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Stress impairs response to antidepressants via HPA axis and immune system activation

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ABSTRACT

Childhood trauma as well as severe events occurring later in life have been associated with the development of major depressive disorder (MDD). However, the interaction of early and later occurring adverse events in patients with MDD is understudied. This study aims to disentangle this interaction by investigating the effects on two of the main stress-response systems of the body, the hypothalamic–pituitaryadrenal (HPA-) axis and the immune system in depressed patients.

The function of the HPA-axis was assessed by measuring FKBP5, SGK1 and NR3C1 mRNA-expression in peripheral blood after an in vivo glucocorticoid receptor (GR) challenge with 1.5 mg dexamethasone in 150 depressed in-patients (47.4% females). Childhood trauma was evaluated using the Childhood Trauma Questionnaire (CTQ), severe life events occurring one year prior to hospital admission were assessed with the List of Threatening Experiences (LTE).

Multiple childhood traumata, i.e. ≥ 3, were present in 68 (45.5%) patients, 59 (39.3%) experienced ≥ 3 severe recent life events. The history of ≥ 3 severe recent life events was associated with an impaired GR-induction of SGK1 ($F = 10.455$; $df = 1$; $p = 0.002$) and FKBP5 mRNA expression ($F = 8.720$; $df = 1$; $p = 0.004$), and with elevated measures of the immune system such as CRP and lymphocyte count. In addition, severe recent life events were associated with a substantially impaired treatment response to antidepressants ($F = 7.456$; $df = 1$; $p = 0.008$). These effects could not be observed in relation to childhood trauma.

Severe life events occurring prior to MDD development substantially impaired the stress-response systems and the response to treatment with antidepressants. This finding may indicate the need to employ additional treatment options such as psychotherapy right at the beginning of treatment or immune-modulating approaches.

1. Introduction

Stress is usually defined as a condition of real or perceived threat to homeostasis (Smith and Vale, 2006). The brain identifies threatening stressors and activates behavioral and physical responses that do not only promote the adaption to the stressors, the allostasis, but also contribute to the development of stress-related disorders when these systems are overused or dysregulated, named as allostatic load (McEwen, 2013, 2017). Thus, stress has undoubtedly been associated with substantial detrimental effects on mental and physical health. Especially stress in the form of childhood trauma may lead to a plethora of health conditions across the lifespan (Nusslock and Miller, 2016). Individuals

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subject to childhood trauma have a higher risk to develop stress-related mental disorders such as major depressive disorder (MDD) and substance abuse (Teicher and Samson, 2013), but also have an increased prevalence of medical conditions such as coronary heart disease, metabolic syndrome and autoimmune conditions (Danese and McEwen, 2012; Shonkoff et al., 2009). These observations have been supported by a wealth of experimental research with preclinical models of early life adversity showing long-term consequences of early stressor exposure (Avitsur et al., 2006; Lupien, 2009; Andersson et al., 2009). These long-term sequelae of early life adversity may derive from a dysregulation of the stress-responding systems in the body, with a possible impaired crosstalk of the neuro and immune systems (Nusslock and Miller, 2016; McEwen, 2004; Kuhlman, 2017). In fact, an up-regulation of the stress-hormone-system, i.e. the hypothalamic–pituitaryadrenal (HPA) axis (Heim and Nemeroff, 2001; Gunnar and Quevedo, 2007) and the inflammatory arm of the immune system (Carpenter, 2010; Danese, 2007) are thought to be responsible for the development of stress-related illness (McEwen, 2013).

Childhood trauma has also been associated with severe life events later in life (Muller, 2019). Several studies suggested that childhood trauma increases vulnerability to the effects of stressful events occurring later in life, in terms of a stress sensitization model (Hammen et al., 2000; Espejo, 2007; Kendler et al., 2004). In addition, converging evidence links severe life events such as loss of employment, chronic or life-threatening health problems, separation, financial insecurity, exposure to violence or bereavement with the development of stress-related mental disorders, with and without a history of childhood trauma (Motrico, 2013; Kessler, 1997). Generally, a dose–response relationship between severity and number of adverse life events and the risk, severity and response to treatment of MDD has been reported (Li et al., 2016).

While it is likely that childhood trauma and severe life events later in life both contribute to the development and treatment responsiveness of stress-related disorders, especially MDD, the mechanisms of this interaction are severely understudied (Ebner and Singewald, 2017). Next, individual differences in subjects exposed to adverse events that arise from a genetic susceptibility further complicate the disentanglement of specific single contributions (Ebner and Singewald, 2017; Halldorsdottir and Binder, 2017).

