maltreatment (CTQ quartiles), N = 682. SNP, single nucleotide polymorphism; BDI, Beck Depression Inventory; CTQ, Childhood Trauma Questionnaire; SLERS, Cross-cultural Stressful Life Events Rating Scale; TEI, Trauma Exposure Inventory. Means are adjusted for sex, age, lifetime adult trauma (TEI quartiles), and stress exposure in past year (SLERS tertiles).

reflect an increased GR (glucocorticoid receptor) sensitivity in this group. This finding was strongly significant ($p < 0.001$).

A possible mechanism for depression and CAR findings is that the AA genotype may represent greater sensitivity to early environmental conditions that lead to adult patterns of cortisol secretion (Boyce & Ellis, 2005). Among individuals with the AA genotype, early life trauma may preferentially trigger epigenetic changes modulating HPA axis regulation such as up-regulation of *FKBP5* persisting into adulthood reflected by an altered CAR.

In the AA-genotype group with low trauma, a high CAR and possibly overall hypercortisolism may reflect the increased GR resistance observed in controls with this genotype (Binder et al., 2004, 2008) which leads to impaired negative feedback of the HPA axis when confronted with traumatic events (Ising et al., 2008), and thus to prolonged exposure to cortisol. This may represent a process at work in children carrying the AA genotype who experience prolonged maltreatment and chronic high cortisol exposure, leading to processes that produce a negative CAR and hypocortisolism as adults. Meaney and colleagues have demonstrated how early abuse alters glucocorticoid receptor activity in adults (McGowan et al., 2009). *FKBP5* may be one of many components contributing to this developmental trajectory. Individuals with G alleles may be less responsive to early environmental conditions, at least with regard to depression and CAR.

These findings replicate the enhanced GR sensitivity associated with *FKBP5* risk genotypes as previously observed among adults with PTSD and current major depression (Binder et al., 2004, 2008; Mehta et al., 2011). A number of studies have also shown

Table 6. Cortisol awakening response crude values and times by *FKBP5* genotype and childhood maltreatment.

Group characteristics rs9296158 genotype	G-carrier		AA		
Childhood maltreatment (CTQ score)	Below median	Above median	Below median	Above median	Total sample all groups
Participants, N	50	57	7	4	118
Total cortisol samples	297	341	39	22	699
Female, N (%)	23 (46)	29 (51)	5 (71)	3 (75)	60 (51)
Caste, N (%)					
Dalit	4 (8)	17 (30)	0 (0)	1 (25)	22 (18)
Chhetri	18 (36)	22 (39)	5 (71)	0 (0)	45 (38)
Brahman	25 (50)	18 (31)	2 (29)	3 (75)	48 (41)
Janajati	3 (6)	0 (0)	0 (0)	0 (0)	3 (3)
Time of collection (SD)					
Waking, collection 1	5:42 (0:35)	5:47 (0:37)	5:50 (0:24)	6:19 (0:36)	5:46 (0:36)
Post-waking, collection 2	6:14 (0:36)	6:17 (0:38)	6:20 (0:24)	6:48 (0:35)	6:17 (0:37)
Time between waking and first collection	0:06 (0:13)	0:10 (0:23)	0:06 (0:11)	0:06 (0:11)	0:08 (0:18)
Cortisol values, μg/dL (SD)					
Waking, Collection 1	0.17 (0.12)	0.15 (0.14)	0.11 (0.11)	0.12 (0.12)	0.16 (0.13)
Post-waking, collection 2	0.18 (0.14)	0.15 (0.14)	0.17 (0.11)	0.07 (0.06)	0.16 (0.14)
Delta, collection 2–collection 1	0.01 (0.15)	0.00 (0.14)	0.08 (0.14)	−0.07 (0.15)	0.00 (0.14)
Estimated marginal means[a] (95% CI)					
CAR	0.08 (−0.08–0.24)	0.06 (−0.13–0.25)	0.45 (0.12–0.79)	−0.66 (−1.07–−0.25)	

N = 118; CAR, cortisol awakening response; SD, standard deviation; CI, confidence interval.
[a]Estimated marginal means for CAR for difference log time 1 and log time 2, based on linear mixed models evaluated at the following values: sleep = 8.6 h, time between waking and first collection = 8 min, and clock time of first collection = 5:46 am.

hypocortisolism related to childhood maltreatment (Gunnar & Donzella, 2002; Gunnar & Vazquez, 2001; Shea et al., 2007; Yehuda et al., 2001) and among individuals with disorders reflecting stress and trauma in adulthood (Chrousos, 1998; Heim et al., 2000; Juruena & Cleare, 2007; Van den Bergh et al., 2008).

