Psychoneuroendocrinology (2014) 43, 62—70

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Elevated systemic expression of ER stress related genes is associated with stress-related mental disorders in the Detroit Neighborhood Health Study

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Received 24 September 2013; received in revised form 22 January 2014; accepted 22 January 2014

KEYWORDS
Endoplasmic reticulum stress;
Unfolded protein response;
Epidemiology;
Matched-pair analysis;
Case—control studies;
Gene expression pattern analysis;
Cardiovascular diseases;
Metabolic diseases

Summary
Background: The role of endoplasmic reticulum (ER) stress response in mental illness is not well understood. Human studies and animal models of depression show elevated brain ER stress response. In addition, some ER stress associated disorders (e.g. cardiovascular disease) show higher rates of depression compared to the general population, raising the possibility that ER stress response contributes to depression risk. It remains unknown, however, if ER stress response is present among individuals suffering from other stress-related mental illness, and whether such a response would be evident in a non-clinical sample. This study tests for systemic changes in ER stress response associated with Major Depressive Disorder (MDD) or post-traumatic stress disorder (PTSD) among community-dwelling individuals.
Methods: We analyzed expression of *BiP*, *EDEM1*, *CHOP*, and *XBP1*, the major indicators of ER stress response, with real-time PCR in leukocyte-derived RNA samples from 86 participants of the Detroit Neighborhood Health Study. Participants were selected based on the presence of either past year MDD or past year PTSD; controls were age and sex matched.

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0306-4530/$ — see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psyneuen.2014.01.013

Results: Relative to controls, MDD is associated with a 1.34-fold increase in *BiP* ($P = 0.004$), 1.35-fold increase in *EDEM1* ($P = 0.001$), 1.68-fold increase in *CHOP* ($P = 0.002$), and 1.60-fold increase in *XBP1* ($P = 0.004$). These results remained significant after correction for multiple testing. In contrast, PTSD is associated with a 1.27-fold increase in *EDEM1* expression only ($P = 0.027$), a result that is attenuated to non-significance following adjustment for multiple testing; however, a subsample of participants with past month PTSD showed elevated expression of *BiP* and *EDEM1* (uncorrected *P* value 0.049 and 0.017, respectively).
Conclusions: These data indicate systemic and persistent activation of the ER stress response pathway in MDD among community-dwelling individuals. Systemic activation of the ER stress response may also occur in PTSD among persons with more recent symptoms.
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1. Introduction

Several human studies and animal models suggest that the endoplasmic reticulum (ER) stress response may play a role in psychiatric disease (Bown et al., 2000; Gold et al., 2013). The ER is an intracellular organelle that is responsible for protein folding and assembly, calcium storage, and lipid and sterol biosynthesis (Back et al., 2005). A variety of pharmacological, pathophysiological, and environmental stimuli can impose stress on the ER and subsequently interrupt the protein folding process in the ER, leading to accumulation of unfolded or misfolded proteins in the ER lumen (Zhang and Kaufman, 2004). This condition is referred to as ''ER stress'' (Zhang and Kaufman, 2004). To cope with ER stress, highly specific signaling pathways localized to the ER have evolved, which are collectively called the ER stress response or the ''Unfolded Protein Response (UPR)''. The primary function of the UPR is to restore ER homeostasis and help the cells adapt to ER stress conditions. However, when ER stress is prolonged or the degree of ER stress is too severe, UPR signaling can initiate programmed cell death by activating stress-induced pro-apoptotic factors (Zhang and Kaufman, 2004). Dysregulation or hyper-activation of the UPR pathway is critically involved in the initiation and progression of a variety of life-threatening diseases, such as cardiovascular disease, metabolic disease, neurodegenerative disease, and cancer (Zhang and Kaufman, 2008).

