# Psychological resilience and the gene regulatory impact of posttraumatic stress in Nepali child soldiers

Brandon A. Kohrt[a,b,c,1], Carol M. Worthman[d], Ramesh P. Adhikari[c], Nagendra P. Luitel[c], Jesusa M. G. Arevalo[e,f], Jeffrey Ma[e,f], Heather McCreath[g], Teresa E. Seeman[g,h], Eileen M. Crimmins[i], and Steven W. Cole[e,f]

[a]Department of Psychiatry and Behavioral Sciences, Duke University School of Medicine, Durham, NC 27708; [b]Duke Global Health Institute, Duke University, Durham, NC 27710; [c]Transcultural Psychosocial Organization Nepal, Kathmandu 44616, Nepal; [d]Department of Anthropology, Emory University, Atlanta, GA 30322; [e]Division of Hematology-Oncology, Department of Medicine, University of California, Los Angeles School of Medicine, Los Angeles, CA 90095; [f]Department of Psychiatry & Biobehavioral Sciences, University of California, Los Angeles School of Medicine, Los Angeles, CA 90095; [g]Division of Geriatrics, Department of Medicine, University of California, Los Angeles School of Medicine, Los Angeles, CA 90095; [h]Department of Epidemiology, University of California, Los Angeles School of Public Health, Los Angeles, CA 90095; and [i]Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089

Edited by Cynthia M. Beall, Case Western Reserve University, Cleveland, OH, and approved June 6, 2016 (received for review January 26, 2016)

**Adverse social conditions in early life have been linked to increased expression of proinflammatory genes and reduced expression of antiviral genes in circulating immune cells—the conserved transcriptional response to adversity (CTRA). However, it remains unclear whether such effects are specific to the Western, educated, industrialized, rich, and democratic (WEIRD) cultural environments in which previous research has been conducted. To assess the roles of early adversity and individual psychological resilience in immune system gene regulation within a non-WEIRD population, we evaluated CTRA gene-expression profiles in 254 former child soldiers and matched noncombatant civilians 5 y after the People's War in Nepal. CTRA gene expression was up-regulated in former child soldiers. These effects were linked to the degree of experienced trauma and associated distress—that is, posttraumatic stress disorder (PTSD) severity—more than to child soldier status per se. Self-perceived psychological resilience was associated with marked buffering of CTRA activation such that PTSD-affected former child soldiers with high levels of personal resilience showed molecular profiles comparable to those of PTSD-free civilians. These results suggest that CTRA responses to early life adversity are not restricted to WEIRD cultural contexts and they underscore the key role of resilience in determining the molecular impact of adverse environments.**

biocultural anthropology | child abuse | global mental health | low-income countries | social genomics

Exposure to social adversity during childhood and adolescence predicts a host of physical and mental health problems across the life span (1, 2). One hypothesized mechanism of such effects involves the impact of adverse environmental conditions on development of bidirectional regulatory interactions between the nervous and immune systems (3, 4). For example, chronic exposure to adverse social environments is associated with activation of a conserved transcriptional response to adversity (CTRA) involving up-regulation of proinflammatory genes and down-regulation of type I IFN- and antibody-related genes in myeloid lineage immune cells (5–7). These molecular dynamics have been implicated in a diverse array of disease processes, including cardiovascular and neurodegenerative diseases (8, 9), metastatic cancer (10), viral infection (6, 11), and psychiatric conditions such as anxiety and depression (12). In nonhuman primates, exposure to unstable social conditions for the first 4 mo of life is sufficient to induce the CTRA profile (13), and longitudinal human studies suggest that the molecular residue of such effects can persist into middle and late adulthood (14, 15). Research has begun to clarify the basic neuroimmune signaling pathways involved in CTRA development (6, 7, 9, 16).

Mapping the sources of molecular resilience to early adversity is complicated by the fact that all previous research in this area has been conducted in Western, educated, industrialized, rich, and democratic (WEIRD) populations (17). Non-WEIRD societies differ in the degree and type of exposure to stressful and traumatic life events. For example, non-WEIRD populations are more likely to be exposed to war and political violence (18, 19). Children in non-WEIRD populations are also more likely to be exposed to war as both civilians and combatants (20). WEIRD cultures may also atypically influence the nature and sources of psychological resilience to early life adversity. Previous studies have identified some resilience factors that may protect against CTRA emergence in midadulthood, such as early-life maternal relationship quality (21) and a sense of personal purpose in life (22–24). These studies were conducted in WEIRD cultures with comparatively extreme individualistic orientations, in which resilience is seen as an attribute of the person. It is unclear whether such individualized conceptions of resilience would pertain in non-WEIRD populations that are more collectivistic in orientation (25, 26), particularly if the nature, intensity, or context of adversity also differs in these environments. In contrast to individualistic interventions, in non-WEIRD populations with high trauma exposure, social ecological resilience interventions promoting family and community recovery have been considered culturally consistent and have shown positive outcomes (27). Non-WEIRD environments may also differ from WEIRD environments in the nature of microbial exposures and

## Significance

**Adverse life conditions are linked to increased expression of proinflammatory genes and reduced expression of antiviral genes. However, these findings have come from Western, educated, industrialized, rich, and democratic (WEIRD) societies. Therefore, we evaluated adversity-related gene regulation among former child soldiers in Nepal—a non-WEIRD population. We found that posttraumatic stress disorder (PTSD) and resilience were inversely and independently associated with gene regulation among a population exposed to war during childhood. The results suggest that gene regulation responses to adversity are not restricted to WEIRD contexts and they underscore the role of psychological resilience in determining the molecular impact of traumatic experiences. Promoting resilience, even in the absence of PTSD symptom reduction, may have benefits for physical and mental health.**

Author contributions: B.A.K., C.M.W., and S.W.C. designed research; B.A.K., R.P.A., and N.P.L. performed research; H.M., T.E.S., and E.M.C. contributed new reagents/analytic tools; B.A.K., J.M.G.A., J.M., and S.W.C. analyzed data; and B.A.K., C.M.W., and S.W.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE77164).

