Transcriptional Modulation of Stress-Related Genes in Association with Early Life Stress Exposure and Trauma-Focused Psychotherapy in Treatment-Resistant Depression Patients

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Early life stress (ELS) is associated with treatment-resistant depression (TRD), and trauma-focused psychotherapy benefits TRD patients exposed to ELS. We explored peripheral modulations of stress-response genes (nuclear receptor subfamily 3 group C member 1 *[NR3C1]*, FK506-binding protein 5 *[FKBP5]*, and serum/glucocorticoid-regulated kinase 1 [*SGK1*]) in relation to ELS and symptom changes during psychotherapy. Forty-one TRD patients participated and 21 patients underwent trauma-focused psychotherapy, comprising eye movement desensitization and reprocessing or trauma-focused cognitive behavioral therapy. We used the Montgomery-Åsberg Depression Rating Scale, the Beck Depression Inventory-II and the Beck Anxiety Inventory for symptom evaluation, the Childhood Experience of Care and Abuse Questionnaire for ELS assessment, and the quantitative reverse transcription polymerase chain reaction (RT-qPCR) for transcript analysis. We found higher *NR3C1* and *FKBP5* baseline mRNA levels in

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patients with maternal neglect. Trauma-focused psychotherapy induced modifications in transcripts' levels and symptom amelioration along psychotherapy correlated with genes' modulations. Transcript levels for all genes were higher in patients relapsing after 24 weeks.

Keywords: stress-related gene expression; EMDR; TF-CBT; early life stress; treatment-resistant depression

Major depressive disorder (MDD) is the most prevalent psychiatric disorder, being responsible for a great deal of disability around the world. Despite advances in pharmacological therapy, this strategy is often ineffective: only about one-third of patients are effectively treated with the first antidepressant trial (Trivedi et al., 2006) and several patients, approximately 18%–55%, are defined as having treatment-resistant depression (TRD; Jaffe et al., 2019; Thomas et al., 2013). Many prognostic variables predict outcomes in MDD and factors associated with unfavorable responses include longer duration of untreated disease, longer duration of depressive episodes, later response to antidepressant treatment, earlier disease onset, greater disease severity, psychiatric comorbidities (anxiety disorders, post-traumatic stress disorder [PTSD], obsessive-compulsive disorder), physical comorbidities, suicidal behaviors, and stressful life events, particularly those occurring early in life (Kautzky et al., 2017; Kraus et al., 2019; Tunvirachaisakul et al., 2018). With respect to this last factor, early life stress (ELS), which includes physical, sexual, and emotional abuse, and childhood neglect, has a particular importance, since it is reported by 46%–62% of MDD patients (Jansen et al., 2016; Nelson et al., 2017; Williams et al., 2016). ELS is associated with TRD and worse treatment outcomes, along with the above-mentioned negative response predictors (Infurna et al., 2016; Kraus et al., 2019; Minelli et al., 2019; Nanni et al., 2012). Indeed, compared to those without a history of childhood maltreatment, individuals exposed to ELS have an earlier onset of depressive symptoms and are three times more likely to develop MDD, twice as likely to present recurrent depressive episodes and chronic disease course, and about 1.5–2 times as likely to develop TRD (Nanni et al., 2012; Nelson et al., 2017).

The relation between ELS and MDD seems to have its biological bases in neuroendocrine, immune, and inflammatory activity dysregulations that have been suggested to mediate the effects and outcomes of trauma on psychological health, increasing the risk for MDD and influencing its course (Park et al., 2019; Silva et al., 2021). For example, genes like Solute Carrier Family 6 Member 4 (*SLC6A4*; Fleurkens et al., 2018) in the serotonin system, dopamine transporter (*DAT1*; D'Souza et al., 2016) and dopamine D2 receptor (*DRD2*; Hayden et al., 2010) in the dopamine system, Brain-Derived Neurotrophic Factor (*BDNF*; Peng et al., 2018) in the neurotrophic system, oxytocin receptor gene (*OXTR*; Thompson et al., 2011) in the oxytocin system, and inflammatory markers including C-reactive protein (Chamberlain et al., 2019) and Interleukin-6 (Munjiza et al., 2018) are known to be involved in the mediation between ELS and MDD.

In particular, the hypothalamic-pituitary-adrenal (HPA) axis is physiologically activated by stress exposure, leading to glucocorticoid release by the adrenal gland, activation of glucocorticoid receptors (GR) in target organs, including brain and peripheral tissues, and the start of a cascade of transcriptional responses inducing changes in gene expression. On these bases, altered patterns of gene expression in stress-related genes have been studied to understand the underlying molecular mechanisms and players that can possibly mediate the effect of childhood maltreatment on MDD and antidepressant response.

Due to their role in the biological mechanisms involved in stress exposure, selected stress-related genes involved in the HPA axis, including nuclear receptor subfamily 3 group C member 1 *(NR3C1)*, FK506-binding protein 5 *(FKBP5)*, and serum/glucocorticoid-regulated kinase 1 *(SGK1)* genes, have been studied as possible candidates mediating the effect of childhood abuse on MDD risk.

The GR, encoded by *NR3C1*, is a leading actor in the physiological responses to stress exposure. Several pieces of evidence support the hypothesis that ELS alters *NR3C1* expression by epigenetic mechanisms, increasing MDD susceptibility (Holmes et al., 2019; Watkeys et al., 2018). The heat shock protein-90-associated co-chaperone *FKBP51*, encoded by *FKBP5*, regulates GR functionality, and several pieces of evidence also support its involvement both in ELS and MDD (Binder, 2009; Matosin et al., 2018). Transcriptional changes in *FKBP5*, including epigenetic mechanisms, may mediate the relation between ELS and MDD (Klengel et al., 2013; Tozzi et al., 2018). Also, *SGK1*, a serine/threonine kinase implicated in

cellular stress response and neuronal functions, is involved in stress-related changes, including MDD, in preclinical models and clinical findings (Anacker et al., 2013; Cattaneo & Riva, 2016; Dattilo et al., 2020). Importantly, these three genes, *NR3C1*, *FKBP5,* and *SGK1,* are intrinsically related and act together to mediate stress responses in a coordinated fashion (Anacker et al., 2013; Cattaneo & Riva, 2016; Menke et al., 2021). Recently, researchers assessed *FKBP5, NR3C1,* and *SGK1* expression levels in the peripheral blood of depressed patients after pharmacological GR activation and found altered expression patterns of *FKBP5* and *SGK1* and increased levels of inflammatory markers following multiple severe recent life events. Also, severe recent life events were related in the same study to an impaired antidepressant treatment response (Menke et al., 2021).