Cross-cultural interpretations and implications

Interpretation of these findings in light of the South Asian cultural context and population should take into account a range of factors. Frequencies of exposure of interest, prevalence of psychiatric phenotype of interest, and frequency of polymorphisms of interest can all impact statistical associations identified across different cultural groups and settings. These three types of frequencies interact, leading to wide variation in significance of results at different sample sizes, which has been demonstrated graphically by Caspi and colleagues (2010). In general, the genotype and allele frequencies in this South Asia sample were comparable to other populations studied. The homozygous genotype frequencies for common *FKBP5* SNPs of interest have been approximately 10% in most of the populations studied to date, i.e. groups of European descent (Appel et al., 2011; Mahon et al., 2013; Zimmermann et al., 2011); and the frequencies were also approximately 10% in this Nepali sample. The only population with greater homozygous prevalence was the African American sample in the original *FKBP5* and PTSD study in which the prevalence rates were approximately two times greater at 20% (Binder et al., 2008). Minor allele frequencies varied from 0.29 to 0.50 in these samples, and our Nepali population was within this range.

The cultural validity of the BDI is an important issue to consider. We have extensively employed systematic transcultural translation processes to adapt and then validate North American, high-income country psychiatric measures for Nepali populations in order to achieve semantic, technical, criterion, and construct equivalence (Kohrt et al., 2011). Moreover, the BDI was specifically validated with this population in Jumla, Nepal, and therefore the psychometric properties are representative of this population (Kohrt et al., 2002). However, there are numerous factors that influence psychometric properties and performance of English speaking, high-income country-developed instruments. Cultural significance and meaning may influence self-disclosure of a psychiatric phenotype. The term 'category fallacy' refers to the assumption that psychiatric symptoms when observed across different cultural groups have the same meaning and significance with regard to pathology and social consequences (Kleinman, 1988).

It is crucial to consider processes that may influence cross-cultural differences in endorsement. We have found that stigma related to PTSD is likely greater than stigma associated with depression in Nepali culture because of beliefs related to karma

Table 7. Linear mixed models for cortisol awakening response[a].

		Main effects only model		Main effects and CTQ-*FKBP5* interaction model	
Fixed effects	No.	β (95%CI)	p-value	β (95%CI)	p-value
Sex					
Male	58	Ref.[c]	0.11	Ref.	0.18
Female	60	0.13 (−0.03–0.29)		0.11 (−0.05–0.26)	
Age					
18–33 years	35	Ref.	0.32	Ref.	0.30
34–43 years	42	0.02 (−0.15–0.18)		−0.01 (−0.17–0.15)	
44 + years	41	0.12 (−0.05–0.30)		0.11 (−0.06–0.28)	
Ethnicity					
Chhetri	45	Ref.	0.30	Ref.	0.17
Brahman	22	0.19 (−0.02–0.39)		0.22 (0.02–0.42)	
Dalit	48	0.05 (−0.11–0.20)		0.12 (−0.03–0.28)	
Janajati	3	−0.12 (−0.54–0.30)		−0.03 (−0.45–0.38)	
Vegetarian					
Non-veg	107	Ref.	0.10	Ref.	0.02
Veg	11	0.21 (−0.04–0.46)		0.29 (0.04–0.53)	
Last menstrual period					
Not menstruating[b]	93	Ref.	0.18	Ref.	0.58
Pre/menstrual	25	−0.13 (−0.33–0.06)		0.11 (−0.24–0.14)	
CTQ					
Low	57	Ref.	0.17	Ref.	0.83
High	61	−0.10 (−0.24–0.04)		−0.02 (−0.16–0.12)	
rs9296158					
G-carrier	107	Ref.	0.89	Ref.	0.02
AA	11	−0.02 (−0.27–0.24)		0.38 (0.07–0.69)	
Interaction					
Other groups	114	N/A		Ref.	< 0.001
High CTQ, AA	4			−1.10 (−1.62–0.58)	