ER stress related protein expression has been shown to be elevated within the temporal cortex among individuals who had Major Depressive Disorder (MDD) and died of suicide compared with individuals who had MDD and died of other causes (Bown et al., 2000). A recent paper suggests that ER stress and parainflammation — i.e. a tissues' stress response with features intermediate between a normal state and an acute inflammatory state — are interrelated processes each influencing many phases of the stress response pathway and as such these pathways may be valid targets for intervention in MDD and bipolar disorder (Gold et al., 2013). Some pharmacological interventions used to treat affective disorders target genes which interact with the UPR pathways (e.g. Wang et al., 1999). For example, valproate and carbamazepine are mood stabilizing drugs which increase expression of 78-kDa glucose-regulated protein (*GRP78*), also known as Binding immunoglobulin protein (*BiP*) (Wang et al., 1999). *BiP*/*GRP78* is a member of the ER stress gene family believed to inhibit ER stress response activation and to inhibit UPR induced apoptosis (Reddy et al., 2003). Furthermore, lithium is a treatment employed for some mood disorders and is known to directly target and inhibit the protein encoded by Glycogen synthase kinase-3 (*GSK3*); GSK3 has been shown to regulate ER stress induced apoptosis in neuronal cells (Meares et al., 2011). Several mouse models of depression-like behavior implicate genes which interact with the UPR pathways, e.g. Calreticulin (*CALR*) (Liu et al., 2011), Bax inhibitor 1 (*BAX*) (Hunsberger et al., 2011), Glycogen synthase kinase-3 (*GSK3B*) (Mines et al., 2010; Meares et al., 2011), Interferon-gamma (*IFNG*) (O'Connor et al., 2009), and tumor necrosis factor-α (*TNF*) (Kaster et al., 2012). These human studies and animal models suggest that the UPR pathways may be activated or up regulated within brain tissue in the presence of some affective disorders.

Despite the suggestive evidence of ER stress in mental disorders in both animal (Hunsberger et al., 2011; Liu et al., 2011) and human (Bown et al., 2000; So et al., 2007) studies, to date there has to our knowledge been no report of ER stress and the UPR among living individuals suffering from such disorders. More specifically, it is not known whether the ER stress response pathway is activated at a systemic level among community-dwelling individuals suffering from MDD. Here we test whether ER stress-related gene expression is elevated in leukocytes derived from individuals diagnosed with MDD in the Detroit Neighborhood Health Study (DNHS). The DNHS is a longitudinal community-based study of mental and physical health and their interaction with social-environmental factors (Uddin et al., 2010; Goldmann et al., 2011). Elevated ER stress response may be specific to MDD or it may be associated with additional mental disorders in which stress plays a contributing role. To test this, we also investigate whether ER stress response-related gene expression is elevated in leukocytes derived from individuals diagnosed with post-traumatic stress disorder (PTSD) in the Detroit Neighborhood Health Study (DNHS). To our knowledge this is the first consideration of whether PTSD is associated with elevated ER stress response and is facilitated by the relatively high burden PTSD in the DNHS population (Uddin et al., 2010). Results from this work may provide insight into biological processes relevant to psychological stress that occur in non-clinical settings.

2. Methods

2.1. Sample

Details of the Detroit Neighborhood Health Study (DNHS) have been previously reported (Uddin et al., 2010; Goldmann et al., 2011; Uddin et al., 2011). The University of Michigan

IRB reviewed and approved this study. Our study sample consists of a subset of the DNHS study participants with previously extracted RNA from leukocytes (described in more detail below). We employed a matched pair study design based on past year MDD cases (n = 18) and their age- and gender-matched controls (n = 18) and past year PTSD cases (n = 26) and their age and gender-matched, trauma-exposed controls (n = 26). Note that two participants serve the role of matched control in both the MDD and PTSD control samples (total sample n = 86).

2.2. Assessment of PTSD and MDD

Past year MDD was assessed by a structured telephone interview using validated instruments based on DSM-IV criteria (APA, 1994). The patient health questionnaire (PHQ-9) (Kroenke et al., 2001) was used to assess depression symptoms. The nine items on the PHQ-9 were scored from 0 (not at all) to 3 (nearly every day), with scores ranging from 0 to 27. In our MDD positive cases, the respondent experienced at least 5 symptoms more than half the time; this corresponds with a score of 10 out of 27 possible. Additional questions were added to these measures to determine the timing, duration and severity of illness and also symptom-related disability. We selected participants diagnosed with MDD during their lifetime who have also experienced symptoms during the past year. We selected age and sex matched control cases who had no lifetime history of MDD. We further excluded participants who had a lifetime history of PTSD (as described below) within both the MDD case and control group.