Therefore, this study aims to investigate the specific contributions of severe recent life events and childhood trauma on two of the main stress-response systems of the body, i.e. the HPA axis and the immune system (Nusslock and Miller, 2016; Danese and Lewis, 2017), in a sample of patients with MDD. The HPA axis is activated when a subject is exposed to a threat, which leads to the release of glucocorticoids (GCs) from the adrenal gland. GCs reach via the systemic blood flow every organ of the body, including the brain. GCs exert their effects by activating glucocorticoid receptors (GRs, encoded by *NR3C1*), that serve as transcription factors and bind to specific DNA sequences and thus regulate transcriptional responses (McEwen, 2004; Leistner and Menke, 2018). The heat shock protein-90-associated co-chaperone FK506 binding protein 51 (FKBP51), encoded by *FKBP5*, substantially regulates the sensitivity of GR and has been implicated in both sequelae of childhood trauma and MDD (Matosin et al., 2018; Binder, 2009). Downstream from GR the serum glucocorticoid kinase-1 (SGK1) has been attributed to stress-related changes in preclinical and clinical observations (Cattaneo and Riva, 2015; Anacker, 2013). Although GR, FKBP51 and SGK1 form a molecular hub responding to stress, the interplay of these components in dependency of adverse events has not been investigated in clinical samples of depressed patients. Therefore, we analyzed the gene expression of NR3C1, FKBP5 and SGK1 before and after a pharmacological stress challenge in vivo with dexamethasone (Leistner and Menke, 2018) in depressed patients in dependence of psychological stress, i.e. childhood trauma and severe life events one year prior to development of depression. In addition, we investigated the immune system by analyzing the C-reactive protein (CRP) and differential blood count before and after dexamethasone ingestion.

2. Material and methods

2.1. Recruitment of patients

We recruited 150 patients who were admitted as inpatients to the Department of Psychiatry, Psychosomatics and Psychotherapy of the University Hospital of Wuerzburg, Germany, for treatment of a depressive episode. All patients were of Caucasian descent, mean age was 45.86 ± 14.67 SD, 47.4% were female. Diagnosis of MDD was determined by a structural clinical interview according to DSM-IV criteria [SCID-I, 32]. Study participants were enrolled within the first 2–5 days after admission. They were treated with antidepressants according to doctor‘s choice within a naturalistic setting. Severity of depressive symptoms was evaluated at admission and then weekly by trained raters using the 21-item Hamilton Depression Rating Scale (HAMD). Patients who fulfilled the criteria for at least a moderate depressive episode (HAMD $\geq$ 14) were eligible. Response to treatment was defined as HAMD reduction $\geq$ 50% after 4 weeks of treatment. Exclusion criteria were severe neurological or general medical conditions, or treatment with systemic glucocorticoids. Blood was collected for the measurement of inflammatory markers, liver enzymes, renal function and coagulation. Vital signs such as ECG, pulse and blood pressure were also monitored. This study was approved by the ethics committee of the University Hospital of Wuerzburg (vote no. 128/15), written informed consent was obtained from all participants.

2.2. Evaluation of adverse events

Adverse events one year prior to admission were assessed with the List of Threatening Experiences (LTE) of Brugha et al. (Brugha, 1985). The questionnaire includes 12 items covering adverse events such as illness or injury, separation or marital difficulties, unemployment and financial problems. Adverse events in childhood were evaluated using the childhood trauma questionnaire (CTQ). This scale comprises 5 subscales for physical, sexual and emotional abuse as well as physical and emotional neglect (Hauser, 2011). According to Häuser et al. childhood trauma was acknowledged with mild/moderate severity (Hauser, 2011). Recent data suggested that 3 and more adverse events lead to substantial detrimental sequelae compared to individuals exposed to<3 events, therefore we compared patients exposed to 3 and more adverse events (i.e. multiple events / trauma respectively) with patients exposed to <3, childhood trauma as well as adverse events one year prior to admission (McLaughlin, 2010).

2.3. Study design

The pharmacological GR challenge test was performed within the first 7 days after admission in n = 150 patients. Unstimulated peripheral blood samples were collected at 18:00 after 2 h of fasting and abstention from caffeine containing beverages and physical activity, then 1.5 mg of dexamethasone was ingested orally as previously described (Leistner and Menke, 2018; Menke, 2012, 2016). The second blood sample was obtained 3 h later at 21:00. Blood was taken for the measurement of differential blood count, cortisol, ACTH and RNA retrieval using PAXgene™ tubes. Cortisol and ACTH concentrations were assessed by Immulite 2000 (Immulite 2000, Siemens, Erlangen, Germany) and differential blood counts were performed by flow cytometry in the central laboratory of the University Hospital of Wuerzburg. This routine was repeated 4 weaks after treatment.

2.4. RNA extraction and quality assessment

Total RNA was isolated from PAXgene™ whole blood samples by PAXgene™ blood RNA kits (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. RNA concentrations and quality were evaluated using NanoDrop 1000 (Peglab, Erlangen, Germany) and

electrophoresis with 1 kb ladder (Fermentas, ThermoFisher, Scientific, Waltham, Massachusetts, USA) as well as Bio-Rad Experion™ automated electrophoresis system using Experion™ RNA analysis kit (Bio-rad, Hercules, CA, USA).

