RESEARCH ARTICLE

Transgenerational effects of the genocide against the Tutsi in Rwanda: A post-traumatic stress disorder symptom domain analysis [version 1; peer review: 2 approved with reservations]

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Abstract

Background: A number of studies have investigated transgenerational effects of parental post-traumatic stress disorder (PTSD) and its repercussions for offspring. Few studies however, have looked at this issue in the African context.

Methods: The present study addresses this gap, utilizing confirmatory factor analysis (CFA), to investigate symptom severity within the three Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) PTSD symptom domains in mothers exposed to the genocide against the Tutsi in Rwanda (n=25) and offspring (n=25), and an ethnically matched control group of mothers (n=25) and offspring (n=25) who were outside of Rwanda during the genocide. All mothers were pregnant during the time of the genocide with the offspring included in the study. Missing data were excluded from the analyses.

Results: We found that among the three symptom domains of PTSD, the re-experiencing symptom domain loaded most strongly onto PTSD among mothers directly exposed to the genocide (Beta = 0.95). In offspring of exposed mothers, however, the three symptom domains of PTSD yielded almost equal loading values (Beta range = 0.84-0.86). Conversely, among non-exposed mothers and their offspring, the hyperarousal symptom domain of PTSD loaded most strongly onto PTSD (Beta = 1.00, Beta = 0.94, respectively). As a secondary analysis, we also explored the relation between DNA methylation in the glucocorticoid receptor (NR3C1) locus, an important stress modulating gene, and individual PTSD symptom domains, finding a strong association between DNA methylation and re-experiencing among genocide-exposed mothers that exceeded any other observed associations by approximately two-fold.

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1 Amit Shira, Bar-Ilan University, Ramat-Gan, Israel

2 Andreas Maercker, University of Zurich, Zurich, Switzerland

Any reports and responses or comments on the article can be found at the end of the article.
Conclusions: This is the first report, to our knowledge, of a symptom-based analysis of transgenerational transmission of PTSD in Africa. These findings can be leveraged to inform further mechanistic and treatment research for PTSD.

Keywords
Prenatal Stress, Mental Health, Tutsi, Glucocorticoid Receptor, Confirmatory Factor Analysis

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Author roles: Rudahindwa S: Conceptualization, Formal Analysis, Methodology, Writing – Original Draft Preparation; Mutesa L: Conceptualization, Funding Acquisition, Investigation, Resources, Writing – Review & Editing; Rutembesa E: Data Curation, Investigation, Writing – Review & Editing; Mutabaruka J: Data Curation, Investigation, Writing – Review & Editing; Qu A: Formal Analysis, Methodology, Supervision, Writing – Review & Editing; Wildman DE: Funding Acquisition, Resources, Writing – Review & Editing; Jansen S: Funding Acquisition, Investigation, Project Administration, Writing – Review & Editing; Uddin M: Conceptualization, Funding Acquisition, Methodology, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: Dr. Wildman is Editor in Chief of Molecular Phylogenetics and Evolution, an Elsevier Journal, for which he receives an honorarium. All other authors have no competing interests to disclose.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction
The 1994 genocide perpetrated against Tutsi in Rwanda is one of the most horrific events in recent history responsible for roughly one million deaths during a short 3-month period. The genocide was a result of tension between the two artificially divided ethnic groups of Rwanda: Hutu and Tutsi. Hutu extremists, alongside coerced and willing civilians, carried out a mass genocide against the minority ethnic Tutsi of Rwanda4–6. Throughout the region, high rates of post-traumatic stress disorder (PTSD) and other psychological and physical disorders have been observed following the 1994 genocide perpetrated against Tutsi4–7. A recent countrywide study concluded that 79.41% of Rwandan individuals (age >16) experienced one or more traumatic events, including threats of death, bodily injury to a person or member of his family, being a witness of killing of a family member or another member of the community, and rape8. Women were to a higher extent exposed to traumatic events (83.6%) than men (73.4%)9. More than two decades later, the long-term impact of the genocide is evident in the prevalence of PTSD – more than 26% among Rwandans (age>16) – and even higher rates (41%) among women survivors5,7. Work published in 2013 analyzing PTSD prevalence among Rwandan widows, prisoners of war, and their descendants reported that high exposure to war and genocide was one of the greatest predictors of PTSD9.