Past year PTSD was assessed in the following manner: Participants were asked to identify potentially traumatic events (PTEs) from a list of 19 categories of events. PTSD symptoms were assessed in reference to both the traumatic event the participant regards as their worst and one randomly selected traumatic event from the remaining traumas the participant has experienced. Lifetime PTSD cases met all six DSM-IV criteria in reference to either the worst or random traumatic event. After determining that a participant had a lifetime history of PTSD, we asked a follow up question regarding the length of time since an individual experienced any symptoms. We included only those individuals who had also experienced PTSD symptoms within the past year. We excluded participants with a history of lifetime MDD as described above. Finally, we identified a subset of these past year PTSD cases (and matched controls) who had experienced PTSD symptoms within the past month (n = 32). PTSD and depression diagnoses have been validated in a random subsample of participants via in-person clinical interview using the clinician-administered PTSD scale for DSM-IV and the structured clinical interview for DSM-IV disorders, respectively, as previously described (Uddin et al., 2010).

2.3. Expression levels of ER stress related genes

Eighteen ml of whole blood was collected from consenting DNHS participants. Total leukocyte RNA was isolated using the LeukoLOCK Fractionation and Stabilization kit (Ambion) using the manufacturer's alternative protocol. Quantitative reverse-transcriptase real time PCR (qRT-PCR) was used to quantify mRNA expression of ER stress-related genes (Zhang and Kaufman, 2004). These genes included: (1) *BiP*/*GRP78*, an abundant ER chaperone protein which has been recognized as a reliable ER stress marker and a master regulator of the UPR pathways (Zhang and Kaufman, 2004); (2) the growth arrest and DNA damage-inducible protein *GADD34*/*CHOP*, an ER stress-induced pro-apoptotic factor under the UPR pathway mediated through *PERK*/*eIF2α* (Zhang and Kaufman, 2004). Induction of *CHOP* can represent induction of the *PERK*/*eIF2α* UPR pathways and ER stress-induced apoptosis (Zhang and Kaufman, 2004); (3) ER degradation enhancer, mannosidase alpha-like 1 (*EDEM1*), an ER stress-inducible factor that is involved in ER-associated degradation (ERAD) of misfolded proteins (Molinari et al., 2003); and (4) The spliced form of X-box binding protein 1 (*XBP1*), an ER stress-induced bZIP transcription factor that is under the *IRE1α*-mediated UPR pathways. Induction of the spliced *XBP1* mRNA (*XBP1s*) is an indication of activation of the *IRE1α*/*XBP1* UPR pathway.

First strand cDNA was synthesized from 500 ng of total RNA using the SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen). Primers targeting the following loci were used:

BiP-F: 5′-CCTGGGTGGCGGAACCTTCGATGTG-3′
BiP-R: 5′-CTGGACGGGCTTCATAGTAGACCGG-3′
CHOP-F: 5′-GCCTTTCTCCTTTGGGACACTGTCCAGC-3′
CHOP-R: 5′-CTCGGCGAGTCGCCTCTACTTCCC-3′
EDEM1-F: 5′-GCTACGACAACTACATGGCTC-3′
EDEM1-R: 5′-GACTTGGACGGTGGAATCTTT-3′
XBP1s-F: 5′-CCGC AGCAGGTGCAGG-3′
XBP1s-R: 5′-GAGTCAATACCGCCAGAATCCA-3′
beta-actin-F: 5′- AGCCTCGCCTTTGCCGATCCG-3′
beta-actin-R: 5′-ACATGCCGGAGCCGTTGTCGA-3′

Note that the sense primer for *XBP1* includes 4 bp before and 12 bp after a 26 bp region which is removed from exon 4 through non-conventional splicing during *XBP1* activation. Therefore this primer pair is expected to anneal only to the spliced form of the gene; a similar strategy has been employed to investigate the function of the spliced form of *XBP1* in the literature (Back et al., 2005; Hirota et al., 2006). The real-time PCR reaction mixture (10 μl) containing 125 nM primers, cDNA template and SYBR Green PCR Master Mix (Invitrogen #4385612) was amplified in a 7500 Fast Real-time PCR System (Applied Biosystems). Cycling conditions included: holding stage at 95 °C for 20 s; 40 cycling stages at 95 °C for 3 s, 60 °C for 30 s. Negative reverse transcriptase samples were used to ensure the absence of contaminating genomic DNA. All reactions were performed in triplicate. Each set of triplicates was checked to ensure that the threshold cycle (Ct) values are all within 1Ct of each other. Fold changes were determined after normalizing to beta-actin as the internal control. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008) was employed to determine the relative expression values of the cases compared with age and sex matched controls. Normality was tested using a Shapiro—Wilk test. We tested for significant differences between case and control in the $2^{-\Delta Ct}$ values using the non-parametric paired sample Wilcoxon signed rank test ($P \leq 0.05$) or the parametric paired samples T test, as appropriate. We also tested which genes show a significant difference between cases and controls when a Bonferroni correction for multiple comparisons is employed ($P \leq 0.0125$).