Evidence-based trauma-focused psychotherapies have been proposed as beneficial approaches for MDD management and TRD patients exposed to ELS, showing evidence of positive therapeutic responses (Yan et al., 2021). There are not many studies assessing potential biological effects and putative biomarkers of therapeutic response in trauma-focused psychotherapies for MDD and TRD patients. Existing literature focuses primarily on biomarkers, mostly cortisol measures, of trauma-focused psychotherapies applied to patients diagnosed with PTSD (Nijdam et al., 2015; Rapcencu et al., 2017; Zantvoord et al., 2019). In MDD patients, to date, there is only one study focusing on molecular biomarkers of therapeutic response in trauma-focused psychotherapies (Maffioletti et al., 2021), while other studies involve neurophysiological approaches (Marwood et al., 2018; Rubart et al., 2018). A recent study from our group showed the involvement of microRNAs, small non-coding RNAs that act as post-transcriptional regulators and are involved in neurological and psychiatric disorders, in the biological alterations of trauma-focused psychotherapy in MDD patients. Expression levels of inflammatory-related microRNAs were associated with recent stressful events in TRD patients and their expression profile was modified by psychotherapy (Maffioletti et al., 2021). Concerning neurophysiological evidence, there was a tendency toward restoration of altered brain functional connectivities and impaired brain regions' activity following psychotherapy (Marwood et al., 2018; Rubart et al., 2018). Gene expression modulations were studied in depressed patients before and after cognitive behavioral therapy (CBT) and different transcript panels distinguished depressed patients from controls and helped in predicting and monitoring therapeutic response to psychotherapy (Redei et al., 2014). Besides the paucity of studies relating biomarkers and trauma-focused psychotherapies in MDD, the effects of ELS are equally poorly studied, with the exception of the above-mentioned study (Maffioletti et al., 2021).

Based on the presented literature, this study aimed at exploring peripheral transcriptional levels of stress response-related genes (*NR3C1*, *FKBP5,* and *SGK1*) in a cohort of TRD patients characterized for ELS. Moreover, we investigated transcripts modulations induced by trauma-focused psychotherapy and associations with symptom improvement.

Materials and Methods

Study Participants and Clinical Assessment

Forty-one adult TRD patients were voluntarily enrolled in the study. The inclusion criterion was a diagnosis of MDD according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*) classification (American Psychiatry Association, 1994). Current psychiatric clinical practice in Italy uses the World Health Organization Tenth Revision of the International Classification of Diseases (*ICD-10*) for MDD diagnosis, which displays a close correspondence with *DSM-IV*.

Patients presenting the following diagnosis or comorbidities were excluded: (a) cognitive disorder or mental retardation; (b) previous history of bipolar disorder, schizophrenia, or schizoaffective disorder; (c) substance abuse, alcohol abuse or dependency, obsessive-compulsive disorder, personality disorder, or PTSD as a primary diagnosis; and (d) comorbidity with eating disorders. All patients were referred to the Psychiatric Hospital "Villa Santa Chiara" in Verona, Italy.

TRD was defined as the failure to respond to at least two adequate trials with two or more different classes of antidepressant drugs and to an adequate trial with a tricyclic drug (TCA), which corresponds to stage III or above, in accordance to the Thase and Rush (Thase & Rush, 1997) staging classification system. The Italian version of the Childhood Experience of Care and Abuse Questionnaire, a validated self-report questionnaire (Bifulco et al., 2005), was used to assess ELS exposure in all patients. This questionnaire assesses different kinds of ELS: lack of parental care (neglect and antipathy), parental physical abuse, and sexual abuse, and shows satisfactory reliability and validity

for ELS measurement (Bifulco et al., 2005). Neglect refers to a parent's disinterest in material care, health, schoolwork, and friendships, and this scale is assessed for each parent and caregiver. Antipathy refers to hostility, coldness, or rejection shown to the child by the parent or caregiver, being assessed for their role as a mother or father figure. Physical abuse refers to hitting by parents or another house member and is separately rated for mother and father. Sexual abuse includes physical contact or approach of sexual nature by any adult to the child (Bifulco et al., 2005).

Italian versions of the Paykel Scale of stressful life events and the Holmes–Rahe Life Stress Inventory (Baratta et al., 1985; Holmes & Rahe, 1967) were used to evaluate the presence of recent stressful life events, occurring one year before the assessment.

Trauma-Focused Psychotherapy

The study was performed with two independent cohorts. For the baseline analyses, all the recruited patients, comprising both cohorts (n = 41), were analyzed with gene transcript measurements and clinical and stress exposure assessments. One of the cohorts, composed of 21 patients, underwent a trauma-focused psychotherapy program due to clinical needs. This cohort was longitudinally evaluated, with clinical assessments and transcript measurements. Within this cohort, 9 patients underwent trauma-focused CBT (TF-CBT) and 12 patients received eye movement desensitization and reprocessing (EMDR) therapy. Each patient received 24 sessions of TF-CBT or EMDR carried out by highly experienced psychotherapists. Psychotherapy sessions were performed in three individual sessions per week, each lasting 60 minutes, for a period of 8 weeks, which corresponded to the total period of hospitalization. Clinical evaluations, with symptom measurements, and blood sampling were conducted at four time points: baseline (T0), after 4 weeks of treatment (T4), after 8 weeks of treatment, which represented the end of psychotherapy sessions and of hospitalization (T8), and 4 weeks after the end of treatment, when patients were referred to the hospital for a follow-up visit (T12). Pharmacological treatment (patients receiving first-generation antipsychotics: 4.8%; second-generation antipsychotics: 33.3%; selective serotonin reuptake inhibitors: 38.1%; serotonin-norepinephrine reuptake inhibitors: 38.1%; tricyclic antidepressants: 4.8%; noradrenergic and specific serotonergic antidepressants: 33.3%; other classes of antidepressants: 38.1%; mood stabilizers: 33.3%; benzodiazepines or hypnotic drugs: 100%) was not substantially changed during the assessment period, although slight adjustments were possible according to clinical needs. Symptoms assessments were made using Montgomery–Åsberg Depression Rating Scale (MADRS; Montgomery & Asberg, 1979) and relative dimensions according to the three-factor model (MADRSF1: mood symptoms; MADRSF2: cognitive symptoms; MADRSF3: neurovegetative symptoms; Suzuki et al., 2005), Beck Depression Inventory-II (BDI-II; Beck, 1993), Beck Anxiety Inventory (BAI; Beck & Steer, 2013), and Pittsburgh Sleep Quality Index (PSQI; Curcio et al., 2013). To assess psychosocial functioning, the Mini-instrument for the observer rating according to International Classification of Functioning, Disability and Health of Activities and Participation in Psychological Disorders (MINI-ICF-APP; Balestrieri et al., 2013) was used. Response to psychotherapy was defined as a reduction greater than 50% in the MADRS score at the T12 assessment.

The study was approved by the Local Ethics Committee (Ethics Committee for Clinical Trials of the province of Verona and Rovigo N: 234777/11.05.16). All participants received a full explanation of study details and procedures, and gave written informed consent to participate. The clinical trial and related assessments were described in detail elsewhere (Minelli et al., 2019).

Blood Collection and mRNA Isolation from Blood

Peripheral venous blood samples were collected in the morning between 8:00 and 9:00 a.m., after an overnight fast, in PAXGene Blood RNA tubes (Cat. 762165, Qiagen) for RNA isolation. PAXGene Blood RNA tubes were kept at room temperature for 2 hours, then frozen at −20°C for 24 hours, and finally stored at −80°C until RNA isolation. Total RNA was extracted from 2.5 ml of blood with the PAXGene Blood miRNA Kit (Cat. 763134, Qiagen), following the manufacturer's instructions. RNA quantification and quality control were carried out using spectrophotometric analysis (NanoDrop 2000, Thermo Scientific).

