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Endoplasmic reticulum stress in bipolar disorder? – *BiP* and *CHOP* gene expression- and *XBP1* splicing analysis in peripheral blood

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ABSTRACT

Background: Endoplasmic Reticulum stress activates the Unfolded Protein Response, which is partially impaired in Bipolar Disorder (BD) according to previous *in-vitro* studies. Thus, *BiP* and *CHOP* gene expression and *XBP1* splicing were analyzed in peripheral blood of study participants with BD and controls.

Methods: RNA was isolated from fasting blood of study participants with BD (n = 81) and controls (n = 54) and reverse transcribed into cDNA. *BiP* and *CHOP* gene expression was analyzed with quantitative RT-PCR. Atypical splicing of *XBP1* mRNA was measured by semi-quantitative RT-PCR, gel-electrophoresis and densitometry. ANCOVAs with the covariates age, BMI, sex, lithium and anticonvulsants intake were used with SPSS. Bonferroni correction was used to correct for multiple testing (adjusted $p = 0.0083$).

Results: *BiP* gene expression was significantly higher in BD than in controls ($F(1/128) = 10.076$, $p = 0.002$, Partial $\eta^2 = 0.073$). Total *XBP1* ($F(1/126) = 9.550$, $p = 0.002$, Partial $\eta^2 = 0.070$) and unspliced *XBP1* ($F(1/128) = 8.803$, $p = 0.004$, Patial $\eta^2 = 0.065$) were significantly decreased in BD. Spliced *XBP1* ($F(1/126) = 5.848$, $p = 0.017$, Partial $\eta^2 = 0.044$) and the ratio spliced *XBP1*/ unspliced *XBP1* did not differ between BD and controls ($F(1/126) = 0.599$, $p = 0.441$, Partial $\eta^2 = 0.005$). Gene expression did not differ between euthymia, depression and mania.

Discussion: *BiP* gene expression was significantly higher in BD compared to controls. Total and unspliced *XBP1* were significantly lower in BD than in the control group. Thus, both genes may be considered as putative trait markers. Nevertheless, *XBP1* splicing itself did not differ between both groups.

1. Introduction

Bipolar Disorder (BD) is characterized by mood swings between euphoria and depressed mood (Rothenhäusler, 2007). The exact mechanisms of these mood swings are still cryptic and must be further elucidated. There is growing evidence from previous *in-vitro* studies that the Endoplasmic Reticulum (ER), the protein folding factory of the cell, plays a major role in BD (Pfaffenseller et al., 2014; Hayashi et al., 2009; So et al., 2007). The membranous ER network is essential, because it shelters ribosomal protein synthesis, stores calcium for signal transduction and is responsible for the correct folding of proteins. Misfolding or unfolding of proteins induce toxic ER stress in the cell and is principally provoked by several conditions. ER stress inducing conditions include depletion of calcium, increased reactive oxygen species (ROS), folding-defective gene polymorphisms, viral infection, hypoxia, energy depletion or hyperglycaemia and obesity (Volk et al., 1997; Gregersen, 2010). ER stress was furthermore associated with a variety of medical diseases e.g. neurodegenerative disorders (Zhang, 2008; Hetz, 2014).

To avoid blockage of the ER by unfolded protein chains and to restore normal cell functions, rescue mechanisms of the Unfolded Protein Response (UPR) are activated by ER stress. UPR rescue mechanisms either restore the functionality of the ER or induce cell death as fatal consequence if cell damage is irreversible (Zhang, 2008). The UPR