2.4. Prevalence of cardiovascular disease associated with ER stress in cases vs. controls

Recent work has demonstrated that ER stress, and the consequent induction of the UPR, plays a major role in the pathophysiology of cardiovascular (CVD)-related disorders such as cardiac hypertrophy, heart failure, atherosclerosis, and ischemic heart disease (Minamino and Kitakaze, 2010; Jiao et al., 2012). Therefore, as a secondary analysis, we tested whether there was a significant difference between cases and controls with respect to the prevalence of CVD. The prevalence of CVD was determined by assessing the presence/absence of conditions previously identified as indicative of CVD in the DNHS cohort (Keyes et al., 2013) and which have been previously associated with the ER stress response, including: hypertension (i.e. high blood pressure) (Sun et al., 2008), myocardial infarction (i.e. heart attack) (Thuerauf et al., 2006), stroke (Chae et al., 2004), chest pain due to heart disease (angina pectoris) (Myoishi et al., 2007), and congestive heart failure or heart disease (Hamada et al., 2004). Presence/absence of these diseases was assessed with a structured phone interview survey in which respondents were asked to respond ''Has a doctor ever told you that you have [ITEM]?'', with [item] referring to the four CVD-related conditions listed above. Cases meeting at least one of these criteria were identified as positive for CVD. Differences in the prevalence of CVD were tested using a McNemar's test ($P \leq 0.05$), a paired version of the Chi-square test.

3. Results

Table 1 describes the demographic profile of the study participants. A Chi squared test shows no difference between case and control groups with respect to age, sex, or race/ethnicity.

3.1. Past year MDD is associated with induction of the ER stress response and activation of multiple UPR pathways in leukocyte samples (Fig. 1)

Among past year MDD cases, there is a 1.34-fold increase in expression of the ER chaperone *BiP* ($P = 0.004$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0025 ± 0.0014 and controls 0.0019 ± 0.0009). The *ERAD* mediator *EDEM1* also shows a 1.35-fold increase ($P = 0.001$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0125 ± 0.0036 and controls 0.0092 ± 0.0026) among MDD cases. In addition, there is a 1.68-fold increase in the expression of the ER stress-induced pro-apoptotic factor *CHOP* ($P = 0.002$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0011 ± 0.0007 and controls 0.0006 ± 0.0005). Finally, MDD cases show a 1.60-fold increase in expression of the spliced (i.e. activated) form of the UPR trans-activator *XBP1* ($P = 0.004$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0327 ± 0.0366 and controls 0.0205 ± 0.0245). Each of these genes remains significantly differently expressed between cases and controls when adjusted for multiple comparisons using the stringent Bonferroni correction ($P \leq 0.0125$). Because our study sample included only 10 individuals with past month MDD, we did not assess ER stress activation for this phenotype.

3.2. Past year PTSD is associated with elevated expression of genes involved in ER-associated degradation (ERAD) of misfolded proteins in leukocyte samples (Fig. 1)

Among past year PTSD cases, *EDEM1* shows a 1.27-fold increase ($P = 0.027$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0054 ± 0.0039 and controls 0.0036 ± 0.0020) in expression compared to controls. Expression of *BiP* also shows a 1.16-fold increase in cases that approaches significance ($P = 0.069$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0048 ± 0.0034 and controls 0.0042 ± 0.0028). In contrast to MDD cases, past year PTSD cases do not show a statistically significant increase in the expression of *CHOP* or *XBP1* in cases compared with controls ($P > 0.05$). When a conservative adjustment is made to account for multiple comparisons, i.e. the Bonferroni correction, *EDEM1* expression is no longer significantly different between cases and controls.