[1]To whom correspondence should be addressed. Email: brandon.kohrt@duke.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1601301113/-/DCSupplemental.

resulting immune system regulatory dynamics (28, 29). Such considerations suggest that assessments of resilience developed for use in WEIRD contexts may not associate with immune cell molecular markers in other cultural and ecological contexts. Given the population bias in existing CTRA research, it remains unclear whether early life social adversity would affect CTRA gene expression similarly in a non-WEIRD low-/middle-income country (LMIC), which is more comparable with the context in which the majority of the world's children develop (30).

Therefore, the present research examined how early life social adversity and individual psychological resilience interact with CTRA gene expression in a non-WEIRD/LMIC population exposed to adversity: specifically, former child soldiers and other war-affected youth in Nepal.

## Results

To assess the roles of early adversity and self-perceived resilience in the development of CTRA gene expression profiles in a non-WEIRD/LMIC culture, we evaluated 154 former child soldiers (age 16–26 y) and 136 matched civilians (15–24 y) who grew up amid war but were not combatants (Table 1). The participants were part of a 5-y longitudinal study that began in 2007, a year after peace accords ending the decade-long People's War in Nepal (31, 32). Two hundred fifty-eight former child soldiers were enrolled in the study before participating in nongovernmental reintegration services provided by United Nations Children's Emergency Fund (UNICEF), and a cohort of 258 civilian children matched on demographics were enrolled in the study but did not receive intervention services. Twelve months later, 222 of the former child soldiers (86% of original sample) and 234 of the matched civilians (91%) were reinterviewed. For the current study, in 2012, 154 of the former child soldiers (60% of original participants) and 136 of the matched civilians (53%) were traceable for interviews, at which time blood spots were collected for gene expression profiling ($n = 282$). In 2012, posttraumatic stress disorder (PTSD) was evaluated with the Child PTSD Symptom Scale (CPSS) (33), which was culturally and clinically validated in Nepal (34). Self-perceived psychological resilience was measured using a culturally adapted abbreviated version of Wagnild and Young's Resilience Scale (35). The prevalence of PTSD in 2012 was 16.2% among former child soldiers compared with 5.9% among matched civilians [odds ratio (OR) = 3.10, 95% confidence interval (CI) (1.35, 7.13), $P = 0.008$]. The mean resilience score was 12.55 (SE 0.30) among child soldiers and 13.15 (SE 0.33) among matched civilians ($t = 1.35$, df = 288, $P = 0.18$).

Among the 282 participants from whom dried blood spot samples were collected, 254 (90.1%) participants' samples yielded valid transcriptome profiles and had data available for all other measures analyzed. Expression of the CTRA profile was assessed using an a priori-defined contrast among 53 gene transcripts (52 of which were detectable in this sample) comprising up-regulation of 19 proinflammatory genes and down-regulation of 34 genes involved in type I IFN and antibody responses. Mixed-effect linear model analyses found CTRA gene expression to vary systematically as a function of an a priori-specified set of demographic characteristics [age, sex, ethnic minority status, low caste status, and educational attainment; $F(5, 240) = 57.55$, $P < 0.0001$] and an a priori-specified set of RNA transcripts marking the relative prevalence of major leukocyte subsets [$CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, natural killer cells, and monocytes; $F(8, 240) = 123.74$, $P < 0.0001$]. Parameter estimates relating CTRA gene expression to individual demographic and leukocyte subset dimensions are reported in Table 2, model 1.

Beyond the effects of demographic characteristics and leukocyte subset distributions, CTRA gene expression was also significantly up-regulated in former child soldiers compared with civilians [$F(1, 239) = 4.88$, $P = 0.0281$] (Table 2, model 2). Subsequent analyses examining a 2D representation of war-related adversity that included current PTSD symptoms as well as child soldier status (i.e., soldier vs. civilian) found CTRA expression to vary significantly within this space [$F(2, 238) = 7.64$, $P = 0.0006$]. Dimension-specific parameters (Table 2, model 3) showed limited CTRA association with child soldier status per se [$F(1, 238) = 2.90$, $P = 0.0901$] but substantial CTRA up-regulation among those experiencing PTSD [$F(1, 238) = 10.35$, $P = 0.0015$] (Fig. 1).

Above and beyond the effects of demographic characteristics, leukocyte subset distributions, and the 2D representation of war-related adversity, CTRA gene expression was also down-regulated in direct proportion to Resilience Scale scores [$F(1, 237) = 5.54$, $P = 0.0194$] (Table 2, model 4 and Fig. 1). To determine how fully