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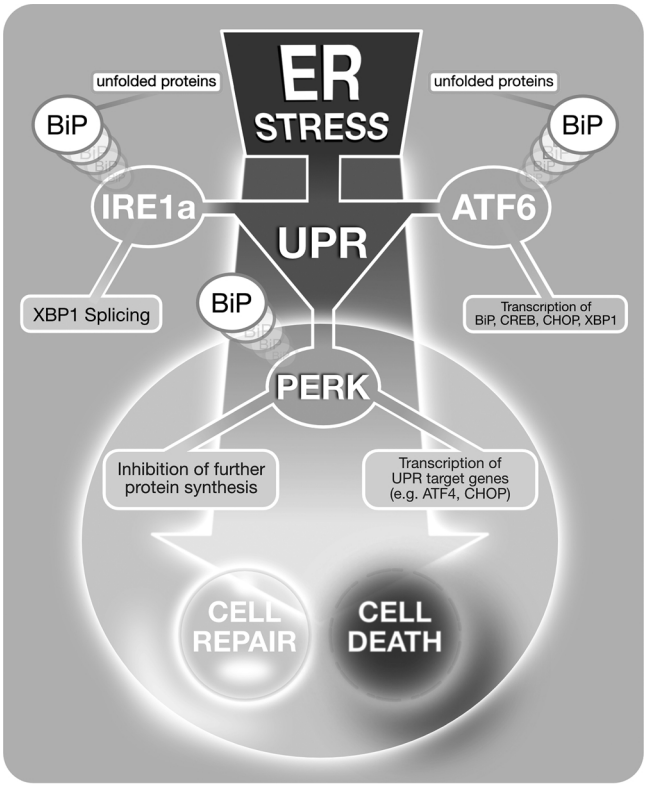

Fig. 1. Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR). Diverse unfavorable conditions induce unfolding or misfolding of proteins resulting in toxic ER stress in the cell. ER stress activates rescue mechanisms of the UPR that either save the cell or activate apoptosis. The major players of the UPR include *ATF6, BiP*, *CHOP, IRE1α*, *PERK* and *XBP1*. BiP is associated with the three important ER transmembrane proteins IRE1α, PERK and ATF6. The chaperone BiP is able to bind unfolded protein chains and acts as an "ER stress sensor". Then unfolded proteins induce dissociation of BiP from the transmembrane proteins. Dissociation of BiP activates the proteins IRE1α, PERK, ATF6. The three activated UPR pathways induce *XBP1* splicing, inhibition of further protein synthesis and transcription of UPR target genes (e.g. *ATF4*, *BiP*, *CHOP*, *CREB*, *XBP1*). All mentioned rescue mechanisms give the cell a break to recover from ER stress; this either saves the cell or activates cell death. Abbreviations: *ATF4*… Activating transcription factor 4; *ATF6*… Activating transcription factor 6; *BiP*… Binding immunoglobulin protein, syn. *HSPA5*, *GRP78*; *CHOP*… encodes a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors, syn. *GADD153*, *DDIT*; *CREB*… cAMP responsive element binding protein; ER… Endoplasmic Reticulum; *IRE1α*… Inositol Requiring Enzyme 1 α, splices and activates *XBP1*; *PERK*… PKR-like ER kinase, *XBP1*… X-box binding protein 1; UPR… Unfolded Protein Response. Gene… italic, Protein… not italic. Copyright: The Fig. was designed by Wolfgang Krasser.

comprises several pathways, which consequently degrade unfolded proteins, inhibit translation and upregulate protective protein folding chaperones. Those rescue mechanisms give the cell a break to recover from ER stress (Han et al., 2013; Li et al., 2013a). The UPR comprises three major signaling cascades initiated by three ER transmembrane receptors- see Fig. 1 (Bengesser et al., 2016a; Zhang, 2008; Roussel et al., 2013; Ron, 2007), namely the Inositol-Requiring Protein-1α (IRE1α), PKR-like ER kinase (PERK) and Activating Transcription Factor 6 (ATF6). The three ER stress receptors IRE1α, PERK and ATF6 are principally associated with the chaperone and "ER stress sensor" BiP (Binding immunoglobulin protein), which keeps the receptors in an inactive state. ER stress leads to dissociation of BiP from the ER transmembrane receptors, which activates the three UPR rescue pathways. As mentioned above, *IRE1α*, *PERK* and *ATF6* activated pathways either subsequently protect the cell by restoring the functionality of the ER or induce cell death (Zhang, 2008).