Determination of Candidate mRNA Expression Levels by the Quantitative Reverse Transcription Polymerase Chain Reaction

Expression levels of the target genes *NR3C1* (Hs00353740_m1), *FKBP5* (Hs01561006_m1), *SGK1* (Hs00178612_m1), and the housekeeping gene *B2M*

(Hs99999907_m1) were analyzed using TaqMan Assays (Thermo Fisher, Waltham, MA, USA) and iTaq Universal Probes One-Step Kit (Bio-Rad Laboratories, Hercules, CA, USA) on the CFX384 Real-Time PCR instrument (Bio-Rad Laboratories, Hercules, CA, USA) following the manufacturer's instructions. All real-time PCR reactions were carried out with the following steps: 10 minutes at 50°C, 5 minutes at 95°C followed by 39 cycles of 10 seconds at 95°C, and 30 seconds at 60°C. All reactions were run in triplicate. The Ct values were normalized according to ΔCt method on the housekeeping gene *B2M*, which was stably expressed across samples.

Statistical Analysis

Meeting relative assumptions, differences between groups for continuous measures were analyzed using a non-parametric Mann–Whitney *U* test. The Kendall rank coefficient was used to evaluate bivariate correlations. These analyses were conducted with the software IBM SPSS Statistics. To assess variations over time in candidate mRNAs levels, a linear mixed model was applied, considering as the dependent variable the difference between each time point and baseline measurements, and time as the within-subject factor. For these analyses, data were log-transformed in order to assume a normal distribution. In a first step, estimation of changes between longitudinal and baseline measurements over time was assayed by analysis of variance (ANOVA). Pairwise contrasts between the mean value at different time points were estimated by using a repeated measures ANOVA analysis. In a second step, the analyses were extended by estimating a model including the "response status" (responders versus non-responders) as the between-subjects factor and the interaction of this variable with time. Comparisons between relative groups (responders versus non-responders) were performed with a 95% confidence interval, computing FDR-adjusted *p*-values by the Benjamini–Hochberg procedure. These analyses were conducted with the software R version 3.6.1.

Results

Baseline Transcript Levels of Stress-Related Genes in Relation to Early Life Stress and Recent Stressful Events

To assess whether ELS affected gene expression, transcriptional levels of *NR3C1, FKBP5,* and *SGK1* were evaluated in the blood samples of the 41 recruited TRD patients. We first checked whether the presence of specific subtypes of ELS and recent stressful events was associated with changes in the blood basal transcriptional levels of these genes. We found higher mRNA levels of *NR3C1* ($z = -2.64$; $p = .008$) and *FKBP5* ($z = -2.33$; $p = .02$) in patients exposed to maternal neglect ($n = 22$) compared to those without this exposure ($n = 19$; Figure 1A and B). No significant results were obtained for *SGK1* expression levels and the presence of ELS or recent stressful events. Recent stressful events, analyzed with the Paykel Scale of stressful life events and Holmes–Rahe Life Stress Inventory, did not present significant associations with gene expression analyses.

Effects of Trauma-Focused Psychotherapy in Transcriptional Changes of Stress-Related Genes

Second, we aimed to investigate whether transcriptional levels of stress-related genes could be modified by trauma-focused psychotherapy. To identify expression changes, mRNA levels of the three target genes were evaluated at baseline (T0), after 4 weeks (T4), at the end of psychotherapy (T8), and at follow-up (T12) in the whole blood of patients undergoing trauma-focused psychotherapy ($n = 21$). We found significant changes over time in blood mRNA levels of *NR3C1* ($p = 3.5 \times 10^{-2}$; $F = 3.03$), *FKBP5* ($p = 1.93 \times 10^{-8}$; $F = 17.2$), and *SGK1* ($p = 2.86 \times 10^{-3}$, $F = 5.14$). As shown in Figure 2, a slight but not significant decrease in mRNA levels of all three genes was observed at T4. Instead, the reduction appeared more pronounced at T8 compared to baseline and it was significant for both *SGK1* ($p = 3.8 \times 10^{-2}$) and *FKBP5* ($p = 5.46 \times 10^{-3}$), but not for *NR3C1* levels. Conversely, these alterations were restored at follow-up compared to the end of psychotherapy ($p = 2.20 \times 10^{-2}$ for *NR3C1*, $p = 1.42 \times 10^{-11}$ for *FKBP5,* and $p = 6.37 \times 10^{-4}$ for *SGK1*) and to baseline levels for *FKBP5* ($p = 2.34 \times 10^{-5}$; Supplementary Table 1). Furthermore, we found significant correlations between changes in transcriptional levels at T8 and T12 compared to baseline, expressed as percentage changes (%Δ) between %Δ*NR3C1* and %Δ*SGK1* ($\tau = .743$ and $p < .001$ at T8, and $\tau = .581$ and $p < .001$ at T12), %Δ*NR3C1* and %Δ*FKBP5* ($\tau = .733$ and $p < .001$ at T8, and $\tau = .590$ and $p < .001$ at T12), and %Δ*SGK1* and %Δ*FKBP5* ($\tau = .724$ and $p < .001$ at T8, and $\tau = .419$ and $p < .01$ at

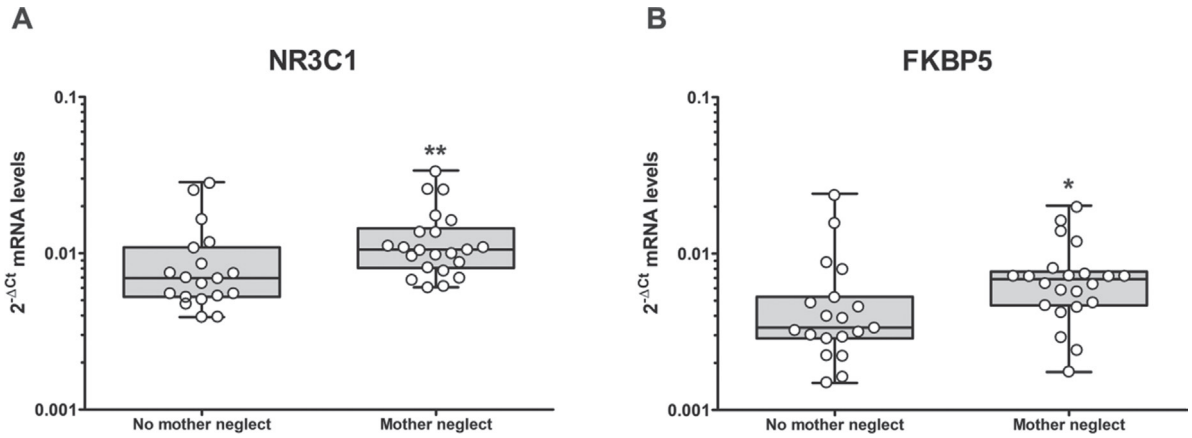

Figure 1. Baseline transcript levels of stress response-related genes in relation to early life stress exposure. Higher peripheral levels of *NR3C1* (**A**) and *FKBP5* (**B**) mRNAs were found in patients exposed to maternal neglect, in comparison with those without. Values are represented in whisker–scatter plots (log_{10} scale) as $2^{-\Delta Ct}$. Statistical significance is indicated at the top of the graph. *$p \leq .05$; **$p \leq .01$.