2.5. Quantitative real-time polymerase chain reaction (PCR)

After quality control RNA from n = 145 patients was reverse-transcribed using the Bio-Rad iScript™ cDNA synthesis kit (Biorad, Hercules, CA, USA) to 1 mg RNA of each sample. cDNA was quantified in duplicates using the Bio-Rad CFX384 real-time PCR detection system, with the iQTM SYBR green supermix from Bio-Rad as well as specific FKBP5, NR3C1 and SGK1 Primer from Sigma-Aldrich, Merck, Darmstadt, Germany. Relative mRNA expression levels from FKBP5, NR3C1 and SGK1 were calculated with mean efficiencies obtained from the LinRegPCR program (Ruijter, 2013) and the normalization factors based on the reference genes RS27 A, and ALAS and SDHA.

2.6. DNA preparation, SNP selection and genotyping

DNA was extracted from EDTA blood. Quality and concentration were assessed with spectrophotometric measurement with Nanodrop 1000 (Peglab, Erlangen, Germany). The *FKBP5* SNP rs1360780 was selected as the tag SNP for the reported association of a *FKBP5* haplotype with stress-related diseases and evidence that this SNP functionally influences FKBP5 expression according to our previous work (Klengel, 2013; Menke, 2013, 2018). All primer sequences and assay protocols are available upon request.

2.7. Statistical analysis

General linear models (GLM) with and without repeated measures analysis were employed to assess the effects of adverse events 1 year prior to admission and in childhood and their interaction on FKBP5, NR3C1 and SGK1 gene expression levels as well as cortisol and ACTH before and after stimulation with dexamethasone. For the repeated measures ANOVAs the statistics are reported for the Greenhouse-Geisser test. All analyses were adjusted for age and gender, as well as childhood trauma or severe life events as applicable. FKBP5 mRNA expression was also adjusted for the rs1360780 risk allele carrier status due to the previously observed association with the GR-induced FKBP5 RNA expression (Klengel, 2013; Menke, 2013, 2018). Demographic data, medication and other clinical variables were compared between both groups with Fisher exact, Pearson chi-square or t tests. All analyses were performed using SPSS for Windows (Release 25, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Sample

Multiple, i.e. ≥ 3 life events 1 year prior to admission were reported by 59 patients (39.3%), 91 patients (60.7%) reported<3 severe life events 1 year prior to admission (see Fig. 1 A). Multiple (≥3) childhood traumata were discerned in 68 patients (45.5%), 82 patients reported<3 different childhood traumata (54.5%; see Fig. 1 B). Table 1 depicts the comparison of sociodemographic and clinical characteristics between patients with multiple severe recent life events / childhood trauma against patients without multiple events. Patients with multiple severe recent life events displayed more suicide attempts (44% vs. 24%, p = 0.032) and a higher BMI (31.3 vs. 27.9; p = 0.024) than patients without multiple events. Severity of depression was significantly correlated with severe recent life events, as measured with the BDI (r = 0.358; p < 0.001) and the HAMD (r = 0.199; p = 0.004). The CTQ score was also correlated with the severity of depression (BDI: r = 0.295; p < 0.001 / HAMD r = 0.146; p = 0.039).

3.2. Relation between severe life events and childhood trauma

There was a modest correlation of the total CTQ with LTE score (R = 0.186; p = 0.012). When observing the subtypes of the CTQ, there were correlations of physical neglect (r = 0.287; p < 0.001), emotional neglect (r = 0.148; p = 0.026) and emotional abuse (r = 0.239; p = 0.001) with the LTE score. Overall, patients with multiple childhood traumata experienced more severe life events than patients without multiple childhood traumata, however, this difference was not significant (2.56 vs. 2.04; p = 0.063). To identify factors influencing the variance of the number of severe life events, we used a stepwise regression analysis with the independent variables sex, age, CTQ total score, number of different traumata, history of physical or emotional neglect, history of physical, sexual or emotional abuse, duration of illness, number of episodes, age at onset, living status and employment status. Employment status significantly contributed and explained 14.5% of the variance of severe life events (R = 0.381;p < 0.001). In addition, age explained 5.4% of the variance (p = 0.006) and physical neglect explained 5.2% of the variance (p = 0.005).

3.3. Impact of severe recent life events on the molecular stress-hub FKBP5, SGK1 and GR

A repeated measures GLM adjusted for age, sex and childhood trauma revealed a significant interaction effect of multiple severe recent life events and *FKBP5* rs1360780 carrier status on FKBP5 mRNA expression after dexamethasone administration (F = 8.720; df = 1; p =