N = 118;
CI, Confidence Interval; CTQ, Childhood Trauma Questionnaire.
[a]Cortisol awakening response is the delta of $\log_{10}$ waking and $\log_{10}$ 30 min.
[b]Includes male participants.
[c]Ref. (reference) indicates comparison group for estimation of effect size.

and personal culpability related to experiencing trauma (Kohrt & Harper, 2008; Kohrt & Hruschka, 2010). This may lead to under-reporting PTSD symptoms compared with depression symptoms. This may represent a cultural difference: PTSD is assumed to be less stigmatized in American and European populations than depression (Summerfield, 2001) whereas PTSD is more stigmatized than depression in regions such as South Asia and West Africa (Fox, 2003; Kohrt & Hruschka, 2010). Therefore, we may have failed to find an association between PTSD and *FKBP5* because of cultural differences in discussing trauma and disclosing post-traumatic psychological distress.

Sociopolitical factors will influence the frequency of stressful and traumatic exposures, with exposures such as political violence and war more common in low-income countries (Tol et al., 2011). Via pathways of social determinants of mental health such as poverty and war the prevalence of psychiatric phenotypes will also vary by culture and setting (Lund et al., 2010; Steel et al., 2009, 2014). The prevalence of both psychiatric problems and negative exposures were high in this population. This is a region with high rates of poverty, physical health problems, lack of education and health infrastructure, and exposure to both domestic and political violence. Rates of depression were high even before the 10-year civil war with almost one third of the population meeting criteria for treatment based on a culturally validated cut-off on the BDI (Kohrt et al., 2012).

Regarding exposures to child maltreatment, prevalence rates of child traumas are influenced not only by population variation but also by the type of tool selected to assess maltreatment or other stressors. CTQ scores are based on adult recollections of trauma exposure and thus influenced by current mental health. Prior GxE studies have found different outcomes when subjective versus objective measures of trauma are employed. For example, in the studies that showed a CRHR1 interaction effect with child maltreatment among African American women the measure was a subjective retrospective recall using the CTQ (Bradley et al., 2008; Kranzler et al., 2011). The British study that found an association also used the CTQ, but the New Zealand replication

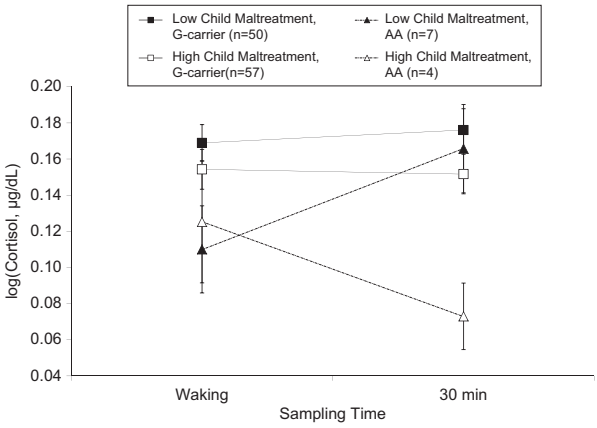

Fig. 3. Diurnal salivary cortisol by child maltreatment and *FKBP5* SNP rs9296158 genotype. Log cortisol (error bars SEM) measured at waking, 30 min after waking, and evening (approximately 7 pm). Four groups represent high and low child maltreatment status (CTQ total score median split) and *FKBP5* SNP rs9298158 genotype (G-carrier versus AA homozygote), N = 118, total cortisol samples = 699. SNP, single nucleotide polymorphism; CTQ, Childhood Trauma Questionnaire; SEM, standard error of the mean.

study used court reports of abuse and did not find an association (Polanczyk et al., 2009). In Germany the polymorphism showed an association with subjective recall of physical neglect on depression, but not on any other child maltreatment measures (Grabe et al., 2010). This highlights the problem of retrospective assessment of childhood trauma. To unequivocally prove the causal relationship to childhood trauma, longitudinal studies will be needed, with birth cohorts starting in pregnancy being an optimal design to elucidate GxE interactions.

