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Elevated expression of unfolded protein response genes in the prefrontal cortex of depressed subjects: effect of suicide

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Abstract

Background: Major Depressive Disorder (MDD) is a leading cause of mental disability worldwide. Despite many studies, the pathophysiology associated with MDD brain is not very clear. It is reported that cellular stress is related to depressive symptoms. Under stressful conditions, intracellular homeostasis processes can be disrupted, which can induce a process of unfolded protein response (UPR) in the subcellular lumen of endoplasmic reticulum (ER). The purpose of this study is to elucidate whether UPR is active in the depressed brain.

Methods: The dorsolateral prefrontal cortex (dlPFC) was used from 23 non-psychiatric controls and 43 MDD subjects. The expression levels of UPR associated genes (GRP78, GRP94, XBP-1, CHOP, ATF4C, and ATF6C) were measured by qRT-PCR.

Results: The level of mRNA expression in MDD subjects was significantly higher for GRP78 ($p=0.008$), GRP94 ($p=0.018$), and ATF4C ($p=0.03$) compared to non-psychiatric controls. Further analysis suggested that changes in the expression of these genes were specifically higher only in those MDD subjects who died by suicide but not in those who died by causes other than suicide when compared with non-psychiatric controls (GRP78, $p=0.007$; GRP94, $p=0.041$; ATF4C, $p=0.037$).

Limitations: This study was performed only in MDD subjects who had died by suicide. Suicide subjects with other psychiatric illnesses need to be included.

Conclusions: Given that UPR is involved in many physiological processes in the brain, including inflammatory response as well as apoptosis, increased expression of UPR genes indicates that ER stress and mediated UPR may be critical factors in suicidality among depressed patients.

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Authorship contribution statement

YY generated and analyzed the data and wrote manuscript. YD conceptualized, co-wrote, reviewed, interpreted the data and edited the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Keywords

Postmortem brain; dorsolateral prefrontal cortex; major depressive disorder; suicide; ER stress; UPR system; gene expression

Introduction

MDD is a commonly occurring mental disorder with an estimated lifetime prevalence of 10.8% in general population across the world (Lim et al., 2018). According to Diagnostic and Statistical Manual of Mental Disorders (DSM-V), to be diagnosed with MDD, a patient requires to have at least five symptoms including depressed mood, suicidal ideation, psychomotor agitation, fatigue or loss of energy, and insomnia within a 2-week period (DSM-5 book); among these, suicide attempt is an absolutely severe symptom. Large proportions of MDD patients (about 40%) have suicidal ideation (Kessler et al., 2005). It reportedly costs \$93.5 billion loss of productivity per year in the United States (Shepard et al., 2016), thus significantly increasing the nationwide economic burden.

Frontal lobe plays an important role in emotion processing and regulation as well as cognitive control. Recent MRI studies have shown that recurrent or long duration of MDD episodes can induce the gradual loss of grey matter in prefrontal cortex (Wise et al., 2017; Peng et al., 2016; Zhang et al., 2016). On the other hand, antidepressants such as fluoxetine or sertraline increase the volume of dorsolateral prefrontal cortex (dlPFC) in MDD patients (Kong et al., 2014, Smith et al., 2013). Recently, several reports have shown that repetitive transcranial magnetic stimulation (rTMS) in the dlPFC is effective in improving depressive symptoms and cognitive functions (Teng et al., 2017, Salehinejad et al., 2017). In addition, the brain SPECT study reported that dlPFC connectivity is enhanced after rTMS in treatment-resistant depressed patients (Richieri et al., 2017). Altogether, the converging evidence suggests the significant role of prefrontal cortex in MDD pathogenesis.