3.3. Time since participant experienced PTSD symptoms does not alter the expression pattern of the ER stress-related genes (Fig. 1)

The expression levels of *BiP* and *EDEM1* are elevated in past month PTSD cases; similarly, *BiP* and *EDEM1* expression levels are elevated in past year PTSD. Among PTSD cases who experienced symptoms recently (within the past month), there is a 1.25-fold increase in *BiP* expression ($P = 0.049$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0036 ± 0.0020 and controls

Table 1 Demographic characteristics of the study samples ($N = 86$).

	Total study ($n = 86$)[a]	MDD ($n = 36$)		PTSD ($n = 52$)		Past Mo PTSD ($n = 32$)	
	Mean (SD)	Mean (SD)	*P*-value[*]	Mean (SD)	*P*-value[*]	Mean (SD)	*P*-value[*]
Mean age	48 years (SD 11.2)	49 years (SD 11.7)	1.00	49 years (SD 10.0)	0.99	51 years (SD 8.2)	1.00
Percent female	61%	50%	0.99	69%	1.00	75%	1.00
Race/ethnicity							
African American	81%	81%	0.99	79%	0.99	78%	0.99
White/non-Hispanic	13%	8%		17%		19%	
Other	6%	11%		4%		3%	

[a] Two participants were used as controls in both MDD and PTSD.
[*] Comparison of case vs. control.

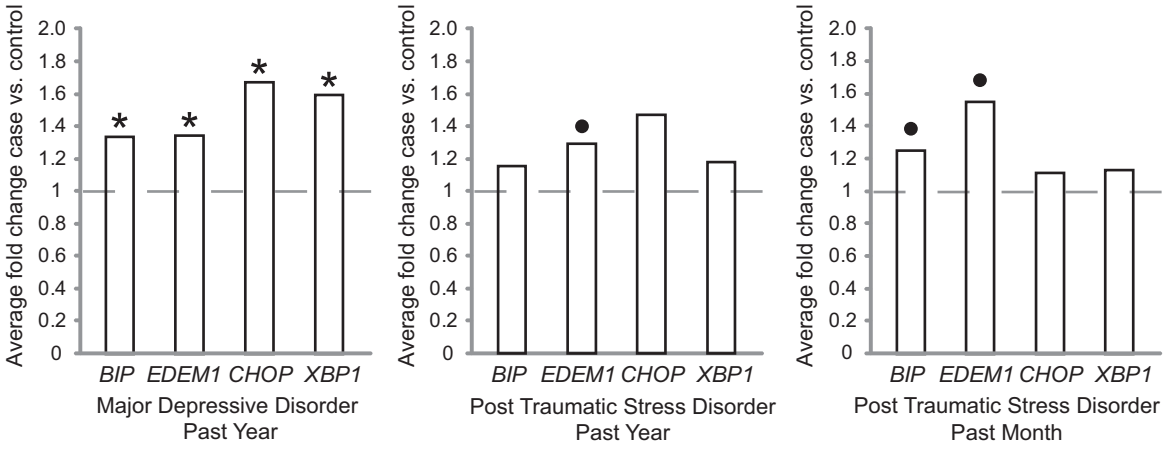

Figure 1 Average fold change between cases and controls for Major Depressive Disorder and Post Traumatic Stress Disorder. The closed circle indicates a statistically significant increase in expression in cases compared with controls at (uncorrected $P < 0.05$). The star symbol indicates a statistically significant increase in expression in cases compared with controls at ($P < 0.0125$; i.e. $\alpha \leq 0.05$ adjusted for multiple comparisons with the Bonferroni correction).

0.0029 ± 0.0017), and *EDEM1* also shows a 1.55-fold increase ($P = 0.017$; mean $\pm$ s.d. $2^{-\Delta CT}$ for cases 0.0054 ± 0.0050 and controls 0.0034 ± 0.0028). The expression levels of *CHOP* and *XBP1* are not significantly different in cases compared with controls among either past month or past year PTSD cases. When a conservative adjustment is made to account for multiple comparisons, i.e. the Bonferroni correction, *BiP* and *EDEM1* expression is no longer significantly differently expressed between cases and controls.