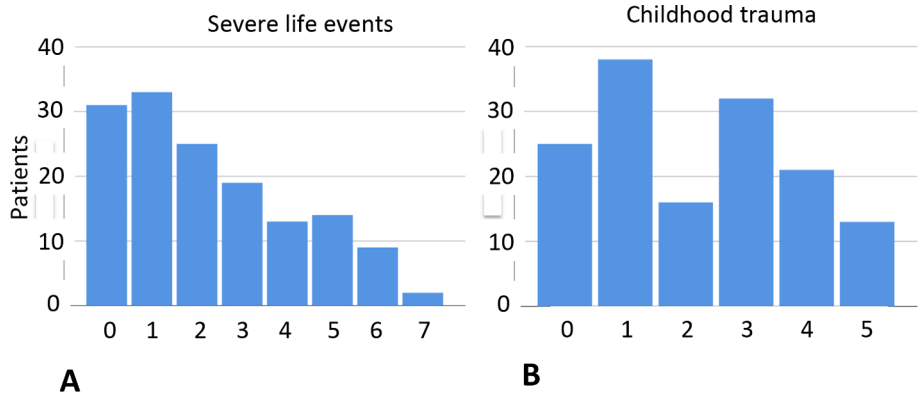

Fig. 1. (A) Distribution of the severe life events one year prior to hospital admission, n = 59 patients experienced 3 and more life events, n = 91<3 or none. (B) Distribution of different types of childhood trauma (i.e. sexual abuse, physical abuse, emotional abuse, physical neglect and emotional neglect), n = 68 experienced 3 and more different types of childhood trauma, n = 82<3 or none.

Table 1
Sociodemographic and clinical characteristics of patients (n = 150) and their association with severe recent life events and childhood trauma. Patients with 3 or more events were classified as subject to multiple events.

Characteristics	Multiple severe life events					Multipe childhood trauma				
	Yes		No		p-value	Yes		No		p-value
	n = 59 (39.3%)		n = 91 (60.7%)			n = 68 (45.5%)		n = 82 (54.5%)		
Sex and age					NS					0.016
Female N (%)	26	(44.1)	49	(53.8)		41	(60.3)	33	(40.2)	
Male N (%)	33	(55.9)	42	(46.2)		27	(39.7)	49	(59.8)	
Age (±SD)	43.0	(±14.5)	47.9	(±14.0)	NS	45.8	(±13.8)	46.3	(±15.0)	NS
Marital Status					NS					NS
Marrried N (%)	22	(37.3)	42	(46.2)		28	(41.2)	36	(43.9)	
Partnership N (%)	7	(11.9)	8	(8.8)		5	(7.4)	10	(12.2)	
Single N (%)	16	(27.1)	24	(26.4)		16	(23.5)	24	(29.3)	
Separated N (%)	12	(20.3)	15	(16.5)		17	(25.0)	10	(12.2)	
Unknown N (%)	2	(3.4)	2	(2.1)		2	(2.9)	2	(2.4)	
Employment Status					0.001					NS
Employed N (%)	20	(33.8)	56	(61.5)		36	(52.9)	40	(48.8)	
Unemployed N (%)	17	(28.8)	2	(2.2)		12	(17.6)	7	(8.5)	
Retired N (%)	8	(13.6)	19	(20.9)		13	(19.1)	14	(17.1)	
Education N (%)	5	(8.5)	11	(12.1)		6	(8.9)	10	(12.2)	
Unknown N (%)	9	(15.3)	3	(3.3)		1	(1.5)	11	(13.4)	
Clinical variables										
Age at onset (±SD)	28.5	(±13.8)	33.7	(±14.9)	NS	29.5	(±14.0)	33.7	(±15.1)	NS
Number of episodes (±SD)	20.0	(±33.2)	12.6	(±25.6)	NS	19.4	(±31.9)	12.3	(±26.0)	NS
Number of hospitalizations (±SD)	1.8	(±2.1)	1.4	(±2.1)	NS	2.0	(±2.4)	1.1	(±1.6)	0.046
History of suicide attempts N (%)	26	(44.1)	22	(24.2)	0.032	24	(35.3)	24	(29.3)	NS
Length of illness in years (±SD)	14.0	(±10.9)	14.0	(±12.0)	NS	15.8	(±11.2)	12.6	(±11.6)	NS
Family history of mental disorders N (%)	42	(71.2)	66	(72.5)	NS	52	(76.5)	56	(68.3)	NS
Family history of depression N (%)	36	(61.0)	61	(67.0)	NS	46	(67.6)	51	(62.2)	NS
Smoking N (%)	22	(37.3)	23	(25.3)	NS	21	(30.1)	24	(29.3)	NS
Body Mass Index (BMI)	31.3	(±11.7)	27.9	(±12.9)	0.024	29.7	(±8.3)	28.1	(±7.6)	NS

0.004; see Fig. 2, A,B). A post hoc analysis revealed that CC homozygotes exposed to multiple severe recent life events showed significantly lower FKBP5 mRNA expression after dexamethasone administration than those without multiple severe recent life events ($F = 12.388$; $df = 1$; $p = 0.001$). This pattern could not be observed for T allele carriers. Post dexamethasone, FKBP5 mRNA expression was negatively correlated with the LTE score ($r = -0.150$; $p = 0.038$) and positively correlated with SGK1 mRNA expression ($r = 0.339$; $p < 0.001$).