T12), indicating a joint effect of psychotherapy on transcript modulations. Since some patients had PTSD comorbidity as a secondary diagnosis (n = 7) and since these patients could benefit more from trauma-focused psychotherapy, leading to possible confounding effects in transcript modulations, longitudinal analyses excluding these patients were conducted. The results confirmed for all the genes significant modulations over time observed in the whole patient cohort ($p <$ .0001 and F = 10.18 for *FKBP5*, p = .044 and F = 2.77 for *NR3C1*, p = .009 and F = 4.43 for *SGK1*), suggesting that psychotherapy modulates gene expression independently of PTSD comorbidity. Furthermore, we performed longitudinal analyses on two subgroups of patients identified as responders (n = 16, 76.2%) or non-responders (n = 5, 23.8%) to psychotherapy at follow-up (Supplementary Figure 1, left column). We found significant transcriptional modulations exclusively in responders ($p <$ .0001 and F = 13.96 for *SGK1*, p = .0027 and F = 5.47 for *FKBP5*, p = .0143 and F = 2.71 for *NR3C1;* Supplementary Figure 2) and, only *FKBP5* confirmed the significant effect already observed in the cohort of

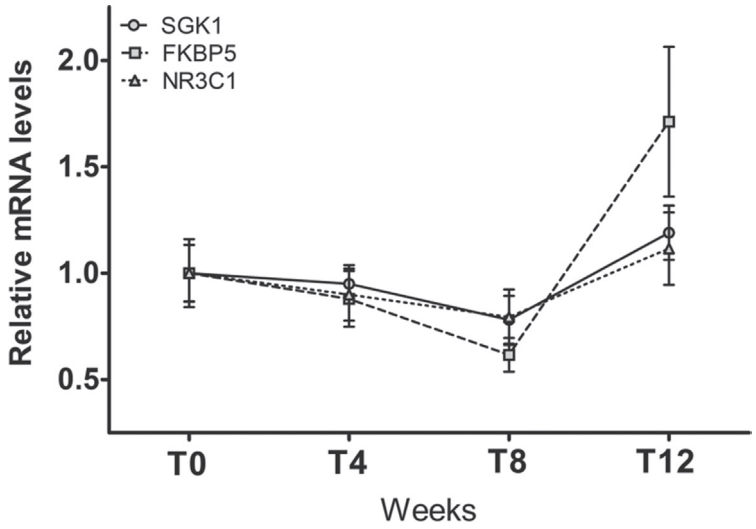

Figure 2. Longitudinal analyses of transcriptional changes in stress response-related genes during the 8 weeks of psychotherapy program and at the follow-up (T12). For each time point, the results are represented as relative (to baseline) mRNA levels ± s.e.m. The variations for the T0 point represent the s.e.m. of mRNA levels individually calculated for each patient at T0 by referring to the mean of mRNA levels at T0, with the value 1 representing the mean of the individual patients' mRNA levels.

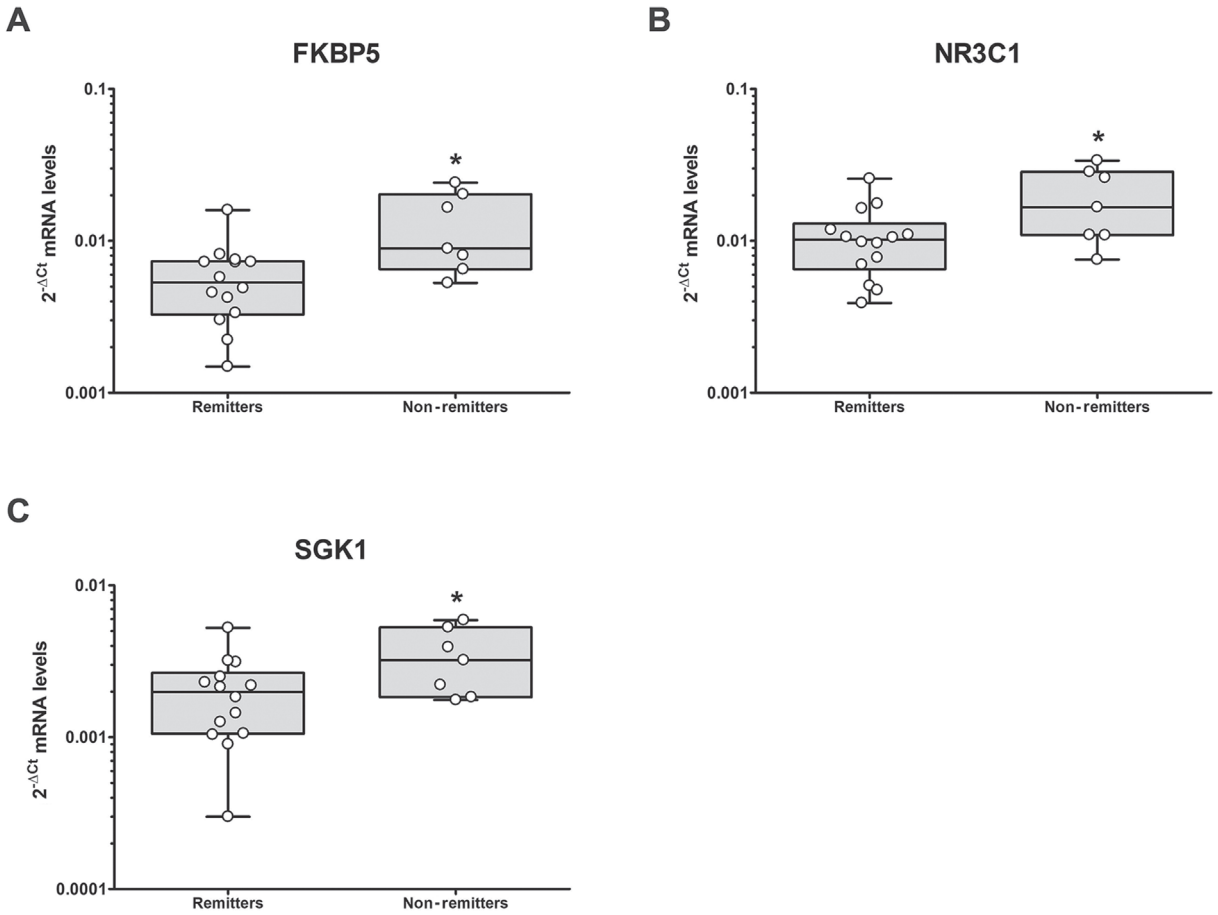

Figure 3. Baseline transcript levels of stress response-related genes and disease relapse. Higher peripheral levels of FKBP5 (**A**), NR3C1 (**B**), and SGK1 (**C**) mRNAs were found in non-remitter patients compared with remitters. Values are represented in whisker–scatter plots ($\log_{10}$ scale) as $2^{-\Delta Ct}$. Statistical significance is indicated at the top of the graph. *$p \leq .05$.

patients receiving trauma-focused psychotherapy at T8 and T12 compared to baseline ($p < .05$ and $p < .01$, respectively). These findings could possibly drive the significant results observed in the whole cohort of patients, albeit it cannot be excluded that the non-significance effect observed in the non-responder subgroup is due to its small sample size. Sociodemographic and clinical characteristics of all patients have been described in a previous publication (Maffioletti et al., 2021).