Another issue relates to the meaning of high scores on exposure measures. GxE studies typically operate with assumptions that exposure categories are cross-culturally equivalent and therefore would be expected to have comparable impact on child development and mental health. However, cross-cultural research suggests that exposures have different social meaning, different consequences, and associate differently with mental health outcomes (Harkness & Super, 1996; Kohrt, 2015; Korbin, 1991, 2002). Measurement of trauma and maltreatment, and quantification of these exposures are complex issues (Kohrt, 2015). For example, in a study of children in China, India, Italy, Kenya, Philippines, and Thailand, there appear to be group differences in aggression and anxiety based on child ratings of normativeness of discipline types (Gershoff et al., 2010).

High scores on CTQ in our study may reflect different processes than occur with elevated CTQ scores in high-income countries. In this Nepali sample, the physical neglect subscale of the CTQ was the most frequently endorsed, and this is likely influenced by poverty and lack of access to resources for appropriate clothing, food, and housing. Lack of appropriate clothing and food is likely mediated by different processes when parents have access to resources but do not use them for child rearing versus settings where the population as whole does not have access to resources to meet basic needs. High CTQ neglect scores in Nepal do not necessarily represent parental pathology or mal-intent as it may in other settings.

Cross-cultural studies are also influenced by how population units are defined. Anthropologists define cultural groups in relation to shared practices and experiences, and these categories may – and often may not – overlap with other markers related to ethnicity, language, and social categories of race (Hruschka & Hadley, 2008). Categories such as caste in South Asia are a product of social processes (Höfer, 2004; Kohrt, 2009) and thus do not represent genetically distinct groups. It is crucial to avoid conflating genetic differences with social categories.

Other aspects of the cultural group need to be considered. Many Hindu subgroups practice vegetarian diets. Although existing literature does not support a relationship between basal cortisol and vegetarian diet (Hung et al., 2006; Remer et al., 1998; Rouse et al., 1984), vegetarianism associated with higher CAR in the study. This may be related to differences in insulin and counter-regulatory hormones after fasting among vegetarians (Hung et al., 2006; Michalsen et al., 2003; Remer et al., 1998). Cortisol levels may vary throughout the menstrual cycle, and this has been proposed as a factor in fluctuating symptom severity of disorders in relation to menstrual phase (Saketos et al., 1993). However, prior studies have not examined how CAR may change across the menstrual cycle.

Analytic and design approaches can be employed to address some of these cross-cultural factors. For example, different exposure to lifetime adult trauma or stressful life events in the past year, were controlled in these analyses. Population stratification is not likely confounding in these analyses because we controlled for caste in the combined analysis and observed the same interactions in analyses restricted only to Chhetri. However, we did find that levels of trauma and stressful life events varied considerably by caste/ethnicity, with Dalit/Nepali groups bearing the greatest burden of life stressors. Therefore, caste/ethnicity captures differences in life experience. Among the Dalit/Nepali groups, the high CTQ scores were also related to physical neglect issues and this is related to Dalit/Nepali groups bearing the greatest burden of poverty in most parts of Nepal. In prior studies with this population, caste differences in depression severity between Dalit/Nepali and high caste groups could be explained entirely by differences in levels of stressful life events and lack of cash income and livestock (Kohrt et al., 2009).

Taken together, these factors demonstrate why the symptom-complex associated with combined genetic and environmental risk will vary depending on other environmental and genetic risk factors or social context. Factors related to population prevalence and cultural meaning may explain why the interaction predicted PTSD symptoms in an urban African American cohort but was associated with depressive symptoms in a rural South Asian cohort. What is striking from the current study is that the biological profile (i.e. cortisol awakening response) showed the most statistically significant GxE interaction effect. This raises interesting questions about cultural variation in self-reported phenotypes in the context of similar trauma burden, genetic risk factors, and biological markers. Increasing attention to studies of biological markers for neuropsychiatric disorders will therefore require culturally and contextually diverse samples to avoid claims of association that may only be relevant to English-speaking residents for North American and Western European HIC settings.