Next, we analyzed SGK1 mRNA expression before and after dexamethasone administration. A repeated measures GLM revealed a significant main effect of multiple severe recent life events on SGK1 expression ($F = 10.455$; $df = 1$; $p = 0.002$; see Fig. 2, C). In patients without multiple life events an induction of SGK1 mRNA expression following dexamethasone intake was observed, but not in patients exposed to multiple life events. A post hoc analysis revealed lower SGK1 mRNA expression before and after dexamethasone administration in patients with multiple severe recent life events compared to those without (18:00 $F = 5.511$; $df = 1$; $p = 0.02$; 21:00 $F = 9.113$; $df = 1$; $p = 0.003$). SGK1 mRNA expression after dexamethasone administration was negatively correlated with the LTE score ($r = -0.228$; $p = 0.003$).

Upstream of SGK1 and FKBP5 we analyzed the NR3C1 mRNA expression before and after dexamethasone administration. However, using a repeated measures GLM we failed to detect significant interaction or main effects of multiple severe life events on the gene expression ($F = 1.000$; $df = 1$; $p = 0.319$).

Significant alterations dependent on multiple severe recent life events could not be observed for cortisol or ACTH concentrations before or after dexamethasone administration.

3.4. Impact of severe recent life events on the molecular stress-hub FKBP5, SGK1 and GR after 4 weeks of treatment with antidepressants

After 4 weeks of treatment with antidepressants the observed alterations of gene expression patterns of FKBP5 and SGK1 at admission had normalized. Using repeated measures GLM, there were no significant effects of multiple life events on FKBP5 or SGK1 gene expression ($F = 2.567$; $df = 1$; $p = 0.112$ and $F = 2.599$; $df = 1$; $p = 0.110$, respectively). Of note, the gene expression patterns of FKBP5 and SGK1 at week 4 were independent of treatment response. In addition, severity of depression measured with HAMD and BDI was not correlated with FKBP5, SGK1 or GR gene expression, neither at baseline nor 4 weeks after treatment with antidepressants. Table 2 depicts a comparison between treatment responders vs. non-responders.

3.5. Impact of severe recent life events on the immune system

Patients exposed to multiple severe recent life events showed significantly higher CRP concentrations than those without multiple life events (0.53 ± 0.81 vs. 0.26 ± 0.34; $F = 10.686$; $df = 1$; $p = 0.001$). Additionally, a repeated measures GLM revealed a significant main effect of multiple severe recent life events on lymphocytes, with higher counts in patients exposed to multiple severe recent life events ($F = 4.647$; $df = 1$; $p = 0.033$; see Fig. 2, D). Overall, we found higher inflammatory markers in patients exposed to severe recent life events, without a present infection.

3.6. Impact of severe recent life events on the immune system after 4 weeks of treatment with antidepressants

Patients exposed to multiple severe recent life events did not show significant higher CRP concentrations than those without multiple severe recent life events anymore (0.40 ± 0.55 vs. 0.34 ± 0.56; $F = 1.590$; $df = 1$; $p = 0.210$). Also, we failed to observe higher lymphocyte counts before or after dexamethasone administration in patients exposed to multiple severe recent life events using a repeated measures GLM ($F = 0.290$; $df = 1$; $p = 0.592$). However, CRP and lymphocyte counts were not related to treatment outcome.

3.7. Impact of severe recent life events on treatment outcome

To assess the clinical impact of severe recent life events and childhood trauma, dimensionally assessed by the total score of LTE and CTQ, on the course of in-patient treatment, i.e. response at 4 weeks, we used a stepwise regression analysis with the independent variables of age, sex and severity of depression. Only severe recent life events significantly explained a variance of treatment response at week 4 (12.8%; $R = 0.357$;