3.4. Prevalence of cardiovascular disease in case and control groups

The ER stress response is associated with CVD (Glembotski, 2007). Moreover, PTSD plays a role in increased risk and severity of cardiovascular disease (Kubzansky et al., 2007; Boscarino and Adams, 2008). Similarly, depression is a well-recognized risk factor for coronary artery disease (Grippo and Johnson, 2002). Consequently, we hypothesized a role for ER stress response in the comorbidity between mental disease and CVD and tested whether there were differences in the prevalence of this disorder among participants with vs. without MDD or PTSD. No significant differences are found between our case and control groups according to a McNemar's Chi-squared test $P > 0.05$ [Table 2]. These data suggest that the differences shown in expression levels between cases and controls are likely due to psychological disease independent of CVD disease.

4. Discussion

Here we report that community-dwelling individuals with a recent history of stress-related mental disorders show activation of the ER stress response and an increase in UPR related gene expression in peripheral leukocytes. The pattern of activation of the UPR pathways differed to some degree between MDD and PTSD, with MDD cases showing evidence of more pronounced ER stress response. In addition, the pattern of ER stress response was generally consistent in cases experiencing PTSD symptoms in the past month and the past year, indicating a persistent activation of the ERAD-associated UPR pathway among those suffering from this disorder. Secondary analyses of the prevalence of ER-stress associated CVD showed no difference between MDD- and PTSD-cases and their respective controls; consequently, differences observed between cases and controls are likely to be due specifically to the mental illness. Taken together, these results suggest that stress-related mental disorders, in particular MDD, are associated with a systemic, persistent ER stress response and activation of the UPR pathways.

The expression profiles observed in these data suggest both commonalities and dissimilarities in the pattern of ER stress response associated with MDD or PTSD. The ER stress marker *BiP* is up-regulated in both MDD and in past month PTSD. *BiP* is transcriptionally activated by several mediators of the UPR, and the high expression level in MDD and past month PTSD indicate an elevation in the level of systemic ER stress. Increased expression of *BiP* suggests induction of ER stress response and activation of the UPR pathways (Bertolotti et al., 2000; Gulow et al., 2002). *EDEM1* directly regulates ER-associated degradation (ERAD) by targeting misfolded glycoproteins for degradation in an N-glycan-independent manner (Ron et al., 2011). Elevated expression of *EDEM1* indicates induction of ER stress response and activation of the UPR that facilitates degradation of misfolded proteins in the ER (Molinari et al., 2003). In both MDD and PTSD, we found *EDEM1* expression is elevated.

In contrast, in MDD — but not PTSD — *CHOP* and *XBP1* expression are elevated. *CHOP* expression is one downstream indicator of the *PERK*-mediated UPR pathway (Zhang and Kaufman, 2004). Additionally, *CHOP* is also recognized as a pro-apoptotic factor in ER stress-induced apoptosis (Zhang and Kaufman, 2004). Up-regulation of *CHOP* in MDD but not PTSD cases suggests that the *PERK*-mediated UPR pathway and ER stress-induced apoptosis in MDD may be more prevalent than in PTSD. Finally, *XBP1* is a transcription factor that positively regulates *BiP*, *EDEM1*, *CHOP*, and itself when the UPR is activated (Fig. 2: adapted from Takayanagi et al., 2013). Notably, activation of *XBP1* is mediated through IRE1a,

Table 2 Cardiovascular disease indicators in cases and controls.

	MDD					PTSD					Past Month PTSD				
	Case — percent with diagnosis	Control — percent with diagnosis	McNemar's chi-squared	df	*P*-value	Case — percent with diagnosis	Control — percent with diagnosis	McNemar's chi-squared	df	*P*-value	Case — percent with diagnosis	Control — percent with diagnosis	McNemar's chi-squared	df	*P*-value
High blood pressure or hypertension	39%	33%	0.000	1	1.00	62%	50%	0.083	1	0.77	69%	63%	0.000	1	1.00
Heart attack or myocardial infarction or stroke	13%	38%	0.500	1	0.48	12%	15%	0.000	1	1.00	13%	19%	0.000	1	1.00
Chest pain due to heart disease (angina pectoris)	6%	0%	0.000	1	1.00	8%	8%	0.000	1	1.00	13%	13%	0.000	1	1.00
Congestive heart failure or heart disease	0%	0%	NA	1	NA	0%	4%	0.000	1	1.00	0%	6%	0.000	1	1.00
Cardio vascular disease	39%	39%	0.250	1	1.00	69%	50%	1.231	1	1.00	75%	63%	0.125	1	0.73