Despite significant improvements in our understanding, the neurobiology associated with MDD pathogenesis is still unclear. The monoamine hypothesis, proposed in 1960 (Bunney and Davis, 1965, Schildkraut, 1965), is an old but still a mainstream hypothesis. Based on the monoamine hypothesis, antidepressants have been developed and used clinically. However, approximately 30% of MDD patients do not respond to antidepressant treatment (Rush et al., 2006). This means that there are other mechanisms involved in MDD besides monoamine changes. Recently, it has been reported that microRNAs, a type of non-coding RNA, are associated with nervous system development, physiology, and disease including depression (Serafini et al., 2012; Serafini et al., 2014). In addition, cellular stress has also been shown to be related to many symptoms of depression (Lotrich, 2012). The endoplasmic reticulum (ER), a subcellular organelle, is mainly responsible for cellular stress responses. Under normal conditions, ER has the role of folding and trafficking of protein from the intracellular to the extracellular membrane. Under stressful conditions, intracellular homeostasis processes are disrupted, which can induce protein misfolding, which can lead to functional loss of many cellular phenotypes (Kaufman, 2002). Cellular adaptive system triggers a complex molecular process called unfolded protein response (UPR) to offset the

loss incurred due to protein misfolding. Previously, our laboratory has studied UPR in rat brain that leads to depressive-state through inflammatory changes in the central nervous system (Timberlake and Dwivedi, 2016; Timberlake et al., 2018). Mechanistically, glucose-regulated protein 78 kD and 94 kD (GRP78 and GRP94) present in the ER initiate the UPR and are responsible for triggering the stress-related neural damage (Bown et al., 2000). In response to these changes in ER, X-box binding protein-1 (XBP-1), activating transcription factor 4 and 6 (ATF4 and ATF6), and C/EBP homologous protein (CHOP) induce a pro-apoptotic environment in the nucleus (reviewed in Timberlake and Dwivedi, 2018). We found abnormalities of several of these genes in rat brain which showed depressive phenotype (Timberlake and Dwivedi, 2016; Timberlake et al., 2018). Interestingly, we also found that triggering UPR can lead to depression-like behavior in rats (Timberlake et al., 2019). Whether such changes occur in the brain of depressed individuals is not clear, although one preliminary report states changes in protein levels of a few UPR related genes in temporal lobe of MDD subjects who died by suicide (Bown et al., 2000). We hypothesize that UPR activation may play an important role in depression and ER stress may be one of the causes associated with suicidality among MDD patients. In the present study, we determined UPR activation by examining the expression levels of UPR genes in dlPFC of MDD subjects who died by suicide or by causes other than suicide and non-psychiatric controls.

Subjects and Methods

Human Postmortem Brain:

The study was approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB). DIPFC was obtained from non-psychiatric control (hereafter referred as controls) and MDD subjects from Alabama Brain Bank and Maryland Psychiatric Research Center Brain Collection Program. DIPFC from a total of 27 controls 43 MDD subjects were used in this study. Of the 43 MDD subjects, 15 died by suicide. Detailed demographic and clinical characteristics of subjects are shown in Table 1. Demographic data included age, gender, race, postmortem interval (PMI), RNA integrity number (RIN), brain pH, cause of death, history of drug abuse, alcohol abuse, and antidepressant medication at the time of death.

After removal from the cranium, the brains were cut into six major pieces (four cerebral cortical lobes, basal ganglia-diencephalon, and lower brain stem-cerebellum), rapidly frozen on dry ice, and stored at -70°C until dissection. During dissection, the frontal lobes were sliced into 1-mm to 1.5-mm thick coronal sections at a temperature between 0°C to 10°C . To keep the samples frozen, the dissections were performed on a metal plate over a container filled with dry ice. The prefrontal cortical samples were cut out of the coronal sections by a fine microdissecting (Graefe) knife under a stereomicroscope with low magnification. The dorsomedial prefrontal cortex (Brodmann's area 9) was taken just dorsal to the frontopolar area including the most polar portion of the superior and partly the middle frontal gyrus between the superior and intermediate frontal sulci. In the sections of the dissected cortical area, the gray and white matters were separated. The tissues were chopped into smaller pieces and stored at -80°C until use. All tissues from control subjects and MDD

subjects were screened for evidence of neuropathology by experienced neuropathologists. The presence of Alzheimer disease, infarcts, demyelinating diseases, or atrophy disqualified subjects from the study. Fixed sections of tissue were screened with H & E staining, a monoclonal antibody which recognizes amyloid deposits of Alzheimer's type, and an antibody to glial fibrillary acid protein. Toxicology data were obtained by the analysis of urine and blood samples.