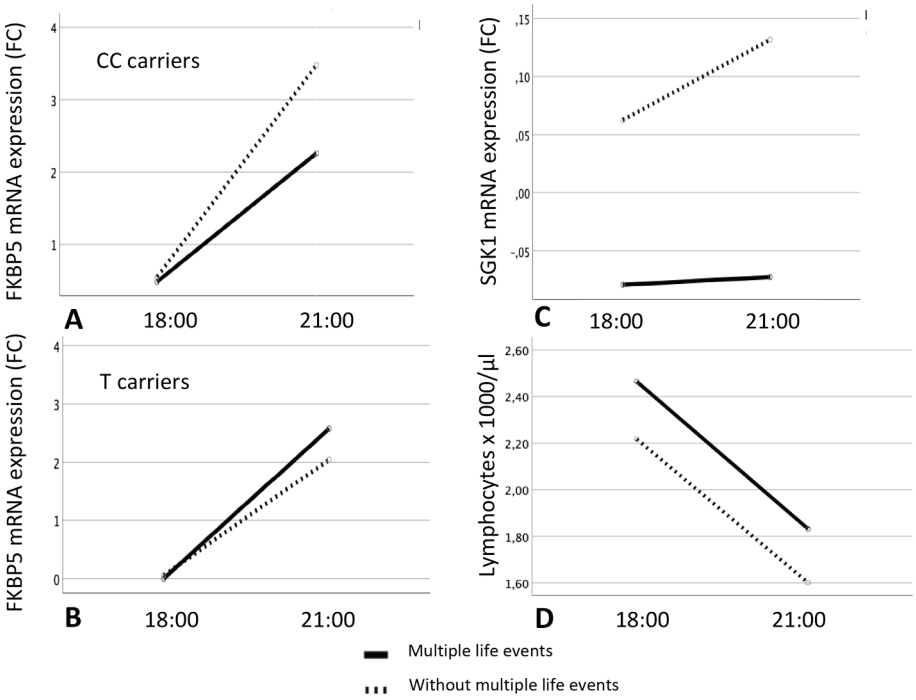

Fig. 2. (A-B) FKBP5 mRNA expression before (18:00) and after GR-stimulation with 1.5 mg dexamethasone (21:00) in patients with (CC n = 40; T n = 19) and without (CC n = 47; T n = 44) multiple severe recent life events stratified according to *FKBP5* rs1360780 genotype. (C) SGK1 mRNA expression before and after GR-stimulation in patients with (n = 59) and without (n = 91) multiple severe recent life events. (D) Lymphocytes concentrations before and after GR-stimulation in patients with (n = 59) and without (n = 91) multiple severe recent life events.

Table 2
Response to treatment with antidepressants and their association with severe recent life events and childhood trauma. Patients with 3 or more events were classified as subject to multiple events.

Characteristics	Multiple severe life events					Multipe childhood trauma				
	Yes		No		p-value	Yes		No		p-value
	n = 59 (39.3%)		n = 91 (60.7%)			n = 68 (45.5%)		n = 82 (54.5%)		
Response										
HAMD admission (±SD)	27.4	(±6.1)	25.6	(±6.7)	NS	27.4	(±6.4)	25.2	(±6.4)	NS
HAMD week 4 (±SD)	17.6	(±5.7)	12.4	(±5.5)	<0.001	15.6	(±6.8)	13.3	(±5.3)	NS
Response week 4 N (%)	5	(8.4)	48	(52.7)	<0.001	19	(27.9)	34	(41.5)	NS
Remission week 4 N (%)	1	(1.7)	22	(24.7)	<0.001	9	(13.2)	15	(18.3)	NS

$p < 0.001$). Next, comparing the response rate, treatment response occurred only in 8.4% of the patients with multiple severe recent life events, but in 52.7% of the patients without multiple severe recent life events. Patients with multiple severe recent life events responded slower to treatment compared to those without. A repeated measures GLM adjusted for age, sex and total CTQ score showed a significant main effect of multiple severe recent life events on treatment response ($F = 7.456$; $df = 1$; $p = 0.008$) and a significant interaction effect ($F = 2.793$; $df = 3$; $p = 0.041$), while CTQ had no significant effect on the treatment course (see Fig. 3). To further clarify the differential impact of severe life events and childhood trauma, we performed the GLM analysis only in patients without any childhood trauma and in patients without any severe recent life events, respectively. For patients without childhood trauma, a repeated measures GLM adjusted for age and sex still showed a significant main effect of multiple severe recent life events on treatment response ($F = 3.096$; $df = 1$; $p = 0.045$). In patients without severe recent life events, a repeated measures GLM did not reveal significant effects.

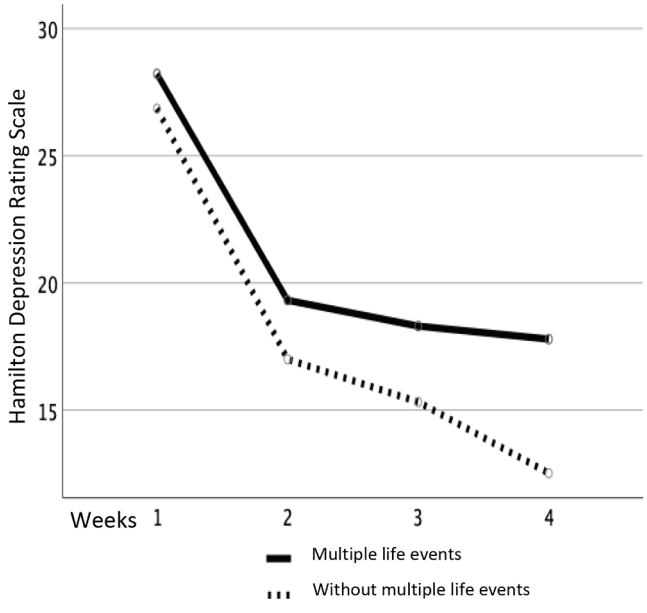

Fig. 3. Hamilton Depression Rating Scale scores over 4 weeks of treatment with antidepressants plotted against experience (n = 59) and no experience (n = 91) of 3 and more severe recent life events. A repeated measures GLM adjusted for age, sex and childhood trauma (CTQ score) showed a significant main effect of the severe recent life events status ($F = 7.456$; $df = 1$; $p = 0.008$) and a significant interaction effect ($F = 2.793$; $df = 3$; $p = 0.041$), while CTQ had no significant effect of the treatment course.