Further study has also uncovered the transgenerational effects of PTSD among genocide-exposed mothers and their offspring9–10. Perroud and his colleagues found that offspring of genocide-exposed mothers had higher rates of PTS and depressive symptom severity than offspring born from non-exposed mothers10. These findings are consistent with research reported on holocaust survivors and their adult offspring, which also depicted higher rates of PTSD among offspring of holocaust survivors compared to those of non-exposed parents11.

While PTSD or PTSD severity is often the primary phenotype of interest in studies of traumatic stress, numerous studies have implemented confirmatory factor analysis (CFA) models of PTSD as a means of testing a hypothesized model that may characterize in more detail potential indicators of PTSD (e.g. 12–14). The purpose of such analysis is to test which set of symptom domains collectively best describe PTSD, by finding the best fit model. Following the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV)13, the first symptom domain is a persisting re-experiencing of the traumatic event, including flashbacks, intrusive thoughts, and nightmares. The second symptom domain is a persistent avoidance of trauma-associated stimuli and a numbing of general responsiveness, which includes feelings of detachment from others, effortful avoidance of thought, feelings, and activities that arouse recollections of the trauma, and a restricted range of affect. The third symptom domain is a persistence of hyperarousal symptoms, which include insomnia, hypervigilance, and exaggerated startle response15.

Traditionally, a CFA is used to test how well a hypothesized model holds and how well the model fits the observed data12,13. In contrast, the purpose of the present study is to apply a CFA analysis in order to determine which symptom domain has the greatest loading on PTSD in the context of the 1994 genocide perpetrated against the Tutsi in Rwanda. The ultimate aim of this study is to elucidate potential transgenerational differences in how exposure to the 1994 genocide perpetrated against Tutsi affects PTS symptom severity domains. More specifically, CFA was used in this study to extract domain loading values on PTSD in order to assess which of the three symptom domains serves as the strongest indicator of PTSD in mothers exposed and pregnant during genocide and their offspring from this pregnancy, as well as in a comparison group of non-exposed mothers (living outside of Rwanda) pregnant in that same period and their offspring from this pregnancy. From here on, we will be referring to these groups as exposed mothers and offspring and non-exposed mothers and offspring. Our primary interest was in the potential differences in loading values observed in exposed mothers compared to their offspring and in non-exposed mothers compared to their offspring, respectively.

As a secondary analysis, we tested how strongly these symptom domains were associated with epigenetic modifications. Epigenetic modifications can be understood as overlying genetic modifications occurring on a gene in a manner that can potentially alter gene expression16. These epigenetic modifications are modulated by personal experiences and can be inherited in some cases17,18. Thus, adversities—particularly early life adversities—experienced throughout one’s life can alter functional properties of a gene through changed epigenetics and, in some instances, may also be observed on the offspring’s epigenome19. Complementing the animal work, which can more directly demonstrate transgenerational epigenetic effects from a mechanistic perspective, a handful of human-based studies has reported stress- and trauma-associated epigenetic patterns in one generation that appear to persist into a subsequent generation, in particular among genes that help to regulate the body’s response to stress20–22. In particular, Perroud et al.9 found that both mothers exposed to the 1994 genocide perpetrated against Tutsi and their offspring had a positive relationship between PTSD symptom severity and blood-derived DNA methylation of the glucocorticoid receptor gene (NR3C1). However, the extent to which NR3C1 DNA methylation associates with particular PTSD symptoms domains remains unknown. Therefore, following our CFA analysis of PTSD symptom domains, we conducted a secondary analysis to assess which symptom domain, as outlined above, has the strongest association with DNA methylation in NR3C1 for both mothers and offspring.

Methods
Participants and measures
This study utilized PTSD Checklist (PCL-17) responses of 50 Tutsi women and their offspring, obtained from previous study, as described by Perroud et al.9. Data were collected from 2011–2012. The PCL-17 questionnaire used in this study was administered by trained psychologists. For the case group, 25 ethnic Tutsi widows exposed to the genocide, and pregnant during the time, were recruited from a Tutsi genocide Widows’ Association and/or psychiatric ambulatory consultations, along with their 25 offspring who were in utero during this period.
(data were missing for two offspring from the exposed group). Participation for mothers in the study was following DSM-IV criterion A, which states that an individual must have witnessed or been threatened by death or serious injury that evoked intense fear, helplessness, or horror. The control group consisted of 25 women of Tutsi ethnicity who pregnant during, but not exposed to the 1994 genocide perpetrated against Tutsi, along with their 25 offspring from this pregnancy. For the 25 non-exposed women, those with a known exposure to traumatic experiences and history of a psychiatric disorder were excluded from the study. All participants provided informed consent.