At least one family member, after giving written informed consent, underwent an interview based on the Diagnostic Evaluation After Death (DEAD) (Salzman et al 1983) and the Structured Clinical Interview for the DSM-IV (SCID) (Spitzer et al 1995). The interviews were done by a trained psychiatric social worker. Two psychiatrists independently reviewed the write-up from this interview, as well as the SCID that was completed from it, as part of their diagnostic assessment of the case. Diagnoses were made from the data obtained in this interview, medical records from the case, and records obtained from the Medical Examiner's office. The two diagnoses were compared and discrepancies were resolved by means of a consensus conference. Control subjects were verified as free from mental illnesses using these consensus diagnostic procedures. Subjects having a history of major medical, neurological, neuropathological disorders, chronic illness (epilepsy, diabetes), or showing positive HIV are excluded. Subjects who died of illness that might have resulted in toxicity (e.g., liver or kidney dysfunction) or who died as the result of fire are also excluded. Antidepressants, alcohol, substance of abuse determined in blood or urine at the time of death (determined by toxicological screening), and alcohol or drug dependence or abuse (determined by psychological autopsy) were used as covariates. To avoid the confound of agonal state, subjects, who were on life-support measures were excluded.

RNA Isolation:

TRIzol® (Invitrogen, Grand Island, NY, USA) was used to isolate total RNA. The detailed method of RNA isolation has been described earlier (Roy et al., 2017). The RNA samples was screened based on their purity (260/280 cutoff ≥ 1.8) as determined with NanoDrop spectrophotometer (ThermoScientific, Waltham, MA, USA) cutoff ≥ 1.9). The integrity of the RNA samples was determined by agarose gel electrophoresis and the quality was confirmed (RNA Integrity Number; RIN ≥ 7) by using Bioanalyzer instrument (Agilent Tech. Inc, Santa Clara, CA, USA).

cDNA Synthesis:

A total of 500 ng RNA was used to synthesize 1st strand cDNA using M-MLV Reverse Transcriptase (Invitrogen, Grand Island, NY, USA) and oligo (dT) primer. Annealing step was conducted by incubating the reaction at 65°C for 5 min with 5 uM concentration of oligo (dT) primer and 1 mM of dNTPs. Thereafter, the reaction was quickly cooled down at 4°C for at least 2 minutes. Then, 1X First strand synthesis buffer, 0.01 mM DTT, 2U of RNaseOut and 200U of M-MLV were added and let the reaction continue at 37°C for 50 min. Subsequently, the reaction was inactivated at 70°C for 15 min.

qPCR Assays:

The relative transcript abundance for each gene was measured following quantitative real-time PCR method by using qPCR instrument (Stratagene MxPro3005, La Jolla, CA, USA). The reaction was performed with 1X EvaGreen qPCR mastermix (Applied Biological Material Inc., Canada), 0.8 μ M each of gene-specific forward and reverse primers (Table 2). A twenty-fold diluted cDNA as template was used to conduct qPCR with the following protocol: initial denaturation at 95°C for 10 min, a repeating 40 cycles of denaturation at 95°C for 10 sec, primer annealing at 60°C for 15 sec, and an elongation at 72°C for 20 sec. To exclude the possibility of primer dimer formation and secondary product amplification, melting curve analysis was done with an initial denaturation at 72°C for 1 min, annealing at 55°C for 30 sec, and repeat denaturation step at 95°C for 30 sec. All human specific primer sequences used in this study are provided in Table 2. The gene specific expression primers were designed with sequence complementarity close to the 3' end of the coding transcript. GAPDH was selected as housekeeping gene. The change in gene expression was analyzed by calculating the fold change (2^{-Ct}) values (Livak KJ and Schmittgen, 2001).

Statistical Analysis:

Statistical analyses were conducted using SPSS 25.0 software (IBM Tech, Armonk, NY, USA). We used the Shapiro-Wilk test to assess the normality of the data. The average difference of age, PMI, brain pH, and RIN were assessed by Student's t-test. Differences in gender, drug abuse, alcohol abuse, antidepressants, and suicide were analyzed by Fisher's exact test. The student's t-test was used to compare the mRNA expression levels between the two groups. The student's t-test was also used to compare the mRNA expression levels according to gender, race, drug abuse, alcohol abuse, and antidepressants. The difference of UPR gene expressions among control, MDD-non suicide, and MDD-suicide was assessed by one-way ANOVA with post-hoc Bonferroni corrections. Correlations of the mRNA expression levels with age, PMI, RIN, and brain pH were conducted with the Pearson correlation coefficient. Statistical significance was set at the 95% level ($p < 0.05$). The sample size was calculated on the basis of two sample t-tests using G*power 3.1.9.4 software (Faul et al., 2009). An effect size of 0.8 and a significance level of $\alpha = 0.05$ were considered.