Symptomatological Changes in Relation to Transcriptional Modulations of Stress-Related Genes

Symptom improvements in patients undergoing trauma-focused psychotherapy ($n = 21$) are summarized in Supplementary Table 2 and Supplementary Figure 3. Based on this clinical evidence, we investigated whether there was any correlation between symptomatology improvement and transcript modulations in association with psychotherapy. Interestingly, the analysis revealed significant correlations between changes in mRNA levels of the stress-responsive genes and symptomatologic changes (Table 1). We found positive correlations between the decrease in *FKBP5, NR3C1,* and *SGK1* expression from T0–T8 and MADRS cognitive dimension symptoms amelioration at the same time ($\tau = .420$, $p = .012$; $\tau = .474$, $p = .005$; $\tau = .366$, and $p = .029$, respectively). Moreover, we found positive significant correlations and a trend between *NR3C1* expression decrease at T8 with depressive symptoms decrease assessed at the same time points with BDI ($\tau = .390$, $p = .013$) and total MADRS ($\tau = .293$, $p = .071$). Furthermore, we found predictive significant or trend-positive correlations between the decrease in *FKBP5, NR3C1,* and *SGK1* expression from T0–T8 and the T0–T12 decrease in MADRS neurovegetative symptom dimension ($\tau = .413$, $p = .012$; $\tau = .423$, $p = .01$; $\tau = .423$, and $p = .01$, respectively), in depressive symptoms assessed by BDI ($\tau = .276$, $p = .080$; $\tau = .325$, $p = .025$; $\tau = 0.381$,

TABLE 1. Kendall's Correlations Between the Reductions in the Blood Transcriptional Levels of Stress Response-Related Genes From T0 to T8 and Symptomatologic Improvements at T8 and T12, as Compared to T0.

Gene	Assessed symptoms (scale)	Symptoms amelioration at time point (compared to baseline)	Kendall's rank correlation coefficient (τ)	Significance (p-value)
FKBP5	Total MADRS	T8	.283	.081
	Mood (MADRSF1)	T8	.246	.147
	Cognitive (MADRSF2)	T8	**.420**	**.012**
	Neurovegetative (MADRSF3)	T8	.243	.148
	Depressive (BDI)	T8	.257	.103
	Anxiety (BAI)	T8	.221	.164
	Sleep Quality (PSQI)	T8	.082	.607
	Psychosocial functioning (MINI-ICF-APP)	T8	−.038	.809
	Total MADRS	T12	.148	.349
	Mood (MADRSF1)	T12	.020	.902
	Cognitive (MADRSF2)	T12	.048	.762
	Neurovegetative (MADRSF3)	T12	**.413**	**.012**
	Depressive (BDI)	T12	.276	.080
	Anxiety (BAI)	T12	.286	.070
	Sleep Quality (PSQI)	T12	.024	.88
	Psychosocial functioning (MINI-ICF-APP)	T12	−.138	.381
NR3C1	Total MADRS	T8	.293	.071
	Mood (MADRSF1)	T8	.235	.166
	Cognitive (MADRSF2)	T8	**.474**	**.005**
	Neurovegetative (MADRSF3)	T8	.232	.167
	Depressive (BDI)	T8	**.390**	**.013**
	Anxiety (BAI)	T8	.163	.304
	Sleep Quality (PSQI)	T8	.129	.414
	Psychosocial functioning (MINI-ICF-APP)	T8	.058	.717
	Total MADRS	T12	.129	.415
	Mood (MADRSF1)	T12	.060	.713
	Cognitive (MADRSF2)	T12	.048	.762
	Neurovegetative (MADRSF3)	T12	.423	.010
	Depressive (BDI)	T12	.352	.025
	Anxiety (BAI)	T12	.267	.091
	Sleep Quality (PSQI)	T12	.062	.694

(*Continued*)

TABLE 1. Kendall's Correlations Between the Reductions in the Blood Transcriptional Levels of Stress Response-Related Genes From T0 to T8 and Symptomatologic Improvements at T8 and T12, as Compared to T0. (*Continued*)

Gene	Assessed symptoms (scale)	Symptoms amelioration at time point (compared to baseline)	Kendall's rank correlation coefficient (τ)	Significance (*p*-value)
	Psychosocial functioning (MINI-ICF-APP)	T12	−.062	.695
SGK1	Total MADRS	T8	.214	.188
	Mood (MADRSF1)	T8	.213	.209
	Cognitive (MADRSF2)	T8	**.366**	**.029**
	Neurovegetative (MADRSF3)	T8	.178	0.289
	Depressive (BDI)	T8	.267	.091
	Anxiety (BAI)	T8	.183	.250
	Sleep Quality (PSQI)	T8	.101	.525
	Psychosocial functioning (MINI-ICF-APP)	T8	−.125	.431
	Total MADRS	T12	.177	.264
	Mood (MADRSF1)	T12	.030	.854
	Cognitive (MADRSF2)	T12	.058	.716
	Neurovegetative (MADRSF3)	T12	**.423**	**.010**
	Depressive (BDI)	T12	**.381**	**.016**
	Anxiety (BAI)	T12	**.352**	**.025**
	Sleep Quality (PSQI)	T12	.034	.832
	Psychosocial functioning (MINI-ICF-APP)	T12	−.072	.650

Note. BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; MADRS = Montgomery–Åsberg Despression Rating Scale; MINI-ICF-APP = Mini-instrument for the observer rating according to the International Classification of Functioning, Disability and Health of Activities and Participation in Psychological Disorders; PSQI = Pittsburgh Sleep Quality Index. Bold characters indicate significant *p*-values and corresponding *t* values.

and $p = .016$, respectively), and in anxiety symptoms assessed by BAI ($\tau = .286$, $p = .070$; $\tau = .267$, $p = .091$; $\tau = .325$, and $p = .025$, respectively).

Baseline Expression Levels of Stress-Related Genes and Treatment Outcomes

Finally, we investigated whether baseline transcriptional levels could predict treatment response or disease relapse. We evaluated differences between responders and non-responders ($n = 16$ [76, 2%] and $n = 5$ [23, 8%], respectively) to psychotherapy at follow-up, as well as between remitters ($n = 14$, 66.7%), identified as patients who did not display a disease relapse at T24, and non-remitters ($n = 7$, 33.3%; Supplementary Figure 1, right column). Although the analysis revealed no predictive power in psychotherapy response, baseline levels of *FKBP5, NR3C1,* and *SGK1* showed a positive association with disease relapse ($\tau = .460$ and $p = .014$, $\tau = .376$ and $p = .044$, $\tau = 0.376$ and $p = .044$, respectively). Moreover, baseline transcript levels were significantly higher ($p = .012$, $p = .046$, and $p = .046$, respectively) in non-remitters compared to remitters (Figure 3A, B, and C), suggesting their predictive power to detect disease relapse.

Discussion

In this article, we have investigated associations between baseline transcriptional levels of the stress-related genes *NR3C1, SGK1,* and *FKBP5*, and

exposure to ELS. Moreover, we have addressed the impact of trauma-focused psychotherapy in terms of symptomatologic changes and gene expression modulations. Finally, we have tested whether our target genes could represent predictive biomarkers of response and relapse by assessing whether their baseline expression levels could correlate with clinical outcomes.