DNA methylation measures DNA methylation measurements of NR3C1 used in this study were previously obtained by Perroud et al. Briefly, DNA was first extracted, bisulfite converted, and amplified via PCR. After pyrosequencing, percent mean methylation values at 10 discrete CpG sites within the exon 1, NR3C1 promoter region were determined for both exposed and non-exposed mothers as well as their offspring; average values across the 10 CpG sites were also determined and used as the primary variable in our secondary analyses reported here.

Statistical analysis To study the relationship between PTSD symptom domains and genocide exposure a confirmatory factor analysis (CFA) model was used testing the individual loading values of each symptom domain onto PTSD (Figure 1). This model is based on Buckley et al., who used a similar approach to study PTSD symptom domains. The model, depicted in Figure 1, was assessed four times: one model each for the exposed mothers and offspring; a third model for non-exposed mothers; and a fourth model for their offspring.

We used analysis of moment structures (AMOS). PTSD was set as the latent variable and the three symptom domains were set as indicators of the latent variable. Beta values derived from the CFA analysis indicate loading values of each symptom domain onto the latent variable PTSD. Results were then used to compare symptom domains among the four different groups (exposed mother and offspring, non-exposed mother and offspring).

For the secondary analyses of DNA methylation in relation to PTSD symptom domains, we implemented for each of the four groups a Bayesian model to construct a matrix that would give us covariant values of how strongly each symptom domain is related to average DNA methylation of the exon 1, NR3C1 promoter region as reported in 9. This analysis was coded in RStudio version 1.1.423 by following the Bayesian equation with normal Wishart distribution. Missing data were excluded from the analyses.

Results Primary CFA analyses of PTSD symptom domains CFA showed that among the genocide-exposed mothers, re-experiencing symptoms had the highest loading value onto PTSD (Beta = 0.95; Table 1; Figure 2). In contrast, the symptoms categorized under hyperarousal had the lowest loading value (Beta = 0.77). Symptoms categorized as avoidance/numbing had the second largest loading value (Beta = 0.87). In offspring of genocide-exposed mothers, re-experiencing symptoms, hyperarousal symptoms, and avoidance/numbing symptoms had almost equal loading values, respectively, on PTSD (Beta = 0.86, Beta = 0.84, Beta = 0.85; Table 1; Figure 3). Among non-exposed mothers, hyperarousal symptoms had the highest loading value on PTSD (Beta = 1.00; Table 2; Figure 4) followed by re-experiencing (Beta = 0.90) and avoidance/numbing (Beta = 0.83). Similarly, in offspring of non-exposed mothers, hyperarousal symptoms loaded on PTSD most strongly (Beta = 0.94; Table 2; Figure 5); but

![Figure 1. Post-traumatic stress disorder (PTSD) model for confirmatory factor analysis (CFA).](image)

Table 1. Symptom domain loading values for exposed mothers and offspring.

<table>
<thead>
<tr>
<th>Latent Factor</th>
<th>Indicator</th>
<th>Beta coefficients for exposed mothers</th>
<th>Beta coefficients for exposed offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTSD</td>
<td>Re-experiencing</td>
<td>0.95</td>
<td>0.86</td>
</tr>
<tr>
<td>PTSD</td>
<td>Hyperarousal</td>
<td>0.77</td>
<td>0.84</td>
</tr>
<tr>
<td>PTSD</td>
<td>Avoidance/Numbing</td>
<td>0.87</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Table 1. Factor Loadings. Latent Factor = PTSD, Indicator = symptom domains, Beta = symptom severity by loading value. PTSD - post-traumatic stress disorder.
Figure 2. Post-traumatic stress disorder (PTSD) Structural Equation Model for exposed mothers. Loading values indicated on leftmost arrows. Recol2 = Re-experiencing. Hyper2 = Hyper arousal. Avoid2 = Avoidance/Numbing. e = error. e1 = .001 e2 = .002 e3 = .003.