Results

Characteristics of Subjects:

The demographic characteristics of control and MDD subjects are provided in Table 1. The power of the study ($1-\beta$) was 0.943. There were no significant differences in age ($t = -0.436$, $df = 56.5$, $p = 0.664$), PMI ($t = 0.587$, $df = 67.4$, $p = 0.559$), brain pH ($t = -0.972$, $df = 37.6$, $p = 0.337$), or race ($p = 0.480$) between control and MDD subjects. There were 16 males and 11 females in the control group and 26 males and 17 females in the MDD group ($p = 1.0$). RIN was also not significantly different between the two groups ($t = 0.041$, $df = 68$, $p = 0.967$). Out of 43, 16 MDD subjects showed positive antidepressant toxicology, 12 had alcohol abuse, and 3 had drug abuse. In addition, 15 MDD subjects had died by suicide.

mRNA Expression of UPR Genes in MDD Subjects:

The level of GAPDH was not significantly different between MDD and control groups ($t = -0.011$, $df = 60$, $p = 0.991$). The mRNA expression levels of GRP78 ($t = -2.719$, $df = 70$, $p = 0.008$), GRP94 ($t = -2.436$, $df = 58.4$, $p = 0.018$), and ATF4C ($t = -2.20$, $df = 55.8$, $p = 0.031$) were significantly higher in dlPFC of MDD subjects compared to control subjects, as shown in Figure 1. The mRNA expression of XBP-1, CHOP, and ATF6C had similar trends as with other three genes, but they did not reach significance level (XBP-1, $t = -1.449$, $df = 54.1$, $p = 0.153$; CHOP, $t = -1.162$, $df = 53.2$, $p = 0.250$; ATF6C, $t = -0.633$, $df = 51.3$, $p = 0.529$). We excluded a few samples because they were identified as potential outliers after conducting qPCR analysis. The number of subjects for each analysis is indicated in Figures 1 and 2.

Effect of Suicide on the Expression of UPR Genes:

To examine the effect of suicide on UPR genes, MDD subjects were divided into those who died by suicide (MDD-suicide) and those who died by causes other than suicide (MDD-non suicide). The three group comparison (MDD-suicide, MDD-non-suicide, and controls) using one-way ANOVA showed overall significant differences in the expression of GRP78 ($F = 5.02$, $df = 64$, $p = 0.010$), GRP94 ($F = 3.35$, $df = 60$, $p = 0.042$), and ATF4C ($F = 3.39$, $df = 67$, $p = 0.040$). Individual comparisons showed that these genes were significantly upregulated in the MDD-suicide group when compared with control group (GRP78: $p = 0.007$; GRP94: $p = 0.041$; ATF4C: $p = 0.037$). No significant differences were noted when MDD-non-suicide group was compared with control subjects (GRP78: $p = 0.449$; GRP94: $p = 0.428$; ATF4C: $p = 0.463$) or MDD-suicide group was compared with MDD-non-suicide group (GRP78: $p = 0.165$; GRP94: $p = 0.633$; ATF4C: $p = 0.491$) (Figure 2).

Effects of Confounding Variables on mRNA Expression Levels of UPR Genes:

None of the UPR genes were affected by race, drug abuse, alcohol abuse, or antidepressant treatment (Table 3). In addition, age, RIN, and brain pH also did not affect UPR genes except there was a negative correlation between age and ATF4C in the MDD group ($r = -0.333$, $p = 0.031$) and positive correlations between RIN and GRP78 in the control group ($r = 0.474$, $p = 0.017$) and brain pH and GRP78 in the MDD group ($r = 0.338$, $p = 0.33$).