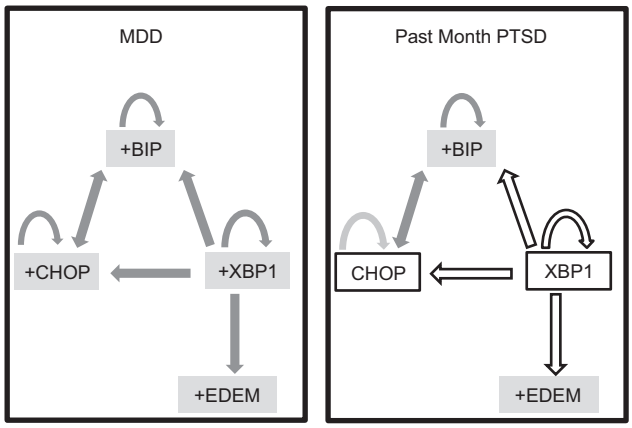

Figure 2 ER stress transcriptional network and dysregulation in Major Depressive Disorder and Past Month Post Traumatic Stress Disorder. Each gray arrow indicates a transcriptional target of a significantly up regulated gene. Each white arrow indicates aspects of the ER stress transcriptional network apparently unaffected in MDD or past month PTSD. Each of the genes tested are a transcriptional target of *XBP1*, therefore the elevated expression observed in MDD may represent a change in ER stress homeostasis. (Adapted from Takayanagi et al., 2013 with kind permission from Springer Science and Business Media).

the proximal UPR transducer (Zhang and Kaufman, 2004). Under ER stress conditions, *IRE1*α is activated to function as an RNase in order to alternately splice *XBP1* mRNA. Spliced *XBP1* mRNA encodes a functional transcription factor that activates expression of the UPR target genes. Changes in the expression level of spliced *XBP1* can therefore reflect ER stress response and activation of the *IRE1*α-mediated UPR pathway, which regulate ERAD and expression of *BiP*. The elevated *XBP1* expression level in MDD may indicate a change in ER homeostasis and activation of the *IRE*α-mediated UPR pathway.

ER stress may provide a mechanistic link between physical and mental diseases. Activation of ER stress and UPR related gene expression in MDD and PTSD may contribute to increased risk of a variety of physical diseases. Elevated ER stress genes in vascular tissue correlate with accelerated atherosclerotic plaque development in *APOE* deficient mice (Khan et al., 2009). Atherosclerosis results from a passive buildup of cholesterol in the artery wall (Libby et al., 2013). Diabetes increases atherosclerosis-related inflammation, and diabetic patients are twice as likely to have a heart attack or stroke. Khan and colleagues showed that hyperglycemia-induced vascular ER stress response plays a role in diabetic atherosclerosis, and that inhibition of ER stress response attenuates diabetic atherosclerosis (Khan et al., 2009). There are significant comorbidities between diabetes and stress-related mental disorders such as MDD and PTSD (Anderson et al., 2001; Simon et al., 2006). It is plausible that increased expression levels of ER stress related genes in peripheral blood in MDD may accelerate or facilitate atherosclerotic plaque development. Moreover, elevated ER stress may play a role in the risk of developing comorbid psychological and cardiovascular disease.

We recently showed that DNHS participants with lifetime depression showed evidence of increased inflammation (Uddin et al., 2010). In addition, we (Uddin et al., 2011)