Figure 3. Post-traumatic stress disorder (PTSD) Structural Equation Model for offspring of exposed mothers. Loading values indicated on leftmost arrows. Recol2 = Re-experiencing. Hyper2 = Hyper arousal. Avoid2 = Avoidance/Numbing. e = error e1 = .004 e2 = .003 e3 = .003.

Table 2. Symptom domain loading values for non-exposed mothers and offspring.

<table>
<thead>
<tr>
<th>Latent Factor</th>
<th>Indicator</th>
<th>Beta coefficients for non-exposed mothers</th>
<th>Beta coefficients for non-exposed offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTSD</td>
<td>Re-experiencing</td>
<td>0.90</td>
<td>0.78</td>
</tr>
<tr>
<td>PTSD</td>
<td>Hyperarousal</td>
<td>1.00</td>
<td>0.94</td>
</tr>
<tr>
<td>PTSD</td>
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Figure 4. Post-traumatic stress disorder (PTSD) Structural Equation Model for non-exposed mothers. Loading values indicated on leftmost arrows. Recol2 = Re-experiencing. Hyper2 = Hyper arousal. Avoid2 = Avoidance/Numbing. e = error e1 = .004 e2 = .003 e3 = .004.
avoidance/numbing symptoms had a higher loading value than re-experiencing symptoms (Beta=0.84 vs. 0.78, respectively).

Secondary analyses of NR3C1 DNA methylation in relation to PTSD symptom domains

Among mothers exposed to the genocide, the re-experiencing symptom domain had the strongest relationship with NR3C1 DNA methylation levels (Covariance = 0.20; Table 3), with the hyperarousal symptom domain and avoidance/numbing symptom domain showing equivalently strong relationships with NR3C1 methylation (Covariance = 0.13 for both domains). Among offspring of exposed mothers, the hyperarousal symptom domain and avoidance/numbing symptom domain also had an equal relationship with methylation of NR3C1 (Covariance = 0.10 for both domains; Table 3); however, in contrast to the pattern observed in the exposed mothers, the re-experiencing symptom domain had the weakest relationship with methylation of NR3C1 (Covariance = 0.06).

Among non-exposed mothers the avoidance/numbing symptom domain had the strongest relationship with DNA methylation in NR3C1 (Covariance = 0.10; Table 4), followed by the re-experiencing (Covariance = 0.08), and hyperarousal (Covariance = 0.06) symptom domains. Like non-exposed mothers, the covariance matrix for offspring of non-exposed mothers showed that the avoidance/numbing symptom domain had the strongest relationship with DNA methylation in NR3C1 (Covariance = 0.11; Table 4), followed closely by the re-experiencing symptom domain (Covariance = 0.10) and, lastly, the hyperarousal symptom domain (Covariance = 0.07).

![Figure 5. Post-traumatic stress disorder (PTSD) Structural Equation Model for offspring of non-exposed mothers. Loading values indicated on leftmost arrows. Recol2 = Re-experiencing. Hyper2 = Hyper arousal. Avoid2 = Avoidance/Numbing. e = error e1 = .006 e2 = .003 e3 = .004.](image)

<table>
<thead>
<tr>
<th></th>
<th>NR3C1 Methylation covariance for exposed mothers</th>
<th>NR3C1 Methylation covariance for offspring of exposed mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-experiencing</td>
<td>0.20</td>
<td>0.06</td>
</tr>
<tr>
<td>Hyperarousal</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Avoidance/Numbing</td>
<td>0.13</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 3. Covariance table for exposed mothers and offspring.

<table>
<thead>
<tr>
<th></th>
<th>NR3C1 Methylation covariance for non-exposed mothers</th>
<th>NR3C1 Methylation covariance for offspring of non-exposed mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-experiencing</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Hyperarousal</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Avoidance/Numbing</td>
<td>0.10</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 4. Covariance table for non-exposed mothers and offspring.
Discussion

Our primary CFA analyses showed that among the genocide exposed mothers, symptoms categorized under the domain of re-experiencing had the highest loading value onto PTSD. These findings suggest that exposed mothers experienced more nightmares, intrusive thoughts, and flashbacks—hallmark symptoms of this domain within the PTSD diagnosis\(^5\). Intuitively, we assume that the high loading value associated with the re-experiencing symptom domain seen in exposed mothers is a consequence of their direct witnessing of the trauma: since the mothers directly witnessed the trauma, they are the ones with the ability to recall the event. In contrast, their children, who were in utero during this time, did not bear witness to the atrocities of the genocide. Therefore, it is not surprising that their children would experience a different level or set of symptom severities. Perhaps more interestingly, offspring of genocide-exposed mothers did not experience one symptom domain more severely than another, suggesting that, at least at the level of symptoms, there is a lack of transgenerational symmetry in the genocide-exposed group.