Discussion

This is the first study to examine the impact of MDD and suicidality on the expression of UPR genes in dlPFC. All the genes analyzed from the UPR system showed upregulation in dlPFC of MDD subjects compared to control subjects; however, expression levels of only GRP78, GRP94, and ATF4C reached significance level. Other UPR genes (XBP-1, CHOP, and ATF6C) showed the same trend, but were not statistically significant. To examine the effect of suicide, we divided the MDD group into MDD-non suicide and MDD suicide and compared them with control subjects. Interestingly, we found that the expression of GRP78, GRP94, and ATF4C was significantly upregulated only in the MDD-suicide group when compared with controls, but no significant differences were noted when compared with MDD-non-suicide group. In addition, no significant changes were noted when MDD-non-suicide group was compared with controls. These findings suggest that the expression

changes in UPR genes may be associated with suicidality in MDD subjects. Previously, a human postmortem study showed that protein expressions of GRP78 and GRP94 are significantly upregulated in the temporal cortex of MDD subjects (Bown et al., 2000). This upregulation occurred in those MDD subjects who had died by suicide when compared with those who had died by causes other than suicide. The authors speculated that it is unlikely that these differences were the result of suicide itself because no differences were found in patients with bipolar disorder or schizophrenia who had died by suicide. Since the present study was performed only in MDD subjects, it will be interesting to further examine if these changes are restricted to MDD suicide or also occur in suicide subjects irrespective of psychiatric illnesses. In an earlier study, mRNA expression levels of XBP-1 and CHOP were examined in leukocytes of MDD subjects and were found to be upregulated (Nevell et al., 2014). In addition, using preclinical models (learned helpless behavioral and chronic restraint stress), our lab recently reported significant expression upregulation of UPR genes in hippocampus (Timberlake and Dwivedi, 2016; Timberlake et al., 2018). Furthermore, we showed that not only is UPR enhanced in depression, but activation of UPR can induce depression-like behavioral changes in rats (Timberlake et al., 2019). Although, suicidal behavior is difficult to capture in pre-clinical models, hopelessness, one of the endophenotypes of suicidality, is characteristic of learned helplessness. Induction of UPR genes in learned helpless rats may indicate possible association of UPR with suicidality as was noted in our human postmortem brain study.

At functional level, dissociation of ER chaperone GRP78 from IRE1, ATF6C, and PERK initiate the downstream signaling cascade (Shen et al., 2002). ATF4C and ATF6C migrate to the nucleus. ATF4C has key roles in enhancing the transcription of the ATF6C gene and contribute to the trafficking of ATF6C from the ER to the Golgi (Teske et al., 2011). In addition, CHOP, one of ATF4C target genes, relates to managing stress or direct cell fate by regulating apoptosis (Marciniak et al., 2004, Marciniak and Ron, 2006). In the present study, both CHOP and ATF6C expression were higher in dlPFC of MDD subjects (although not significant) compared to control subjects. Earlier, it has been reported that chronic psychological stress in rats (forced ice water swimming) can induce CNS physiology and decreased GRP78 expression and concomitant increase in ATF4C and CHOP expression (Yi et al., 2017). Another study has shown that treatment with fluoxetine can decrease mRNA expression of GRP78 and CHOP in the prefrontal cortex of wild type male C57BL/6J mice (Lu et al., 2018). Other than initiating the UPR cascade, GRP94 also serves as a master regulator of Toll-like receptors (TLRs) (Yang et al., 2007). In the present study, mRNA expression of GRP94 was significantly elevated in MDD subjects. Our group previously reported increased protein-protein interaction between GRP94 and TLRs 2, 4, 7 and 9 in rat brain that showed behavioral depression (Timberlake et al., 2018). Another study showed that the mRNA expression and protein expression of TLR3 and TLR4 was increased in postmortem dlPFC of MDD subjects (Pandey et al., 2014). These studies indicate that elevated mRNA expression of GRP94 in dlPFC may be related to the increased expression of TLRs.

It has been long known that ATF6C and ATF4C-CHOP are directly associated with inflammatory response (Rao et al., 2014; Timberlake et al., 2018; Zhang et al., 2014). An association of depression and inflammation was initially reported by Smith (1991), where it

was shown that macrophages affect MDD pathogenesis. Subsequent studies showed an association between altered pro-inflammatory pathways and MDD pathophysiology (Kim and Maes, 2003). Pro-inflammatory cytokines, such as IL-1 β , TNF- α , and IL-6 are increased in MDD subjects (Dowlati et al., 2010, Kim et al., 2007). These pro-inflammatory cytokines impact monoamines such as serotonin, noradrenaline, dopamine, and amino acid glutamate (Miller and Raison, 2016). In fact, several studies have shown that TNF- α and IL-6 were altered in the postmortem brain of suicide victims (Pandey et al., 2012, Wang et al., 2018). Mechanistically, ER stress can induce apoptosis and the release of TNF- α and IL-6 in macrophage (Feng et al., 2003; Li et al., 2005). It is quite possible that UPR genes can cause MDD pathogenesis via changes in pro-inflammatory cytokines.