The UPR comprises several cascades of biological pathways downstream of IRE1α, PERK and ATF6, which are activated by dissociation of BiP. Firstly, activated IRE1α excises a 26 bp atypical intron from the *XBP1* pre-mRNA. Spliced *XBP1* encodes for an active transcription factor controlling the expression of several UPR target genes (Van Schadewijk et al., 2012). Secondly, following the dissociation of BiP, ATF6 induces the transcription of the genes *BiP*, *CREB*, *CHOP* and *XBP1*. Thirdly, PERK activation leads to a protein synthesis break by phosphorylation of the Eukaryotic Initiation Factor of Translation 2α (eIF2α). The break of protein synthesis prevents the ER from blockage by nascent protein chains and unfolded proteins (Zhang, 2008). PERK also activates the transcription of *CHOP* that is a transcription factor controlling the expression of mainly pro-apoptotic factors (Huang et al., 2014). Summarized, the UPR rescue mechanisms give the cell time to recover from ER stress or to perform apoptosis if the cell damage is irreversible.

Dysregulation of ER stress related pathways have been discussed by several authors in BD (Bengesser et al., 2016a). Diverse *in-vitro* studies investigating the UPR at mRNA or protein level have been conducted. Most studies showed an impaired response to ER stress inducing agents in cultured cells derived from individuals with BD. Especially *CHOP* expression and *XBP1* splicing seemed to be impaired in BD after induction of ER stress (Pfaffenseller et al., 2014; Hayashi et al., 2009; So, Warsh et al. 2007). Hypothesis driven gene association studies also underline the importance of ER related genes in BD. One Japanese gene association study showed a nominal significant association of a *BiP* haplotype with BD (Kakiuchi et al., 2005). The *XBP1* (−116C→G) gene variant, located in the promoter region of *XBP1*, was associated with BD as well (Kakiuchi et al., 2003). Another ER related gene, namely *LMAN2L*, is associated with BD pathogenesis. Two *LMAN2L* gene variants (rs2271893, rs67468962) were genome-wide associated with BD ($p < 5 \times 10^{-8}$) in a meta-analysis of genome wide association studies (GWAS) in a combined sample of more than 14 000 subjects of European and Asian-ancestry (Chen et al., 2013). Even though *LMAN2L* was not associated with ER-stress or the UPR so far, polymorphisms in the *LMAN2L* gene could principally favor ER stress, because it encodes for an ER transmembrane protein that transports glycosylated proteins. Putative malfunctions of the transmembrane protein could hypothetically lead to accumulation of glycosylated proteins in the ER (Wang, Groenendyk et al. 2015).

In summary, there are many hints in the literature that the pathogenesis of BD is somehow linked to a disturbed homeostasis of the ER. Nevertheless, gene expression studies of ER stress associated genes in peripheral blood of individuals with BD and controls in a sufficiently large sample are sparse. There are only rare gene expression studies of twins disconcordant for BD, but with rather small sample sizes (Matigian et al., 2007). In order to close this gap, we assessed gene expression of ER-stress associated genes *BiP* and *CHOP* in mononuclear cells of the peripheral blood from patients with BD and controls by qPCR. Furthermore, we assessed atypical splicing of *XBP1* mRNA by classical semi-quantitative RT-PCR. We aimed to investigate differences in ER related mechanisms between individuals with BD and controls (I. trait) as well as between affective states in BD (II. state). To the best of our knowledge, we investigated for the first time whether expression of ER-stress related genes (*BiP*, *CHOP* and *XBP1*) differ between the affective episodes euthymia, depression and mania in BD.

2. Methods

2.1. Description of sample

Austrian Caucasian individuals were recruited within the BIPGEN and BIPFAT studies, which were described previously (Bengesser et al., 2016c, b, 2015, Reininghaus et al., 2013). Gene expression was analyzed in 81 study participants with BD and 54 healthy controls. Individuals with BD were former in- or outpatients of the Medical