In contrast, among ethnic Tutsis not exposed directly to the genocide, the hyperarousal symptom domain loaded most strongly onto PTSD among both the mothers and the children. One possibility is that, within this group, being a member of a minority ethnic group that was the target of genocide promoted vigilant behaviors characteristic of this symptom domain; in other words, these individuals may have been prone to express hyperarousal symptoms as a response to being potential targets of genocide, even though that threat may not have been imminent as these individuals were outside of Rwanda at the time. This could be interpreted as an extreme version of social identity threat theory, in which individuals of minority backgrounds experience heightened vigilance to social environmental cues as they seek information about whether their identity may be a source of potential mistreatment (reviewed in 27).

Results from our secondary analyses showed that DNA methylation on the exon 1\(_p\) promoter region of NR3C1 had the strongest relationship with the re-experiencing symptom domain of PTSD for genocide exposed mothers. This follows the same pattern as the results from our CFA analysis, which indicated that the re-experiencing symptom domain loaded most strongly onto PTSD in genocide exposed mothers. However, unlike their genocide exposed mothers, genocide-exposed offspring did not show associations with DNA methylation that paralleled the primary CFA results (i.e. the three PTSD symptom domains were not equally related to the DNA methylation status of NR3C1). Rather, the hyperarousal and avoidance/numbing symptom domains showed the strongest relationship with methylation of the NR3C1 exon 1\(_p\) promoter region in this group, with equal covariance values; in contrast, the re-experiencing symptom domain of PTSD had the weakest relationship with methylation of NR3C1 for offspring of genocide exposed mothers. Of note, in the Perroud et al. study\(^6\), the offspring of genocide-exposed mothers showed the highest levels of DNA methylation in NR3C1, which were nearly 50% higher than that observed in their mothers. Interestingly, in our CFA-based approach reported here, the mothers demonstrated an association with NR3C1 DNA methylation in relation to the re-experiencing symptom domain that exceeded any observed association between NR3C1 DNA methylation and symptom domains in their offspring (or in any other group) by twofold or greater. This suggests that, while in utero exposure to genocide may have a pronounced effect on overall DNA methylation at this locus, the more subtle relationship between NR3C1 methylation and PTSD symptom domains may be more evident within generations.

A number of limitations should be considered in evaluating the results of the current study. Firstly, the psychopathological assessments and accompanying blood sample collection of offspring included in the study occurred more than a decade following their in utero exposure to the genocide. More specifically, offspring of genocide exposed and non-exposed mothers were already of adult age when recruited into the original study\(^6\), thus results from the present study may be influenced by post-natal factors such as: parental rearing methods, constant exposure to maternal PTSD symptoms, and other stressors acquired throughout the offspring’s lifetime, therefore making it difficult to differentiate pre-natal environmental effects from post-natal factors. Therefore, we cannot attribute the results of this study solely to in-utero exposure to the genocide. Secondly, our secondary analyses utilized group mean DNA methylation values, drawing from previous work\(^2\), instead of individual DNA methylation percentages of each participant, which could add variability to the reported results. To conduct our study using the mean methylation values taken from the previous study, we simulated data that would fall within the expected distribution given the mean and standard deviation of each group. Covariance values indicating the relationship strength between DNA methylation of NR3C1 and each PTSD symptom domain were then presented and discussed in terms of their ranking from highest to lowest. Future studies should extend the approach presented in this study to create a linear regression model of DNA methylation of NR3C1 and PTSD symptom domains given actual percent DNA methylation values for each study participant. Lastly, our study only explored the relationship of DNA methylation on NR3C1 and PTSD symptom domains. Future studies should explore the relationship of PTSD symptom domains on DNA methylation in other genes related to PTSD.