**Table 1. Demographics of former child soldiers and civilian youth**

| Demographic | Former child soldiers ($n = 154$) | Civilian youth ($n = 136$) | Group differences |
|---|---|---|---|
| Age, mean (SE) | 20.43 (0.13) | 19.88 (0.15) | $t = 2.75$, $P < 0.01$ |
| Gender, *n* (%) | | | |
| Female | 77 (50.0%) | 58 (42.6%) | $\chi2 = 1.57$, $P = 0.24$ |
| Male | 77 (50.0%) | 78 (57.4%) | |
| Caste, *n* (%) | | | |
| Upper caste | 37 (24.0%) | 35 (25.7%) | $\chi2 = 0.13$, $P = 0.94$ |
| Lower caste | 38 (24.7%) | 32 (23.5%) | |
| Ethnic minority | 79 (51.3%) | 69 (50.7%) | |
| Education, *n* (%) | | | |
| ≤5th grade completed | 37 (24.0%) | 26 (19.1%) | $\chi2 = 1.40$, $P = 0.50$ |
| 5th–9th grade completed | 59 (38.3%) | 51 (37.5%) | |
| ≥10th grade completed | 58 (37.7%) | 59 (43.4%) | |
| Lifetime traumatic events, *n* (%) | | | |
| ≤1 event | 58 (37.7%) | 93 (68.4%) | $\chi2 = 27.31$, $P < 0.01$ |
| Two or more events | 96 (62.3%) | 43 (31.6%) | |
| Combat exposure, *n* (%) | | | |
| No combat | 116 (75.3%) | 131 (96.3%) | $\chi2 = 25.22$, $P < 0.01$ |
| Any combat | 38 (24.7%) | 5 (3.7%) | |
| PTSD | | | |
| No (CPSS <20) | 129 (83.8%) | 128 (94.1%) | $\chi2 = 7.67$, $P < 0.01$ |
| Yes (CPSS ≥20) | 25 (16.2%) | 8 (5.9%) | |
| Resilience, mean (SE) | 12.55 (0.30) | 13.15 (0.33) | $t = 1.35$, $P = 0.18$ |

ANTHROPOLOGY

**Table 2. Difference in CTRA gene expression as a function of demographic and hematologic covariates, child soldier status, PTSD, and resilience**

| Model | *b* (SE)* | *P* value |
|---|---|---|
| Model 1 (demographic and hematologic control variables only) | | |
| Age, y | −0.066 (0.026) | 0.0125 |
| Sex (female) | −1.315 (0.093) | <0.0001 |
| Ethnic minority (*Janajati*) | 0.114 (0.112) | 0.3100 |
| Low social caste (*Dalit*) | 0.122 (0.136) | 0.3706 |
| Education level (range 0–6) | 0.197 (0.032) | <0.0001 |
| *CD3D* | 2.605 (0.286) | <0.0001 |
| *CD3E* | −6.483 (0.560) | <0.0001 |
| *CD4* | −4.219 (0.369) | <0.0001 |
| *CD8A* | 1.202 (0.220) | <0.0001 |
| *CD19* | 1.833 (0.564) | 0.0013 |
| *NCAM1* | −1.448 (1.067) | 0.1760 |
| *FCGR3A* | 1.155 (0.144) | <0.0001 |
| *CD14* | −1.004 (0.123) | <0.0001 |
| Model 2 (model 1 + child soldier status) | | |
| Child soldier† | 0.198 (0.009) | 0.0281 |
| Model 3 (model 2 + PTSD) | | |
| Child soldier† | 0.154 (0.090) | 0.0901 |
| PTSD† | 0.468 (0.146) | 0.0015 |
| Model 4 (model 3 + resilience) | | |
| Child soldier† | 0.133 (0.091) | 0.1443 |
| PTSD† | 0.455 (0.146) | 0.0020 |
| Resilience (range 2–24) | −0.028 (0.012) | 0.0194 |

*Partial regression coefficient from mixed-effect linear model relating average expression of 52 CTRA indicator genes to listed variables. $n = 254$.
†Child soldier status and PTSD status included as categorical variables: former child soldier = 1, civilian youth = 0; current PTSD (CPSS total score ≥20) = 1; not current PTSD (CPSS total score <20) = 0.

individual psychological resilience might buffer the effects of war-related adversity, we examined predicted CTRA gene expression levels across the observed range of individual differences in Resilience Scale scores (i.e., −2, −1, 0, +1, and +2 SD relative to the sample average). As shown in Fig. 1, buffering effects were pronounced. Among PTSD-affected former child soldiers, those with high resilience scores (+2 SD) showed CTRA gene expression levels markedly below those observed for average- and low-resilience PTSD-affected child soldiers and comparable in magnitude with levels observed in the PTSD-free civilian youth. These buffering effects did not arise from any confounding of high adversity exposure with low self-perceived resilience because Resilience Scale scores in PTSD-affected child soldiers were broadly similar to the remainder of the sample (mean 11.7 ± SD 4.1 vs. 12.9 ± SD 3.8 for the remainder; difference in means, $P = 0.1675$; difference in dispersion, $P = 0.7737$).

## Discussion

These data show the emergence of persistent CTRA gene expression profiles in the aftermath of early life adversity among former child soldiers in a non-WEIRD/LMIC cultural environment. CTRA gene expression profiles were linked to the degree of trauma exposure and associated distress, as measured by PTSD symptom severity above a culturally and clinically validated cutoff point (34), more than to child soldier status per se. These relationships were independent of demographic characteristics (age, sex, ethnic status, low caste, and education) and individual differences in leukocyte subset prevalence. They also emerged in a cultural context that is likely more collectively orientated than populations previously studied in social genomics literature (36–38). Despite this collective social orientation, individual self-perceptions of personal psychological resilience were associated with markedly lower CTRA gene expression profiles, to the extent that PTSD-affected former child soldiers with high levels of self-reported resilience showed molecular profiles comparable to those of PTSD-free civilian youth. These results suggest that CTRA responses to early life social adversity may represent a relatively broad human potential that can be observed in non-WEIRD populations. Our findings echo a recent study in which a molecular association established in WEIRD populations was replicated in a non-WEIRD population: specifically, the association of stressful exposures and telomere shortening among Indian conservation refugees (39).