There are a few limitations in this study. Firstly, we cannot conclude whether the suicidal effect on the expression changes of UPR genes is MDD-specific. Secondly, we cannot directly compare our results with the previous study that refers to protein expression of UPR genes in temporal lobe of MDD subjects (Bown et al., 2000) because we did not measure protein levels of these genes. Lastly, our previous rat studies used hippocampus to measure UPR gene expressions (Timberlake and Dwivedi, 2016; Timberlake et al., 2018; Timberlake et al., 2019). In the future, further studies need to be conducted in subjects with other psychiatric illnesses with and without suicide. Protein levels of these UPR genes also need to be studied. Although the present study used only qPCR approach, in our earlier preclinical studies, we found that both mRNA and protein expression of UPR genes were similarly affected in rats showing depression-like behavior. In addition, other brain areas need to be included to examine specific changes in UPR genes.

In conclusion, the present study shows that mRNA expression of UPR genes is upregulated in dlPFC of MDD subjects. These changes appear to be related to suicidal behavior, suggesting that ER stress may be one of the underlying causes of suicidality among MDD patients. How UPR is involved in suicidal behavior is not clear at present; however, it appears that cellular stress may lead to UPR activation, which ultimately can activate immune response and apoptosis (Fig. 3). Several studies have shown that UPR is involved in inflammation (Garg et al., 2012) either through direct activation of NF- κ B and subsequent inflammatory cytokine production (Yamazaki et al., 2009; Hotamisligil, 2010) via I κ B degradation, through c-Jun N-terminal Kinase (JNK) hyper-activation (Ozcan et al., 2004), or through increased TLR expression and activity (Timberlake and Dwivedi, 2018; Timberlake M. et al., 2018). It is pertinent to mention that recent studies suggest that pro-inflammatory cytokines are elevated in the brain of suicide subjects (Pandey et al., 2012, Wang et al., 2018). Downstream of UPR activation is regulation and facilitation of apoptosis (Yamaguchi and Wang, 2004; Endo et al., 2006; Meares et al., 2011; Zhang et al., 2014; Timberlake and Dwivedi, 2016), which is also implicated in depression (Dwivedi, 2009; Gold et al., 2013; Timberlake and Dwivedi, 2016; Xiang, 2015). In an earlier study, we showed that inducing UPR pharmacologically can cause depression-like behavior in rats (Timberlake et al., 2019). In the future, it may be possible to do early intervention for suicidal ideation or attempt measuring UPR gene expression in peripheral tissues. Further research will be needed in this area.

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Highlights

- Investigated expression levels of UPR genes in dlPFC of MDD subjects.
- Elevated mRNA expression levels of GRP78, GRP94, *and* ATF4C in MDD suicide.
- ER stress may be one of the causes associated with suicidality among MDD patients.

Figure 1:

Expression of UPR genes in dorsolateral prefrontal cortex of MDD and nonpsychiatric control subjects. UPR genes mRNA expression levels between Ct and MDD subjects: a) *GRP78*, b) *GRP94*, c) *XBP-1*, d) *CHOP*, e) *ATF4C*, f) *ATF6C*. The horizontal line and error bars represent the mean $\pm$ SEM. Ct, control subjects; MDD, Major Depressive Disorder

Figure 2:

Effect of suicide on the expression of UPR genes. Comparison of UPR genes among control, MDD-no suicide, and MDD-suicide groups: a) *GRP78*, b) *GRP94*, c) *XBP-1*, d) *CHOP*, e) *ATF4*, f) *ATF6C*. The horizontal line and error bars represent the mean $\pm$ SEM. Ct, control subjects; MDD-NS, Major Depressive Disorder-non suicide; MDD-S, Major Depressive Disorder-suicide.