Limitations

This sample contains participants who were originally recruited in 2000 and then followed up in 2007–2008 and participants who were recruited in 2007–2008. There may be unassessed biases between the two groups recruited at different times. While every effort was made to use the same recruitment processes, changing conditions during the interval, migration patterns, and war exposure may contribute to differences between these two subsets of the sample. Another limitation is that the educational levels of the respondents varied, and this variation was associated with caste/ethnicity. It is possible that literate respondents interpreted questions differently than illiterate respondents, which could lead to different prevalence rates based on literacy status or caste/ethnicity that was a result of interpretation rather than the experience of depression.

The small number of participants with the AA genotype limits the generalizability of findings. The total sample and the Chhetri-only subsample displayed the association of interest for this study. However, analyses were not sufficiently powered to assess for the association within Brahman, Dalit/Nepali, and Janajati groups separately. The association may operate differently within these groups. We did find that Brahman and Janajati groups were genetically very similar at the *FKBP5* locus, despite strong socially prescribed differences in categorizing these groups. Future studies should include larger sample sizes and consider over-recruitment of under-represented groups such as Dalit/Nepali and Janajati.

In the cortisol protocol, AA-genotype participants were more likely to be women. In the AA and high child maltreatment subgroup, Brahman comprised 75% of the sample. However, the sample was dominated by Chhetri participants because it was representative of the population. While these demographic factors were controlled in analyses, there may be other demographic or associated variables that led to the observed group differences in CAR. Furthermore, the individuals in the AA genotype group with high exposure to child maltreatment reported waking times approximately 30 min later than other participants. For future studies, one could explore whether CAR differences are observable if participants are awakened at specific times.

Conclusion

The unique contribution of our study is a partial replication of a known GxE interaction in post-conflict, low-income setting, specifically with a South Asian population in rural Nepal. Moreover, we demonstrated the association of biological variation (cortisol awakening response) associated with the GxE interaction. In consequence of our finding functional effects of the investigated *FKBP5* polymorphism in South Asians, the genetic variant (or variants highly correlated with it) now has been associated with altered GR-responsivity and the development of stress-related psychiatric disorders in South-Asian, European, European American, and African American subjects (Zannas & Binder, 2014). Such persistent associations suggest that the SNP complex tagged by rs9296158 and including rs1360780 may be functionally relevant across populations. In populations studied thus far, this *FKBP5* haplotype is associated with higher GR resistance in healthy subjects and GR super-sensitivity in the presence of a psychiatric disorder and/or early trauma. The psychiatric presentation of these long-term endocrine dysregulations may vary among populations with different genetic and environmental risk factors, as well as other sociocultural conditions, but ultimately, *FKBP5* is an important candidate gene in the development of stress-related psychiatric disorders. Improved representation of diverse populations and settings in GxE research will help to identify potential mechanisms in the generation of psychopathology.

From a public health perspective, an important finding was that even in the presence of high exposure to recent political violence as experienced by the population, child maltreatment has a significant effect on adult psychopathology and long-term effects on HPA regulation. Interventions to reduce child maltreatment can have lasting biological and developmental effects in reducing risk for adult psychopathology, even in – and maybe especially in – the context of high levels of adult exposure to trauma, which is a characteristic of many low- and middle-income settings. We thus advocate for interventions focusing on the prevention of child abuse as a crucial

step towards reducing the adult burden of mental illness. Alongside increasing clinical services and psychosocial programming, prevention of child maltreatment should be a priority in the growing global mental health movement.

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Author contributions: B.A.K. designed, supervised, and implemented the epidemiological study, performed the statistical analyses, and drafted the manuscript. C.M.W. supervised the study design, cortisol analyses, and revised the manuscript. K.J.R., K.B.M., and E.B.B. supervised the genetic study, genetic analyses, and revised the manuscript. N.U., S.K., V.D.S., and M.K.N. contributed to the study design, implementation, and supervision in Nepal. All authors approved the manuscript. The authors alone are responsible for the content and writing of the paper.

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