The present data underscore the importance of recognizing PTSD in non-WEIRD cultural groups because PTSD may herald physical and mental health problems throughout the life course. However, this contrasts with some anthropological and psychiatric perspectives arguing that PTSD is not relevant in non-WEIRD populations (40). We demonstrated an association between CTRA up-regulation and child soldier status and a stronger association between CTRA up-regulation and current PTSD. Given that both child soldiers and matched youth were exposed to war and different forms of trauma (41, 42), it is not surprising that PTSD was a stronger predictor of CTRA up-regulation than child soldier status per se. Our prior research with child soldiers in Nepal indicates that there were protective factors arising from being

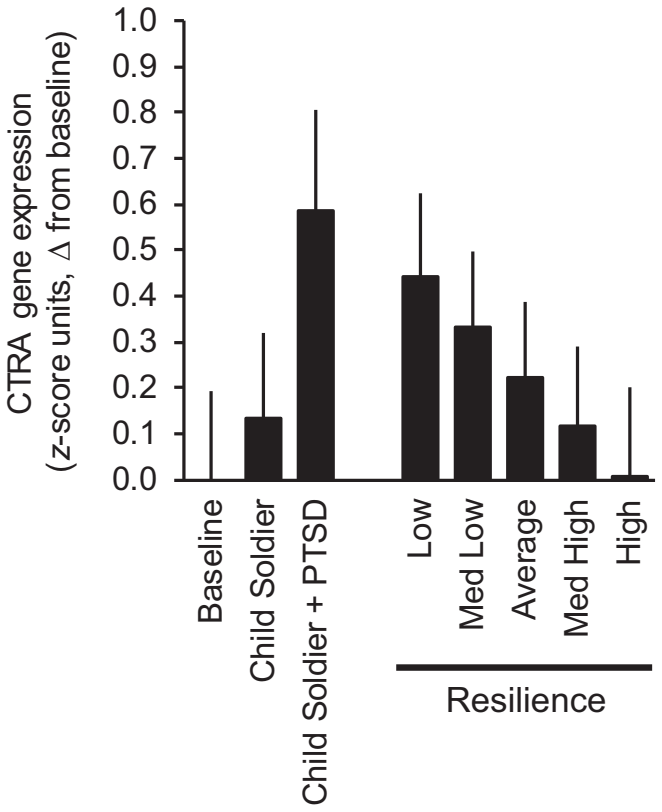


**Fig. 1.** CTRA gene expression as a function of early life adversity and psychological resilience. (Left three bars) Average CTRA gene expression values for civilians, former child soldiers (absent PTSD), and former soldiers suffering significant PTSD symptoms with minimal Resilience Scale scores. (Right five bars) Average CTRA gene expression values for former child soldiers with PTSD and Resilience Scale scores 2 SD below average (Low), 1 SD below average (Med Low), average (Average), 1 SD above average (Med High), and 2 SD above average (High). Estimates come from a mixed-effect linear model controlling for demographic characteristics, leukocyte subset indicators, a 2D representation of war-related adversity exposure (child soldier status and PTSD status), and continuous Resilience Scale scores. Data represent difference (+ SE) in CTRA gene expression relative to baseline.

a child soldier, such as protection against sexual violence among girl soldiers in the People's Army (43, 44). Child soldiers also reported solidarity and feeling supported by Maoists, which was not reported by community members (43). Therefore, despite higher trauma burden among child soldiers, the child soldier experience may confer protective factors. Similar findings of both psychological liabilities and benefits have been reported for adult soldiers in the United States (45).

A more surprising finding was the association between a resilience questionnaire designed in a WEIRD cultural context and CTRA gene expression in this non-WEIRD population. Wagnild and Young's Resilience Scale is dominated by items related to individualistic psychological processes and does not refer to familial, community, or other collective processes (35). Items from their scale used in the Nepali version, for example, included the following: "I am able to depend on myself more than anyone else," "I can be on my own if I have to," and "my belief in myself gets me through hard times." Endorsement of these beliefs was associated with down-regulated CTRA gene expression, which raises the potential that self-efficacy, whether in collectivistic or individualistic societies, could be beneficial for health.

Another important finding was that both PTSD and self-perceived psychological resilience contributed unique variance to this biological process. Critics have argued that resilience is often conflated with the absence of psychological distress after trauma (46): i.e., PTSD symptom severity is inversely proportional to resilience. Our findings support other research arguing that resilience is not merely the flipside of psychological trauma (47). For example, some child soldiers had high PTSD symptom severity, but they also reported high levels of resilience, and these soldiers displayed molecular profiles comparable with civilian children without PTSD. These results suggest that biological sequelae of trauma and adversity might be addressed by promoting resilience, even in the absence of PTSD symptom reduction. From an evolutionary perspective, these findings are important for understanding how humans have dealt with social adversity. Anthropologists suggest that much of human history was characterized by violence and small-scale warfare (48). Resilience may represent a protective psychosocial process evolved to mitigate against the long-term health effects of trauma on health (49).

Given that this study is, to our knowledge, the first study to identify both adversity and protective processes associated with CTRA profiles in a non-WEIRD population, it is important to consider limitations of the methods and analysis. First, the full Wagnild and Young Resilience Scale was not used (35). We selected items through pilot testing to determine cultural salience (50). The full 26-item version may show a different association with CTRA profiles. Second, this study examined average association of an a priori-specified set of genes and was not designed to discover novel genomic profiles or specific individual gene transcripts that show statistically reliable associations with adversity or resilience factors. Additional genomic profiles or individual transcripts may well be found to associate with early life adversity in future exploratory/discovery analyses. Third, differences between child soldiers and civilian youth may be biased by those individuals whom we located for this 5-y follow-up study. Although no differences were found in PTSD at baseline in 2007 between current participants and persons lost to follow-up in 2012 (*Supporting Information*), there may be other domains in which participants and persons lost to follow-up differ. Fourth, because the child soldiers in this study had received an intervention from UNICEF, the limited difference in CTRA expression relative to civilian children could be a result of reintegration services, which emphasized social connectedness and community supports (31) and yielded improvement for PTSD and depression (32).