We found higher baseline *NR3C1* and *FKBP5* mRNA levels among TRD patients exposed to maternal neglect, in comparison to those non-exposed. In a study investigating genes related to the HPA axis, inflammation, neurodevelopment, and neurotransmission in depressed children and adolescents, the authors found that some genes, including *NR3C1,* were expressed at lower levels in the MDD group in comparison with individuals with depressive symptoms but no diagnosis of MDD and with the healthy control group. They also found that the aggregate expression of *NR3C1* and other stress-related genes might underlie the relationship between ELS and MDD (Spindola et al., 2017). Enhanced *FKBP5* expression and reduced cortisol levels after a pharmacological stress challenge test were found in anxious depressed patients in comparison with non-anxious depressed subjects. These alterations were partly dependent on sexual abuse, demonstrating an increased HPA axis sensitivity in anxious depression moderated by ELS (Menke et al., 2018). Menke and colleagues found that exposure to recent stressful events in depressed patients associated with impaired GR-dependent induction of *FKBP5* and *SGK1* levels and with increased inflammatory markers, including C-reactive protein and lymphocyte counts, revealing that stress associates with impaired GR sensitivity and HPA axis dysfunctions (Menke et al., 2021). They did not observe impaired activation of *FKBP5* and *SGK1* expression after the GR challenge test in patients exposed to multiple childhood traumas, contrary to our findings. Regarding post-treatment transcripts modulations, there is a parallel between our results and their findings, although the two studies evaluated different treatment modalities and employed diverse gene expression measures in terms of the use of pharmacological tests. We observed significant *FKBP5* and *SGK1* transcript modifications with 8 weeks of psychotherapy. Similarly, they reported the normalization of *FKBP5* and *SGK1* levels upon pharmacological GR challenge test after 4 weeks of treatment with antidepressants in patients with multiple severe recent life events (Menke et al., 2021).

Addressing the presence of environmental stressors in MDD is relevant since patients with ELS show distinct metabolic profiles when compared to non-traumatized depressed patients and healthy subjects, which may be useful for the diagnosis and prognosis of different MDD phenotypes (Ding et al., 2014). Indeed, HPA axis dysfunctions may be more related to ELS than to MDD itself (Ceruso et al., 2020). Regarding ELS subtypes, we found significant correlations between higher baseline transcript levels and maternal neglect. This result is relevant since more "silent" forms of ELS represent a serious burden, being as harmful as other types of maltreatment and representing important risk factors for psychiatric disorders (Mulder et al., 2018; Stoltenborgh et al., 2013). Understanding the molecular impairments of ELS subtypes is important to clarify their individual impact. Indeed, emotional and physical neglect and emotional abuse confer the highest risk for MDD in adults, while other types of abuse, including sexual and physical abuse, although being related to greater MDD susceptibility, are less specific to this disorder (Infurna et al., 2016; Mandelli et al., 2015).

Our longitudinal analysis revealed that trauma-focused psychotherapy induced modifications in transcript levels. *FKBP5* and *SGK1* decreased after 8 weeks of psychotherapy and at follow-up (T12) there was a significant restoration of all transcripts compared to the end of psychotherapy, probably due to a re-exposure to daily stressors with respect to the hospitalization period. Also, psychotherapy may exert an effect on transcript modulations of genes belonging to the same stress axis. Indeed, *NR3C1, FKBP5,* and *SGK1* are intrinsically coordinated: after a threat exposure, glucocorticoids released by the adrenal gland activate the GR, initiating transcriptional responses. When glucocorticoids bind to GR, *FKBP5* is exchanged for another co-chaperone (*FKBP4*) and GR is phosphorylated by kinases, mainly *SGK1*, allowing its activation and translocation into the nucleus (Cattaneo & Riva, 2016). *SGK1* is a serine/threonine kinase regulating cell survival, proliferation, and differentiation (Amato et al., 2009). An emerging role of this kinase in different pathogenic hypotheses of MDD, including the stress hypothesis, has been reviewed (Dattilo et al., 2020). *SGK1* regulates cellular stress responses and neuronal functions and its expression is modulated by glucocorticoids in human neural stem cells and rodent neurons (Anacker et al., 2013). *SGK1* mRNA is increased in the blood of

MDD patients and in the hippocampus of animal models submitted to stress, indicating that *SGK1* mediates glucocorticoids effects on neurogenesis and increases GR response, which is relevant for stress-related disorders (Anacker et al., 2013). Inside the nucleus, GR binds to glucocorticoid-responsive elements in the DNA, activating *FKBP5, SGK1,* and other genes' transcriptions (Cattaneo & Riva, 2016). *FKBP5* transcription by the active GR inside the nucleus induces negative feedback, inactivating the cytosolic GR. *SGK1* transcription increases phosphorylation GR processes in the cytosol, promoting its activation in a positive feedback mechanism. *SGK1* and *FKBP5* expressions are tightly correlated and their function impairments may be a consequence of altered physiological compensations for environmental stressors (Anacker et al., 2013; Menke et al., 2021).

To our knowledge, this is the first study analyzing gene expression effects of trauma-focused psychotherapy in TRD patients. Existing literature focuses on biomarkers of trauma-focused psychotherapies applied to patients with PTSD, proposing basal cortisol levels (Zantvoord et al., 2019), cortisol awakening response (Rapcencu et al., 2017), and cortisol response after dexamethasone suppression (Nijdam et al., 2015) as markers of treatment outcomes. Findings of transcript modulations in depressed patients and controls, before and after CBT, showed that different transcriptional panels distinguished MDD patients from controls, which may be useful in predicting therapeutic response (Redei et al., 2014). These authors found three transcripts differentially expressed in remitted and non-remitted patients after CBT and equal differences in some transcripts before treatment, indicating that expression profiles could predict treatment responses (Redei et al., 2014).

Interestingly, besides findings on transcriptional modulations in response to psychotherapy, we found positive correlations between transcript variations and symptomatologic improvements. Decreased expression levels correlated with symptom improvement, as assessed by different scales. We performed extensive symptomatologic analyses, including depressive and anxiety symptoms, sleep disturbances, and psychosocial functioning. This broad approach is relevant since the objective of MDD treatment is achieving full symptomatic and functional recovery (Habert et al., 2016; McIntyre et al., 2015). Regarding depressive symptoms, we used both BDI-II (Beck, 1993) and MADRS relative dimensions, comprising mood, cognitive, and neurovegetative symptoms (Suzuki et al., 2005). Considering these relative dimensions is important, since MDD symptoms are composed of independent clusters (Higuchi et al., 2008; Paavonen et al., 2014), each linked to distinct neurochemical disturbances (Suzuki et al., 2005). This independent analysis is clinically useful, since other symptoms besides depressive mood may occur independently and represent a stronger impact on life quality, warranting the importance of the factor analysis of depressive symptoms (Ballard et al., 2018; Bech, 2006).