Despite these limitations, the present study highlights the relationship between specific symptom domains and PTSD within a transgenerational context and, secondarily, with epigenetic modifications in a stress-sensitive gene. The novelty in this study is its transgenerational illustration of PTSD symptom severity domains and their possible relationship to DNA methylation of NR3C1 in the African context. In particular, its goal was to investigate the nature of PTSD symptom severity domains as it relates to genocide exposure against the Tutsi in Rwanda. We found that, among those with direct exposure to the Tutsi genocide in Rwanda, the re-experiencing symptom domain showed the strongest loading onto PTSD. Future studies should investigate whether similar relations hold for individual
symptom domains in other trauma exposed populations, as well as identify potential biomarkers of the individual symptom domains for other trauma exposed populations.

**Conclusion**
Examination of transgenerational effects associated with traumatic event exposure is an active area of research. Results from the present study not only lend another perspective on the relative severity of PTSD symptom domains experienced by trauma exposed individuals, in particular within an African context; they also illustrate how in utero exposure to trauma associates with offspring symptom severities and how such symptoms are, in turn, related to epigenetic modifications in stress related genes. Future work in our ongoing study will examine transgenerational effects of genocide exposure on mental health in a larger sample of study participants and will broaden epigenetic analyses to include a broader suite of genes involved with stress regulation.

**Data availability**
The data underlying this study is available from OSF: http://doi.org/10.17605/OSF.IO/P94TA.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

**References**


The authors responsible for the data described have agreed to make them publicly accessible.

**Competing interests**
Dr. Wildman is Editor in Chief of Molecular Phylogenetics and Evolution, an Elsevier Journal, for which he receives an honorarium. All other authors have no competing interests to disclose.

**Grant information**
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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Acknowledgements**
We thank the study participants for their willingness to participate in this study. We also gratefully acknowledge the UIUC statistical consulting group who provided valuable feedback on our analyses.


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Version 1

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Andreas Maercker
Department of Psychology Psychopathology and Clinical Intervention, University of Zurich, Zurich, Switzerland

Good to have this study. The sample(s) are very important and the general research questions are valueable.

However, some aspects have to be changed before indexing:

1. Too few important statistical data are given. Reporting structural equation model results requires providing tables with its conventional statistical specifications, in the case of CFA: Chi-Squared test, RMSEA, GFI, AGFI, RMR, and SRMR

   The results of these indicators have to be evaluated, i.e. if they are in the sufficient range of values. Otherwise these results cannot be interpreted. Usually, most final CFA model comparisons seem sufficient but with some outliers that have to be interpreted.

2. Other data in the current text are highly repetitive, e.g. the Betas of the CFAs are reported threefold identically: in the text, in the figures, and the tables. This is against the rules of scientific publication which mandates to report all results only once.

   In the discussion the authors interpret their symptom group dominance results "intuitively" (p. 7, 1st paragraph). This is not good science. Why not cross-checking various PTSD models by authors such as Brewin et al., Ehlers & Clark etc. why in one case recollections are most prominent and in others hyperarousal symptoms? Furthermore, why not reflecting on existing research literature that avoidance symptoms are usually the least prevalent in samples from Africa? (see: Hinton, D. E., & LewisFernández, R. (2011). The crosscultural validity of posttraumatic stress disorder: implications for DSM5. Depression and anxiety, 28(9), 783-801)

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Reader Comment 02 Dec 2019

**Monica Uddin**, Genomics Program, College of Public Health, University of South Florida, Tampa, FL, USA

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   We thank the reviewer for their comment. When we attempted to test for measurement invariances across our study groups, by assessing the above data, the results helped us realize that our sample was not large enough to obtain the sufficient range of value needed to do a cross group interpretation using a CFA. Accordingly, we decided to revise our method to test our research questions using a Pearson correlation approach, followed by the Dunn and Clark test for significance between two correlations.

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   We thank the reviewer for their advice and have reported all coefficients only once in our revised manuscript.