The present study was enabled by dried blood spot (DBS) sampling under field conditions in Nepal. However, technical calibration studies suggest that DBS transcriptome profiling is less sensitive to true differences in gene expression than are analyses of immediately processed venipuncture blood samples [peripheral blood mononuclear cells (PBMCs)] (*Supporting Information* and ref. 51). This result implies that the Nepal DBS study may have missed some results that could have been apparent using optimal venipuncture blood samples but that the significant results that were detected are unlikely to be spurious and more likely underestimate the true magnitude of differences that would have been observed using gold standard PBMCs. An additional issue is that early exposure to parasites, which is more prevalent in non-WEIRD settings, may impact immune functioning (29) and thus the relationship of CTRA expression to social adversity. Future research should include measures of parasite burden and immune markers in relation to CTRA expression.

Regarding causal pathways, this study was not able to identify the developmental period most salient in producing current CTRA profiles (e.g., was it early childhood antecedent to becoming child soldiers, experiences during war, or postwar circumstances?). Although self-report measures were conducted longitudinally, CTRA was evaluated only at the third wave of data collection. Future studies should use longitudinal representative samples to identify associations of CTRA with adversity and resilience over the course of child development into adulthood. It is also important to note that this study is an observational analysis and cannot formally define causal relationships among child soldier status, PTSD, resilience, and gene expression.

Keeping these limitations in mind, it is useful to consider implications to improve the lives of persons exposed to early adversity in LMIC settings. The association of psychological resilience with CTRA down-regulation independent of PTSD suggests that promoting resilience, rather than just focusing on PTSD symptom reduction, may have benefits for health. This hypothesis is important because the dearth of mental health experts in LMIC settings limits the feasibility of administering intensive trauma-focused interventions (52). Resilience-promotion interventions may be more easily delivered in such contexts and may have more buy-in from beneficiaries compared with psychiatric interventions (53, 54). For example, child soldiers identified the ability to help others, to help their country, and to reduce discrimination in society as more important mental health goals than reduction of symptoms such as nightmares and flashbacks (55). This aspiration-based approach reflects previous findings in WEIRD populations linking a self-transcendent purpose in life (eudaimonic well-being) to reduced CTRA gene expression (22–24). Ultimately, these advances in understanding the role of social genomics can contribute to reducing the physical and mental health impacts of trauma and adversity worldwide.

## Materials and Methods

**Setting and Participants.** Nepal ranks 142 out of 174 on the human development index—near the bottom of the medium human development category (56). Nepal is characteristic of low-income countries with a high burden of infectious disease, which contributes to high rates of infant and under-5-y-old mortality (57). In addition to high exposure to infectious disease and lack of adequate health care, children in Nepal also experience adversity in the form of political violence (58). From 1996 to 2006 during the People's War in Nepal, the Communist Party of Nepal (Maoists) People's Liberation Army (PLA) and the Royal Nepal Army conscripted children as soldiers, sentries, spies, cooks, and porters (59, 60). Our prior research in Nepal in 2007, conducted a few months after the war ended, found that 55% of child soldiers had PTSD compared with 20% of matched civilian children, in a sample that was unique from the current cohort (41).

The current cohort began in 2007 when we used criterion sampling to select 258 child soldiers and 258 matched civilian children for this study from eight districts across Nepal (see *Supporting Information* for additional information on identification of study participants). In 2008, at 1-y follow-up, child soldiers ($n = 222$, 86% of baseline participants) and matched civilian youth ($n = 234$, 91% of baseline participants) were reinterviewed (32). For the current study in 2012, attempts were made to reinterview all participants from the

original study. For participants over 18 y of age at the time of the current study follow-up in 2012, consent was obtained. For participants under 18 y of age, legal guardians provided consent, and the minors provided assent. The research was approved by the institutional review boards of the Nepal Health Research Council, Kathmandu, Nepal; Emory University; George Washington University; and the University of California, Los Angeles (RNA Comparison Study in *Supporting Information*).

**Variables.** Participants completed 60- to 90-min individual interviews. Due to high illiteracy rates, research assistants read questionnaires in Nepali to the youth. The interview included culturally adapted versions of mental health assessment questionnaires and a 37-question semistructured interview, developed based on findings from qualitative research with child soldiers (43, 61).

***Child PTSD Symptom Scale.*** PTSD was assessed with the Child PTSD Symptom Scale (CPSS) (33), a 17-item scale corresponding to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR) symptoms (scale range 0–68). The range for this sample was 0–48, mean = 5.68 (SE 0.58). The CPSS was transculturally translated and validated in Nepal and has good psychometric properties: area under the curve (AUC) = 0.77, sensitivity = 0.68, specificity = 0.73, cutoff score ≥20, Cronbach's $\alpha = 0.94$ (34). For the current analyses, participants were categorized as PTSD-positive or PTSD-negative based on the validated cutoff (34). Participants lost to follow-up did not differ from participants interviewed in 2012 with regard to baseline CPSS scores in 2007 (*Supporting Information*).

***Wagnild and Young's Resilience Scale.*** Resilience was assessed with eight items from Wagnild and Young's Resilience Scale (35) as follows: "I am able to depend on myself more than anyone else"; "I can be on my own if I have to"; "I usually take things in stride"; "I feel that I can handle many things at a time"; "I can usually find something to laugh about"; "My belief in myself gets me through hard times"; "I have enough energy to do what I have to do"; and "I am resilient." These items were used based on previous piloting of the tool in Nepal to determine which items were culturally salient (50). For the eight-item Nepal version, $\alpha = 0.72$, range = 2–24, mean = 12.83 (SE = 0.22). Continuous *Z*-scores were used for the current analyses.