Finally, mRNA baseline levels were associated with disease relapse, a clinically relevant finding that could potentially be explored in predicting disease outcomes. Identifying biomarkers with predictive power to evaluate disease outcomes is a hallmark in MDD research, helping in clinical management and choice of individualized therapies. To date, there is only one molecular study in TRD patients undergoing trauma-focused psychotherapy, showing an involvement in inflammation-related microRNAs in the effect of this treatment approach (Maffioletti et al., 2021) and our study may shed some light in this field. Moreover, evaluating the effectiveness of trauma-focused approaches in MDD is important, since the presence of ELS confers distinct profiles to this subgroup of patients (Infurna et al., 2016).

Some limitations need to be addressed. The small sample size may have an impact on some results, particularly on the analyses dividing subgroups of responders/non-responders and remitters/non-remitters. We did not separately evaluate the subgroups undergoing different kinds of trauma-focused psychotherapy, which would incur in further sample-size limitations. Factors like pharmacological treatments and other psychosocial support received in the hospital setting could have influenced the results in the cohort undergoing trauma-focused psychotherapy. In particular, pharmacological treatments were maintained or slightly adjusted according to clinical needs and it is not possible to address whether the observed transcriptional variations were due to the single effect of trauma-focused psychotherapy. Although controlling for pharmacological therapy was not within the scope of the present study, future analyses would benefit from the comparison of different types of treatment. Further analyses with a larger sample size, preferably conducted with a previous power analysis, as well as with more follow-up visits, may be needed in future studies to

assess longer-term effects of psychotherapy on gene expression modulations. We did not consider gender as a possible factor influencing the results and this important issue should be addressed in future analyses. Also, comparisons between patients undergoing trauma-focused psychotherapy and a control group of patients not receiving this therapeutic approach, in randomized controlled designs, would provide a better understanding of the effects of psychotherapy on clinical outcomes and gene expression modulations. In particular, the absence of a control group may limit the establishment of causal relationships between the observed clinical outcomes of trauma-focused psychotherapy and the transcriptional modulation of the studied genes, which may have been due to interactions of non-studied factors. Lastly, although the selection of the candidate genes has been performed on the basis of well-established evidence in the literature, other genes belonging to the HPA axis or related to the stress-response system should be mentioned and evaluated in future studies. These include genes known to be involved in MDD pathophysiology and in stress exposure, like the corticotropin-releasing hormone receptor 1 (Laucht et al., 2013), mineralocorticoid receptor (*NR3C2;* Gerritsen et al., 2017), and Spindle and Kinetochore-Associated Complex Subunit 2 (Sadeh et al., 2016).

Conclusion

Our study points to peculiar stress-related gene expression signatures associated with ELS in depressed patients, in particular with childhood neglect. This result is relevant since MDD patients exposed to ELS present a distinct phenotype and may benefit from focused therapies. Our findings also reveal that depressive, anxiety, cognitive, and neurovegetative symptom amelioration observed in TRD patients at the end of trauma-focused psychotherapy and maintained at follow-up correlate with reduced transcriptional levels of stress-related genes. Finally, baseline transcriptional levels of stress genes may predict disease relapse, a clinically relevant finding aiding in the identification of molecular biomarkers of therapy outcomes.

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Authors' Contribution. RCS and VD share the first authorship. RCS, VD, EM and AM conceived of the study, participated in its design and the coordination and acquisition of data, performed the statistical analyses, and co-wrote the manuscript; VD performed the statistical analyses; VD; MM and EM participated in the design of the study and carried out gene expression analyses; AM; MB, RB, GP enrolled and screened patients and were involved in the treatment protocol; MG and AC helped draft the manuscript and critically reviewed it for intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

Transcriptional Modulation of Stress-Related Genes in Association With Early Life Stress Exposure and Trauma-Focused Psychotherapy in Treatment-Resistant Depression Patients

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Supplementary Tables:

SUPPLEMENTARY TABLE 1. Comparisons Between Different Timepoints for mRNAs, Significantly Changing dDring Trauma-Focused Psychotherapy (Adjusted *p*-values)

mRNAs	T0–T4	T0–T8	T0–T12	T4–T8	T4–T12	T8–T12
FKBP5	$p = 5.47^{\star}10^{-1}$	$p = 5.46^{\star}10^{-3}$	$p = 2.34^{\star}10^{-5}$	$p = 3.19^{\star}10^{-2}$	$p = 5.46^{\star}10^{-6}$	$p = 1.42^{\star}10^{-11}$
NR3C1	$p = 4.27^{\star}10^{-1}$	$p = 1.16^{\star}10^{-1}$	$p = 4.27^{\star}10^{-1}$	$p = 3.27^{\star}10^{-1}$	$p = 1.93^{\star}10^{-1}$	$p = 2.20^{\star}10^{-2}$
SGK1	$p = 7.93^{\star}10^{-1}$	$p = 3.8^{\star}10^{-2}$	$p = 1.38^{\star}10^{-1}$	$p = 3.47^{\star}10^{-2}$	$p = 2.11^{\star}10^{-1}$	$p = 6.37^{\star}10^{-4}$

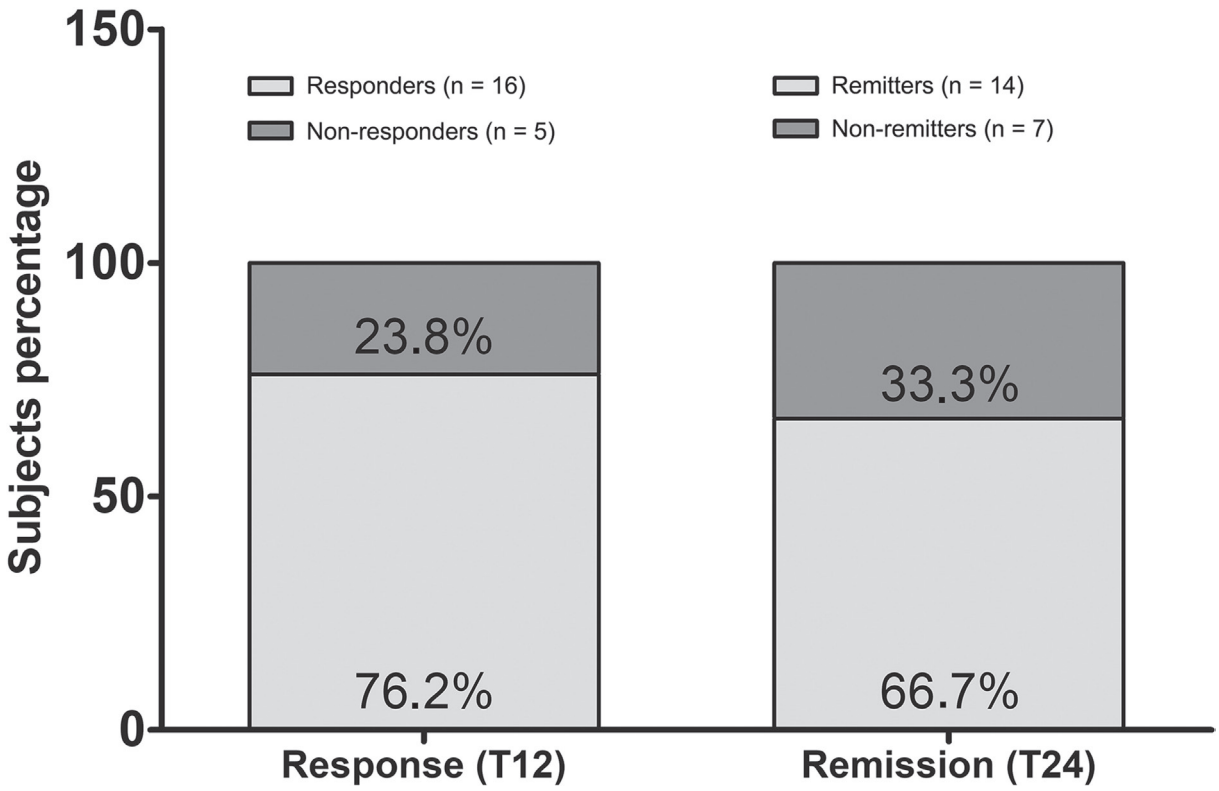
Note. Bold characters indicate significant *p*-values.