3. In the discussion the authors interpret their symptom group dominance results “intuitively” (p. 7, 1st paragraph). This is not good science. Why not cross-checking various PTSD models by authors such as Brewin et al., Ehlers & Clark etc. why in one
case recollections are most prominent and in others hyperarousal symptoms? Furthermore, why not reflecting on existing research literature that avoidance symptoms are usually the least prevalent in samples from Africa? (see: Hinton, D. E., & LewisFernández, R. (2011). The crosscultural validity of posttraumatic stress disorder: implications for DSM5. Depression and anxiety, 28(9), 783-801)

We thank the reviewer for their suggestion and have referenced the above paper on the cross-culture validity of posttraumatic stress disorder on page 15-16, please see excerpt below. As described in above, we have also updated our methods to enable tests for statistical significance and interpret our results accordingly.

“Cross cultural examinations of PTSD have shown that differential clustering of symptoms result in different rates of individual PTSD symptoms (reviewed in 35). Therefore, a symptom-based analysis of PTSD utilizing the DSM-5’s clustering of PTSD may capture differences in PTSD symptoms that we were unable to capture with the three-factor clustering of PTSD in the DSM-IV (12), as assessed in this study.”

**Competing Interests:** No competing interests were disclosed.
Bensimon, M., Bodner, E., Rosenbloom, T., & Yadid, Y. (2017). Parental posttraumatic stress disorder symptoms is related to successful aging in offspring of Holocaust survivors. *Frontiers in Psychology, 8*, 1099) suggest such methylation patterns can explain greater mental as well as physical morbidity across generations in exposed families.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
No

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
No

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Reader Comment 02 Dec 2019**

**Monica Uddin**, Genomics Program, College of Public Health, University of South Florida, Tampa, FL, USA

1. My primary concern regarding this paper is that the conclusions are based on descriptive statistics. It seems the authors did not examine measurement invariance across the study groups. Without such tests, it precludes the interpretation of their findings. The reader is left wondering whether the coefficient for intrusive recollection is indeed significantly different across groups or relative to other coefficients.

We thank the reviewer for sharing their concern. To address this issue, we switched to a Pearson correlation matrix approach which then allowed us to test for significance by utilizing the Dunn and Clark's test for significance between two correlations. The conclusions are now based on results from the significance tests.

2. It is not clear if the covariances between PTSD syndrome and methylation are significant or not. I also wondered why the authors used group means DNA methylation values rather than individual DNA methylation. If that information was not available, the authors should explain what was the reason for this secondary analysis approach.
We thank the reviewer for their comment. We opted to use a Bayesian approach for the DNA methylation analyses because we did not have access to individual methylation values and thus needed to use an analytic approach that would allow us to use prior summary findings, without raw data, to explore how it may associate with new information (i.e. data on symptom severity). Similarly, due to the lack of individual methylation values we found it inappropriate to test for significance and instead presented the results as covariates by rank. The purpose of our secondary analysis was solely to explore any potential relationships that may exist. Unlike the work presented here which was based on a previous pilot study, future work in our ongoing study will use raw data to explore similar questions.

3. Finally, the paper can benefit by relating the current findings with previous ones. For example, the paper by Bowers and Yehuda (Bowers, M. E., & Yehuda, R. (2016). Intergenerational effects of PTSD on offspring glucocorticoid receptor methylation. In D., Spengler, & E. Binder (Eds.), Epigenetics and neuroendocrinology: Clinical focus on psychiatry (vol. 2, pp. 141-155). Switzerland: Springer) reviews additional studies looking at the methylation in NR3C1. Shrira and colleagues (Shrira, A., Ayalon, L., Bensimon, M., Bodner, E., Rosenbloom, T., & Yadid, Y. (2017). Parental posttraumatic stress disorder symptoms is related to successful aging in offspring of Holocaust survivors. Frontiers in Psychology, 8. 1099) suggest such methylation patterns can explain greater mental as well as physical morbidity across generations in exposed families.

We thank the reviewer for their suggestions and have referenced the above-mentioned papers in our revised manuscript on page 14 and 12, respectively.

“For example, a case-control study of successful aging in offspring of holocaust survivors and an ethnically matched control group, described that almost half of the parents not exposed to the holocaust reported a sudden loss of a loved one as a traumatic event (29). This is important to note because although the comparison group was not directly exposed to the holocaust, the tragedy still had some degree of impact on their psyche.”

“Moreover, we did not formally assess secondary traumatization, which has been shown to mediate the relationship between maternal PTS and offspring PTS in Holocaust survivors (11,31,32).”

**Competing Interests:** No competing interests were disclosed.