**CTRA Gene Expression.** Details on blood spot collection, analysis, and assay validation are provided in *Supporting Information*. Briefly, total RNA was extracted from DBS samples (Qiagen RNeasy) in the University of California, Los Angeles (UCLA) Social Genomics Core and converted to fluorescence-labeled cDNA (NuGEN Ovation PicoSL) and hybridized to Illumina HT-12 v4.0 BeadChips in the UCLA Neuroscience Genomics Core following the manufacturer's standard protocol. Data are deposited in the Gene Expression Omnibus database (accession no. GSE77164). Two hundred fifty-four of 282 DBS samples (90.1%) yielded valid results, and CTRA gene expression was analyzed as previously described (6, 22), with data quantile-normalized (62), $\log_2$-transformed, and *z*-score–standardized within gene for mixed-effect linear model analyses (63) quantifying association between expression of 53 CTRA indicator transcripts (with inverse components weighted negatively as described below) and exposure factors including child soldier status (1/0), posttraumatic stress (1/0: CPSS score ≥20), and Resilience Scale score (continuous score). Analyses controlled for a priori-specified covariates, including systematic effects of gene, participant age (years), sex (1/0, 1 = female), ethnic minority status (1/0, 1 = Janajati), low caste (1/0, 1 = Dalit), education level (seven ordinal levels) (see *Supporting Information* for additional information on demographic categories), and expression of eight mRNA transcripts indicating relative prevalence of major leukocyte subsets (*CD14* for monocytes; *CD3D, CD3E, CD4,* and *CD8A* for T-lymphocyte subsets; *CD19* for B lymphocytes; and CD56/*NCAM1* and CD16/*FCGR3A* for natural killer cells). The 53 CTRA indicator genes comprised two a priori-specified gene sets as follows: 19 proinflammatory genes (*IL1A, IL1B, IL6, IL8, TNF, PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA,* and *RELB*) weighted +1 as positive indicators of the CTRA profile and 34 genes involved in type I IFN responses (*GBP1, IFI16, IFI27, IFI27L1-2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1-3, IFIT5, IFIT1L, IFITM1-3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7-8, MX1-2, OAS1-3,* and *OASL*) and antibody synthesis (*IGJ, IGLL1,* and *IGLL3*) weighted −1 as inverse indicators (10, 22–24, 64). Data were unavailable for *IFI16* due to random variations in microarray probe synthesis, leaving a final analyzed set of 52 CTRA indicator transcripts. Models were estimated by maximum likelihood using SAS PROC MIXED, with the 52 indicator genes treated as repeated measures and a heterogeneous compound symmetry covariance structure specified to accommodate correlation and heteroscedasticity across residuals (22).

**ACKNOWLEDGMENTS.** We thank Suraj Koirala, Mark Jordans, and TPO Nepal for assistance in study implementation; and the University of California, Los Angeles Neuroscience Genomics Core Laboratory and Paula Kincheloe (Emory Laboratory for Comparative Human Biology) for assistance in study procedures. This research was supported by the HopeLab Foundation and US National Institutes of Health Grants P30 AG017265 and F31 MH075584.

1. Felitti VJ, et al. (1998) Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med* 14(4):245–258.
2. Van Niel C, Pachter LM, Wade R, Jr, Felitti VJ, Stein MT (2014) Adverse events in children: Predictors of adult physical and mental conditions. *J Dev Behav Pediatr* 35(8):549–551.
3. Nusslock R, Miller GE (June 4, 2015) Early-life adversity and physical and emotional health across the lifespan: A neuroimmune network hypothesis. *Biol Psychiatry*, 10.1016/j.biopsych.2015.05.017.
4. Irwin MR, Cole SW (2011) Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol* 11(9):625–632.
5. Cole SW (2014) Human social genomics. *PLoS Genet* 10(8):e1004601.
6. Cole SW, et al. (2015) Myeloid differentiation architecture of leukocyte transcriptome dynamics in perceived social isolation. *Proc Natl Acad Sci USA* 112(49): 15142–15147.
7. Powell ND, et al. (2013) Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis. *Proc Natl Acad Sci USA* 110(41):16574–16579.
8. Finch CE (2010) *The Biology of Human Longevity: Inflammation, Nutrition, and Aging in the Evolution of Lifespans* (Academic, Burlington, MA).
9. Heidt T, et al. (2014) Chronic variable stress activates hematopoietic stem cells. *Nat Med* 20(7):754–758.
10. Cole SW, Nagaraja AS, Lutgendorf SK, Green PA, Sood AK (2015) Sympathetic nervous system regulation of the tumour microenvironment. *Nat Rev Cancer* 15(9):563–572.
11. Capitanio JP, Cole SW (2015) Social instability and immunity in rhesus monkeys: The role of the sympathetic nervous system. *Philos Trans R Soc Lond B Biol Sci* 370(1669): 20140104.
12. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat Rev Neurosci* 9(1):46–56.
13. Cole SW, et al. (2012) Transcriptional modulation of the developing immune system by early life social adversity. *Proc Natl Acad Sci USA* 109(50):20578–20583.
14. Hostinar CE, Lachman ME, Mroczek DK, Seeman TE, Miller GE (2015) Additive contributions of childhood adversity and recent stressors to inflammation at midlife: Findings from the MIDUS study. *Dev Psychol* 51(11):1630–1644.
15. Levine ME, Cole SW, Weir DR, Crimmins EM (2015) Childhood and later life stressors and increased inflammatory gene expression at older ages. *Soc Sci Med* 130:16–22.
16. Cole SW, Hawkley LC, Arevalo JM, Cacioppo JT (2011) Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. *Proc Natl Acad Sci USA* 108(7):3080–3085.
17. Henrich J, Heine SJ, Norenzayan A (2010) The weirdest people in the world? *Behav Brain Sci* 33(2-3):61–83, discussion 83–135.
18. Steel Z, et al. (2009) Association of torture and other potentially traumatic events with mental health outcomes among populations exposed to mass conflict and displacement: A systematic review and meta-analysis. *JAMA* 302(5):537–549.
19. Bornemisza O, Ranson MK, Poletti TM, Sondorp E (2010) Promoting health equity in conflict-affected fragile states. *Soc Sci Med* 70(1):80–88.
20. Betancourt TS, et al. (2013) Psychosocial adjustment and mental health in former child soldiers: Systematic review of the literature and recommendations for future research. *J Child Psychol Psychiatry* 54(1):17–36.
21. Chen E, Miller GE, Kobor MS, Cole SW (2011) Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. *Mol Psychiatry* 16(7):729–737.
22. Fredrickson BL, et al. (2015) Psychological well-being and the human conserved transcriptional response to adversity. *PLoS One* 10(3):e0121839.
23. Fredrickson BL, et al. (2013) A functional genomic perspective on human well-being. *Proc Natl Acad Sci USA* 110(33):13684–13689.
24. Cole SW, et al. (2015) Loneliness, eudaimonia, and the human conserved transcriptional response to adversity. *Psychoneuroendocrinology* 62:11–17.
25. Brewer MB, Chen Y-R (2007) Where (who) are collectives in collectivism? Toward conceptual clarification of individualism and collectivism. *Psychol Rev* 114(1):133–151.
26. Taylor SE, Welch WT, Kim HS, Sherman DK (2007) Cultural differences in the impact of social support on psychological and biological stress responses. *Psychol Sci* 18(9): 831–837.
27. Tol WA, Jordans MJ, Kohrt BA, Betancourt TS, Komproe IH (2013) Promoting mental health and psychosocial well-being in children affected by political violence: Part II—Expanding the evidence base. *Handbook of Resilience in Children of War* eds Fernando C, Ferrari M (Springer, New York), pp 29–38.
28. Eizaguirre C, Lenz TL, Kalbe M, Milinski M (2012) Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nat Commun* 3:621.
29. Dethlefsen L, McFall-Ngai M, Relman DA (2007) An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* 449(7164):811–818.
30. Worthman CM (2011) Inside-out and outside-in? Global development theory, policy, and youth. *Ethos* 39(4):432–451.