Figure 3: Schematic diagram showing the possible role of ER stress in MDD pathogenesis and suicidality. Elevated expression of GRP78, GRP94, and ATF4 may activate immune response and apoptosis, which may possibly be involved in MDD pathogenesis and suicidality.

Table 1.

Demographic and clinical characteristics of control and MDD subjects

		Control	MDD	P-value
Number of subjects		27	43	N/A
Age		48.4 ± 17.0	50.3 ± 17.5	0.664
Gender	Male	16	26	1.0
	Female	11	17	
Postmortem interval (hour)		18.7 ± 4.9	17.9 ± 7.2	0.559
RNA integrity number		7.9 ± 0.7	8.0 ± 0.5	0.967
Brain pH		6.6 ± 0.7	6.8 ± 0.4	0.337
Race (white: black :other)		26: 1: 0	38: 4: 1	0.480
Drug abuse		0	3	N/A
Alcohol abuse		0	12	N/A
Antidepressant drugs		0	16	N/A
suicide		0	15	N/A
Cause of death		Accidental chest injury, ASCVD, Cancer, Cardiac failure, Cardiomegaly, Complications due to diabetes, CO poisoning, Drowning, Drug overdose, Electrocution, Embolism, Fatty liver, GSW, Hanging, Hemopericardium, ketoacidosis, Leukemia, Liver failure, Lymphoma, Morbid obesity, MVA, Pneumonia, Respiratory failure, Seizure, Undetermined		

Values denote mean ± standard deviation. MDD: Major Depressive Disorder, N/A: not applicable, ASCVD: Atherosclerotic Cardiovascular Disease, CO: carbon monoxide, GSW: gunshot wound, MVA: motor vehicle accident.

Table 2.

Primer sequences for mRNA expression analysis

Primers		Sequences
GRP78	Forward	AGCCACCAAGATGCTGACAT
	Reverse	GGCCTGCACTTCCATAGAGTT
GRP94	Forward	AGACTTGTTTTGGATGCCCC
	Reverse	TCCTGTGACCCATAATCCCAC
XBP-1	Forward	TGGATACGGGGGTTCCTTTT
	Reverse	GGCAAAAGTGTCTCCCAAGA
CHOP	Forward	ACACTACCCACCTTCCCAGA
	Reverse	CTGCGTATGTGGGATTGAGG
ATF4	Forward	TTAAGCCATGGCGCTTCTCA
	Reverse	TCATTTTCGGTCATGTTGCGG
ATF6	Forward	TCAACTTTTGTGAGCGGGGA
	Reverse	AATCTAAGGGGAGTGCTGCG
GAPDH	Forward	CCACATCGCTGAGACACCAT
	Reverse	AGTTAAAAGCAGCCCTGGTGA

Table 3.

Correlations of confounding variables with the expression of UPR genes.

Variables	Group	UPR Genes					
		GRP78	GRP94	XBP-1	CHOP	ATF4C	ATF6C
Age	MDD	0.215	0.991	0.689	0.508	*0.031	0.521
	Ct	0.085	0.857	0.191	0.868	0.113	0.437
Gender	MDD	0.885	0.323	0.701	0.400	0.816	0.247
	Ct	0.635	0.974	0.358	0.658	0.705	0.503
PMI	MDD	0.810	0.644	0.130	0.744	0.282	0.425
	Ct	0.656	0.508	0.832	0.735	0.522	0.532
RIN	MDD	0.115	0.661	0.616	0.800	0.163	0.354
	Ct	*0.017	0.190	0.090	0.156	0.396	0.478
pH	MDD	*0.033	0.456	0.820	0.229	0.179	0.107
	Ct	0.273	0.854	0.346	0.284	0.147	0.795
Race	MDD	0.134	0.348	0.386	0.289	0.264	0.599
Drug abuse	MDD	0.846	0.565	0.903	0.901	0.740	0.876
Alcohol abuse	MDD	0.697	0.463	0.708	0.715	0.669	0.762
Antidepressants	MDD	0.972	0.412	0.983	0.871	0.959	0.667

Values denote p value. PMI: postmortem interval, RIN: RNA integrity number, MDD: Major Depressive Disorder, Ct: control. The asterisk indicates a statistically significant correlation ($p < 0.05$).