3.8. Impact of multiple childhood traumata on the molecular stress-hub FKBP5, SGK1 and GR

We could not observe any significant alterations of the gene expression patterns of FKBP5, SGK1 or GR before and after dexamethasone administration in dependency of multiple childhood traumata.

However, a repeated measures GLM revealed a significant main effect of multiple childhood traumata on cortisol concentrations before and after dexamethasone administration, with lower cortisol concentrations in patients exposed to multiple childhood traumata ($F = 4.779$; $df = 1$; $p = 0.0311$).

3.9. Impact of multiple childhood traumata on the immune system

We could not observe significantly different CRP concentrations between patients with a history of multiple childhood traumata and patients without (0.32 ± 0.45 vs. 0.42 ± 0.69; $F = 0.442$; $df = 1$; $p = 0.507$). Using repeated measures GLM, we could not detect different lymphocyte counts before and after dexamethasone administration comparing patients with and without history of multiple childhood traumata ($F = 1.418$; $df = 1$; $p = 0.236$).

4. Discussion

To our knowledge, this is the first study disentangling the different effects of childhood trauma and severe recent life events on crucial molecular components of the stress response systems in depressed patients. By applying a glucocorticoid receptor challenge test (Leistner and Menke, 2018), we discerned an impaired activation of FKBP5 and SGK1 gene expression only in patients exposed to multiple severe recent life events, while these alterations could not be observed in patients with a history of multiple childhood traumata. FKBP5 gene expression was moderated by the functional *FKBP5* rs1360780 variant that has been robustly associated with GR stimulation (Matosin et al., 2018; Klengel, 2013; Menke, 2013). After 4 weeks of treatment with antidepressants, FKBP5 and SGK1 gene expression patterns normalized in patients exposed to multiple severe recent life events. Interestingly, the normalization was independent of clinical response to treatment.

Early life adversity has become the biological hallmark for the development of several stress-related psychiatric disorders in recent years. It has repeatedly been linked to impaired function of the HPA axis, for example to hypocortisolism and altered cortisol reactivity (Kumsta, 2017; Lovallo, 2012; Koss, 2016; Elzinga, 2008). In the present study, we observed significantly reduced cortisol concentrations in patients subject to childhood trauma in terms of a hypocortisolism. However, we could not detect impaired cortisol reactivity. One reason may be the present study design where only depressed patients were recruited without a comparison group of traumatized “healthy” controls. In

addition, GR challenges different from the present one were used in previous investigations, with most studies analyzing the cortisol awakening response (CAR) or applying psychosocial stress tasks, not a pharmacological GR activation. Strong evidence exists for interactions of *FKBP5* genotype with childhood trauma, with the *FKBP5* minor genotype conferring an increased risk for psychiatric disorders when exposed to childhood trauma. As we recruited only depressed patients and no healthy controls, we could not replicate this finding. Further, we could not detect significant alterations in FKBP5 gene expression moderated by the *FKBP5* genotype when comparing participants with and without childhood trauma. Instead, we observed significant FKBP5 gene expression alterations in depressed patients subject to multiple severe life events one year prior to admission, which was dependent on *FKBP5* genotype. While baseline FKBP5 gene expression was unaltered, FKBP5 gene expression following GR activation was impaired only in patients exposed to severe recent life events and homozygous for the rs1360780 major C allele. The *FKBP5* SNP rs1360780 has been identified as a functional variant that disrupts an intronic TATA-box binding protein site in the vicinity of a glucocorticoid response element (GRE) in intron 2 and has been linked to three-dimensional structural changes in this locus (Matosin et al., 2018; Klengel, 2013). In subjects carrying the minor allele (T) the intronic GRE comes in contact with the transcription start site, whereas this is not the case for subjects without the minor allele. Numerous studies showed that the minor allele leads to an exaggerated gene expression following GR activation, and therefore may compensate the impaired expression by severe life events in those carrying at least one minor allele (Matosin et al., 2018). The interaction of FKBP5 with environmental factors, mainly childhood trauma, has been linked to epigenetic alterations, mainly in intron 2 and intron 7 (Klengel, 2013; Wiechmann, 2019). However, data on interaction of FKBP5 with stressful non-traumatic life events in adulthood is lacking.

In addition, we discerned an impaired GR-activated SGK1 gene expression in patients subject to severe recent life events. Preclinical studies observed higher levels of SGK1 gene expression after chronic stress, which was also reported for drug-naïve depressed patients (Cattaneo and Riva, 2015). However, no study so far investigated the impact of recent stressful life events on the GR-induced SGK1 gene expression in vivo. SGK1 is activated by physiological stressors and mediates some of the effects exerted by glucocorticoids on the brain (Cattaneo and Riva, 2015). It has been implicated in the cellular response to stress, for example, it has been implicated in the negative effects of cortisol on neurogenesis (Anacker, 2013). In addition, there is evidence that SGK1 gene expression is positively correlated with FKBP5 gene expression (Anacker, 2013), which was also observed in our study. Evidence supports the role for SGK1 as crucial sensor for acute and chronic stress, together with other components of the stress-hormone system such as FKBP5, and an impairment of SGK1 function, along with an impairment of FKBP5 function, may thus be a biological correlate of the impaired compensation of environmental stressors. As we recruited only subjects with a depression and no healthy controls, the study participants were likely lacking a sufficient compensation of the previous environmental stressors.