31. Kohrt BA, Jordans MJD, Koirala S, Worthman CM (2015) Designing mental health interventions informed by child development and human biology theory: A social ecology intervention for child soldiers in Nepal. *Am J Hum Biol* 27(1):27–40.
32. Kohrt BA, Burkey M, Stuart EA, Koirala S (2015) Alternative approaches for studying humanitarian interventions: Propensity score methods to evaluate reintegration packages impact on depression, PTSD, and function impairment among child soldiers in Nepal. *Glob Ment Health* 2:e16.
33. Foa EB, Johnson KM, Feeny NC, Treadwell KR (2001) The child PTSD Symptom Scale: A preliminary examination of its psychometric properties. *J Clin Child Psychol* 30(3): 376–384.
34. Kohrt BA, et al. (2011) Validation of cross-cultural child mental health and psychosocial research instruments: Adapting the Depression Self-Rating Scale and Child PTSD Symptom Scale in Nepal. *BMC Psychiatry* 11(1):127.
35. Wagnild GM, Young HM (1993) Development and psychometric evaluation of the Resilience Scale. *J Nurs Meas* 1(2):165–178.
36. Tol WA, Jordans MJD, Regmi S, Sharma B (2005) Cultural challenges to psychosocial counselling in Nepal. *Transcult Psychiatry* 42(2):317–333.
37. Kohrt BA, Maharjan SM (2009) When a child is no longer a child: Nepali ethnopsychology of child development and violence. *Stud Nepali Hist Soc* 14(1):107–142.
38. Kohrt BA, Maharjan SM, Timsina D, Griffith JL (2012) Applying Nepali ethnopsychology to psychotherapy for the treatment of mental illness and prevention of suicide among Bhutanese refugees. *Ann Anthropol Pract* 36(1):88–112.
39. Zahran S, et al. (2015) Stress and telomere shortening among central Indian conservation refugees. *Proc Natl Acad Sci USA* 112(9):E928–E936.
40. Breslau J (2004) Cultures of trauma: Anthropological views of posttraumatic stress disorder in international health. *Cult Med Phsychiatry* 28(2):113–126; discussion 211–120.
41. Kohrt BA, et al. (2008) Comparison of mental health between former child soldiers and children never conscripted by armed groups in Nepal. *JAMA* 300(6):691–702.
42. Kohrt BA, et al. (2010) Social ecology of child soldiers: Child, family, and community determinants of mental health, psychosocial well-being, and reintegration in Nepal. *Transcult Psychiatry* 47(5):727–753.
43. Kohrt BA, Tol WA, Pettigrew J, Karki R (2010) Children and revolution: The mental health and psychosocial wellbeing of child soldiers in Nepal's Maoist army. *The War Machine and Global Health*, eds Singer M, Hodge GD (Rowan & Littlefield, Lanham, MD), pp 89–116.
44. Kohrt BA (2015) The role of traditional rituals for reintegration and psychosocial wellbeing of child soldiers in Nepal. *Genocide and Mass Violence: Memory, Symptom, and Recovery*, eds Hinton AL, Hinton DE (Cambridge Univ Press, Boston), pp 369–387.
45. Fontana A, Rosenheck R (1998) Psychological benefits and liabilities of traumatic exposure in the war zone. *J Trauma Stress* 11(3):485–503.
46. Theron LC, Liebenberg L, Ungar M, eds (2014) *Youth Resilience and Culture: Commonalities and Complexities* (Springer, New York).
47. Punamaki R-L, Qouta S, El-Sarraj E (2001) Resiliency factors predicting psychological adjustment after political violence among Palestinian children. *Int J Behav Dev* 25(3): 256–267.
48. Allen MW, Jones TL (2014) *Violence and Warfare Among Hunter-Gatherers* (Left Coast Press, Walnut Creek, CA).
49. Konner M (2007) Trauma, adaptation, and resilience: A cross-cultural and evolutionary perspective. *Understanding Trauma: Integrating Biological, Clinical, and Cultural Perspectives*, eds Lemelson R, Kirmayer LJ, Barad M (Cambridge Univ Press, Cambridge, UK), pp 300–338.
50. Patel A (2012) Mental health in Jumla, Nepal: A qualitative study examining the effects of war on mental health. Master's thesis (Emory University, Atlanta).
51. McDade TW, et al. (2016) Genome-wide profiling of RNA from dried blood spots: Convergence with bioinformatic results derived from whole venous blood and peripheral blood mononuclear cells. *Biodemogr Soc Biol* 62(2):182–197.
52. Tol WA, et al. (2011) Mental health and psychosocial support in humanitarian settings: Linking practice and research. *Lancet* 378(9802):1581–1591.
53. Tol WA, Jordans MJD, Kohrt BA, Betancourt TS, Komproe IH (2013) Promoting mental health and psychosocial well-being in children affected by political violence: Part I—Current evidence for an ecological resilience approach. *Handbook of Resilience in Children of War* eds Fernando C, Ferrari M (Springer, New York), pp 11–27.