and others (Smith et al., 2011) have reported that participants with lifetime PTSD show epigenetic dysregulation in multiple immune-related genes, and an exaggerated immune response to a common pathogen (Uddin et al., 2010). General inflammation, oxidative stress, and calcium homeostasis share bi-directional interactions with the UPR pathway (Zhang, 2010). There are multiple lines of evidence suggesting that in addition to its roles in the ER stress response, the protein encoded by *XBP1* plays key roles in moderating innate immune response to pathogens. In the absence of *XBP1* expression, pathogen induced inflammation results in an exaggerated ER stress response (Richardson et al., 2010). In the absence of *XBP1* expression, researchers report increased inflammation in the intestinal epithelium (Kaser et al., 2008). Conversely, *XBP1* expression protects the host against ER stress caused by its own secretory immune response to infection (Richardson et al., 2010). Elevated rates of inflammation are a common phenotype associated with mental disorders including MDD and PTSD (Dantzer et al., 2008; Plantinga et al., 2013). It is possible that high expression levels of *XBP1* in MDD cases may represent an adaptive response to elevated levels of inflammation associated with MDD. Up-regulation of *XBP1* also modulates lipid and glucose metabolism, which is associated with cardiovascular and metabolic diseases (Glimcher and Lee, 2009; Lee et al., 2012). Consequently, elevated ER stress conditions and activated *XBP1* expression may also play a role in the risk of developing comorbid psychological, cardiovascular and metabolic disease.

A non-invasive, quantitative clinical test of a single biomarker to aid in the diagnosis and treatment of MDD remains elusive (Lakhan et al., 2010). A variety of peripheral/serum growth factors, cytokines, hormones, and metabolic factors have been shown to be altered in MDD but are not specific to MDD, e.g. BDNF, IL-6, and TNF-a; a multivariate biomarker panel may in combination improve sensitivity and specificity for diagnosis and treatment of MDD (Schmidt et al., 2011). Our data preliminarily suggest that ER stress pathway activation may represent an additional set of biomarkers that may contribute to diagnosis of MDD.

4.1. Study limitations

Our study includes a minimum of four limitations that should be considered. First, the cross-sectional analyses reported here leave us unable to determine whether the differences in expression were a consequence of MDD or PTSD or whether the differences in expression are indicative of biologic vulnerabilities that existed among the MDD-affected or PTSD-affected individuals before the onset of their disorder. Ongoing work using samples from this same longitudinal cohort may be informative regarding this distinction. Second, our focus on peripheral tissues limits the inferences that can be drawn about ER stress in the brain. Third, our sample size (n = 86) is modest. Nonetheless, we did observe statistically significant changes in expression ($P \leq 0.05$) across several members of the ER stress pathway, suggesting that the findings reported here are robust. Moreover, we applied an extremely stringent method to adjust for repeated measures which increases the probability of falsely rejecting a hypothesis, yet the expression data for MDD remained significantly different between cases and controls in each of the ER stress response genes that we tested. Finally, we assessed presence/absence of CVD-related conditions through self-report of a doctor's diagnosis. This may underreport CVD in our control sample. Given these results, replicating this work in independent cohorts is warranted.

5. Conclusion

Two stress-related mental disorders — MDD and PTSD — correlate with elevated expression levels of genes involved in the ER stress pathway. To our knowledge this is the first report associating PTSD or MDD with high systemic levels of ER stress. Patients diagnosed with either MDD or PTSD have an elevated risk of a variety of physical diseases. The ER stress response may contribute to this association, particularly with respect to heart disease and diabetes.

Role of the funding sources

This study was supported by the National Institutes of Health (A.E.A., grant numbers DA022720, DA022720-S1, and RC1MH088283); the Wayne State University School of Medicine Post-Doctoral Research Fellowship (L.N.); the American Association of Anatomists Post-doctoral Fellowship (L.N.); and the National Science Foundation (D.W., grant number BCS 0827546).

Conflict of interest

For work unrelated to this project, Sandro Galea is funded in part by a grant from Merck Pharmaceuticals; Derek Wildman is funded in part from Lung, LLC, receives Honoraria from Elsevier, INC; and Allison Aiello consults for SCA Tork. Lisa Nevell, Kezhong Zhang, Karestan Koenen, Richelo Soliven, Chao Zhang, and Monica Uddin declare no conflict of interest.

Acknowledgments

We would like to thank the participants of the DNHS for their time and generosity; this research would not be possible without their support. We would like to thank Rebecca M. Coulborn, Janie Slayden, and Helen Meier for overseeing DNHS specimen collection and coordinating the overall DNHS project. We also thank Amy Weckle, Alexis Pavle, Andrew Schultz, and Henriette Mair-Meijers for handling the DNHS specimen processing and laboratory technical assistance.

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