Interestingly, we did not find significant alterations upstream or downstream of FKBP5 / SGK1, neither for the GR (NR3C1) gene expression nor for cortisol concentrations. Previous studies reported inconsistent results of GR alterations due to childhood trauma and severe recent life events (Joels et al., 2018; Smart, 2015). In addition, GR activation by dexamethasone may not uncover all subtle GR sensitivity differences (Leistner and Menke, 2018). However, after GR activation we found only main effects on the differential blood count, no interaction effects, which also supports no GR sensitivity differences, along with the unaltered cortisol concentrations. Therefore, the observed differences in FKBP5 / SGK1 signaling may not be due to a disturbed GR sensitivity, but may rather result from alterations directly within the FKBP5 / SGK1 molecular stress hub.

An activated immune system has been robustly shown for patients with a history of childhood trauma (Danese, 2007; Danese and Lewis, 2017). However, there is growing evidence that also severe recent life events are associated with elevated inflammatory markers. For example, adolescents subject to stressful life events were more likely to develop a depressive episode, if the life events produced elevated CRP and cytokines (Kautz, 2019). In women during or after pregnancy, experience of stressful life events was related to elevated inflammatory markers such as CRP (Aas, 2019; Ross, 2019). While we could not detect an altered immune response following GR activation in patients with a history of childhood trauma compared to those without, we found increased levels of lymphocytes before and after GR activation, as well as increased CRP levels. This supports previous evidence that not only childhood trauma impacts the immune system, but also severe recent life events, at least in depressed patients. Again, similar to the FKBP5 / SGK1 response, these inflammatory patterns are likely related to the stressful recent life events, and not to the depression itself.

We observed a normalization of both, FKBP5 and SGK1 gene expression alterations and the alterations of the inflammatory markers after 4 weeks of treatment with antidepressants. However, this effect was not associated with clinical response. Such patterns could also be observed in previous studies, revealing gene expression alterations during antidepressant treatment without a link to clinical response (Cattaneo, 2013). The presently observed patterns are probably due to the stress effects prior to admission as they were not associated with the severity of depression and therefore changes in these patterns are not likely related to clinical response. Moreover, the normalization can be interpreted as a compensated function of the FKBP5 / SGK1 stress-response hub and the immune system through treatment with antidepressants.

The widely accepted sensitization model proposed that exposure to childhood trauma enhances the vulnerability to the effects of later stressful life events and that stressful life events increase the liability to depression especially in these subjects with history of childhood trauma (Hammen et al., 2000; Hammen, 2005). We did not evaluate the risk to develop MDD in our study in the presence of childhood trauma and severe life events as we recruited only depressed patients. However, we did find that the scores for childhood trauma and severe life events were significantly correlated and that participants with a history of childhood trauma experienced more severe recent life events. Using a regression analysis to determine factors explaining the variance of severe recent life events, we observed that employment status (with a high rate of unemployment) could explain the most of the variance with 14.5%, in addition age (5.4%) and physical neglect (5.2%) explained some variance.

Interestingly, we found a significant impaired clinical response to antidepressants in patients subject to multiple severe recent life events, independent of a history of childhood trauma. A regression analysis showed that only the severe recent life events explained a substantial part of the response variance. While four weeks of treatment with antidepressants ameliorated the dysregulated gene expression pattern of the molecular FKBP5 / SGK1 stress-hub, clinical response could not be achieved. Therefore, the presence of multiple severe recent life events may serve as a predictor for an impaired response to treatment with antidepressants and thus may help to stratify patients to facilitate individualized treatment (Menke, 2018). If replicated in prospective studies, patients exposed to multiple severe recent life events should receive an augmented drug treatment right at the beginning of the treatment process or other treatment options such as psychotherapy, electroconvulsive therapy or immune-modulatory approaches.

Several limitations of this study must be acknowledged. Both severe recent life events and childhood traumata were assessed retrospectively. Studies suggest that recall bias of childhood trauma primarily involves under-reporting of childhood trauma, also in individuals with presence of current psychopathology. However, as we detected a history of multiple mild / moderate childhood trauma in 46% of the patients, a possible under-reporting may be negligible. Next, using the LTE we

applied a stressor checklist, not a stressor interview. This does not allow for determining the severity or temporal sequencing of stressors. In addition, we did not evaluate how participants perceived the stressors, which may also effect the impact of stressors on psychopathology and the underlying biological mechanisms (Keller, 2012).

In conclusion, we found a significantly dysregulated gene expression pattern of the molecular FKBP5 / SGK1 stress hub and increased inflammatory markers following multiple severe recent life events, which was independent of GR sensitivity and childhood trauma. These alterations diminished after 4 weeks of treatment with antidepressants. However, independent of this normalization the exposure of multiple severe recent life events predicted an impaired clinical response to treatment with antidepressants.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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