54. Kohrt B (2013) Social ecology interventions for post-traumatic stress disorder: What can we learn from child soldiers? *Br J Psychiatry* 203(3):165–167.
55. Karki R, Kohrt BA, Jordans MJD (2009) Child led indicators: Pilot testing a child participation tool for psychosocial support programmes for former child soldiers in Nepal. *Intervention* 7(2):92–109.
56. United Nations Development Programme (2007) *Human Development Report 2007/2008* (Pallgrave Macmillian, New York).
57. United Nations (2008) *District Health Profiles of Nepal: Nepal Public Health Association Compilation*. Available at un.org.np/data-coll/. Accessed June 27, 2016.
58. Tol WA, et al. (2010) Political violence and mental health: A multi-disciplinary review of the literature on Nepal. *Soc Sci Med* 70(1):35–44.
59. Human Rights Watch (2007) *Children in the Ranks: The Maoists' Use of Child Soldiers in Nepal* (Human Rights Watch, Kathmandu, Nepal).
60. United Nations (2006) *Report of the Secretary-General on Children and Armed Conflict in Nepal* (United Nations Security Council, New York).
61. Morley CA, Kohrt BA (2013) Impact of peer support on PTSD, hope, and functional impairment: A mixed-methods study of child soldiers in Nepal. *J Aggress Maltreat Trauma* 22(7):714–734.
62. Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 19(2):185–193.
63. McCulloch CE, Searle SR, Neuhaus JM (2008) *Generalized, Linear, and Mixed Models* (John Wiley, Hoboken, NJ).
64. Vedhara K, et al. (2015) Personality and gene expression: Do individual differences exist in the leukocyte transcriptome? *Psychoneuroendocrinology* 52:72–82.
65. UNICEF (2007) *Paris Principles: Principles and Guidelines on Children Associated with Children Associated with Armed Forces and Armed Groups* (UNICEF, New York). Available at www.unicef.org/emerg/files/ParisPrinciples310107English.pdf. Accessed June 1, 2008.
66. Adhikari RP, et al. (2014) Protective and risk factors of psychosocial wellbeing related to the reintegration of former child soldiers in Nepal. *Intervention (Amstelveen)* 12(3):367–378.
67. Upadhaya N, et al. (2014) The role of mental health and psychosocial support nongovernmental organizations: Reflections from post-conflict Nepal. *Intervention*. 12(Suppl 1):113–128.
68. Kohrt BA, Jordans MJD, Morley CA (2010) Four principles of mental health research and psychosocial intervention for child soldiers: Lessons learned in Nepal. *Int Psychiatry* 7(3):58–60.
69. Kohrt BA, et al. (2009) Culture in psychiatric epidemiology: Using ethnography and multiple mediator models to assess the relationship of caste with depression and anxiety in Nepal. *Ann Hum Biol* 36(3):261–280.
70. Kohrt BA (2009) Vulnerable social groups in post-conflict settings: A mixed-methods policy analysis and epidemiology study of caste and psychological morbidity in Nepal. *Intervention* 7(3):239–264.
71. Luitel NP, et al. (2013) Conflict and mental health: A cross-sectional epidemiological study in Nepal. *Soc Psychiatry Psychiatr Epidemiol* 48(2):183–193.
72. Kohrt BA, Worthman CM (2009) Gender and anxiety in Nepal: The role of social support, stressful life events, and structural violence. *CNS Neurosci Ther* 15(3): 237–248.
73. Wei C, et al. (2014) Comparison of frozen and unfrozen blood spots for gene expression studies. *J Pediatr* 164(1):189–191.e1.
74. Haak PT, et al. (2009) Archived unfrozen neonatal blood spots are amenable to quantitative gene expression analysis. *Neonatology* 95(3):210–216.
75. Bergen AW, et al. (2012) Chronic psychosocial stressors and salivary biomarkers in emerging adults. *Psychoneuroendocrinology* 37(8):1158–1170.
76. Khoo SK, et al. (2011) Acquiring genome-wide gene expression profiles in Guthrie card blood spots using microarrays. *Pathol Int* 61(1):1–6.
77. Slaughter J, et al. (2013) High correlations in gene expression between paired umbilical cord blood and neonatal blood of healthy newborns on Guthrie cards. *J Matern Fetal Neonatal Med* 26(18):1765–1767.
78. Kanyongo GY, Brook GP, Kyei-Blankson L, Gocmen G (2007) Reliability and statistical power: How measurement fallibility affects power and required sample sizes for several parametric and nonparametric statistics. *J Mod Appl Stat Methods* 6(1):9.
79. Maeno Y, et al. (2003) Utility of the dried blood on filter paper as a source of cytokine mRNA for the analysis of immunoreactions in Plasmodium yoelii infection. *Acta Trop* 87(2):295–300.