SUPPLEMENTARY TABLE 2. Symptomatological Assessments With Diverse Scales

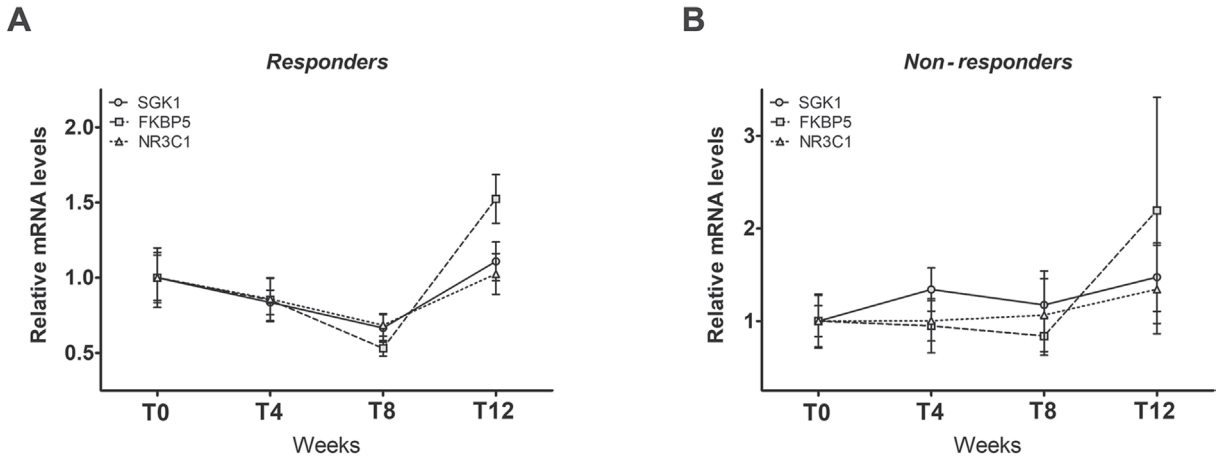
Scale	Time	Time*response	T0–T4	T0–T8	T0–T12	T4–T8	T4–T12	T8–T12
MADRS total score	$p = 1.26*10^{-18}$ F = 61.05	$p = 4.04*10^{-5}$ F = 9.17	T0 = 28 ± 1.54 T4 = 11 ± 1.46 $p < 2*10^{-16}$	T0 = 28 ± 1.54 T8 = 4 ± 0.90 $p < 2*10^{-16}$	T0 = 28 ± 1.54 T12 = 9 ± 1.75 $p < 2*10^{-16}$	T4 = 11 ± 1.46 T8 = 4 ± .90 $p = 9.23*10^{-4}$	T4 = 11 ± 1.46 T12 = 9 ± 1.75 $p = .298$	T8 = 4 ± .90 T12 = 9 ± 1.75 $p = .021$
MADRSF1 score	$p = 5.25*10^{-17}$ F = 51.84	$p = 4.65*10^{-4}$ F = 6.83	T0 = 7 ± .49 T4 = 3 ± .47 $p < 2*10^{-16}$	T0 = 7 ± 0.49 T8 = 1 ± .31 $p < 2*10^{-16}$	T0 = 7 ± .49 T12 = 2 ± .53 p < $2*10^{-16}$	T4 = 3 ± .47 T8 = 1 ± .31 p =.020	T4 = 3 ± .47 T12 = 2 ± .53 $p = .442$	T8 = 1 ± .31 T12 = 2 ± .53 $p = .105$
MADRSF2 score	$p = 9.25*10^{-17}$ F = 50.54	$p = 2.11*10^{-6}$ F = 12.23	T0 = 14 ± 0.72 T4 = 6 ± .88 $p = 4.88*10^{-15}$	T0 = 14 ± .72 T8 = 2 ± .54 $p < 2*10^{-16}$	T0 = 14 ± .72 T12 = 5 ± 1.10 $p < 2*10^{-16}$	T4 = 6 ± .88 T8 = 2 ± .54 $p = 1.81*10^{-4}$	T4 = 6 ± .88 T12 = 5 ± 1.10 $p = .360$	T8 = 2 ± .54 T12 = 5 ± 1.10 $p = .004$
MADRSF3 score	$p = 1.61*10^{-14}$ F = 34.12	$p = .005$ F = 4.64	T0 = 6 ± 0.65 T4 = 2 ± 0.32 $p = 3.27*10^{-13}$	T0 = 6 ± 0.65 T8 = 1 ± 0.31 $p < 2*10^{-16}$	T0 = 6 ± 0.65 T12 = 2 ± .34 $p = 1.33*10^{-15}$	T4 = 2 ± 0.32 T8=1 ± .31 $p = .167$	T4 = 2 ± 0.32 T12=2 ± .34 $p = .451$	T8 = 1 ± 0.31 T12=2 ± .34 $p = .451$
BDI score	$p = 2.08*10^{-13}$ F = 34.88	$p = .018$ F = 3.63	T0 = 36 ± 2.34 T4 = 20 ± 2.53 $p = 1.02*10^{-10}$	T0 = 36 ± 2.34 T8 = 14 ± 1.90 $p < 2*10^{-16}$	T0 = 36 ± 2.34 T12 = 15 ± 2.30 $p < 2*10^{-16}$	T4 = 20 ± 2.53 T8 = 14 ± 1.90 $p = .021$	T4 = 20 ± 2.53 T12 = 15 ± 2.30 $p = .049$	T8 = 14 ± 1.90 T12 = 15 ± 2.30 $p = .672$
BAI score	$p = 1.02*10^{-5}$ F = 10.56	$p = .062$ F = 2.58	T0 = 26 ± 2.73 T4 = 19 ± 2.35 $p = .014$	T0 = 26 ± 2.73 T8 = 12 ± 2.22 $p = 9.41*10^{-7}$	T0 = 26 ± 2.73 T12 = 15 ± 2.23 $p = 4.80*10^{-5}$	T4 = 19 ± 2.35 T8 = 12 ± 2.22 $p = .016$	T4 = 19 ± 2.35 T12 = 15 ± 2.23 $p = .127$	T8 = 12 ± 2.22 T12 = 15 ± 2.23 $p = .352$

Note. BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; MADRS = Montgomery–Åsberg Despression Rating Scale. For each timepoint, scores are represented as mean ± s.e.m. The *p*-values relative to overall variations over time, time*response interactions, and pairwise comparisons (adjusted *p*-values), for all the considered symptomatological scores are shown. Bold characters indicate significant *p*-values and corresponding F values.

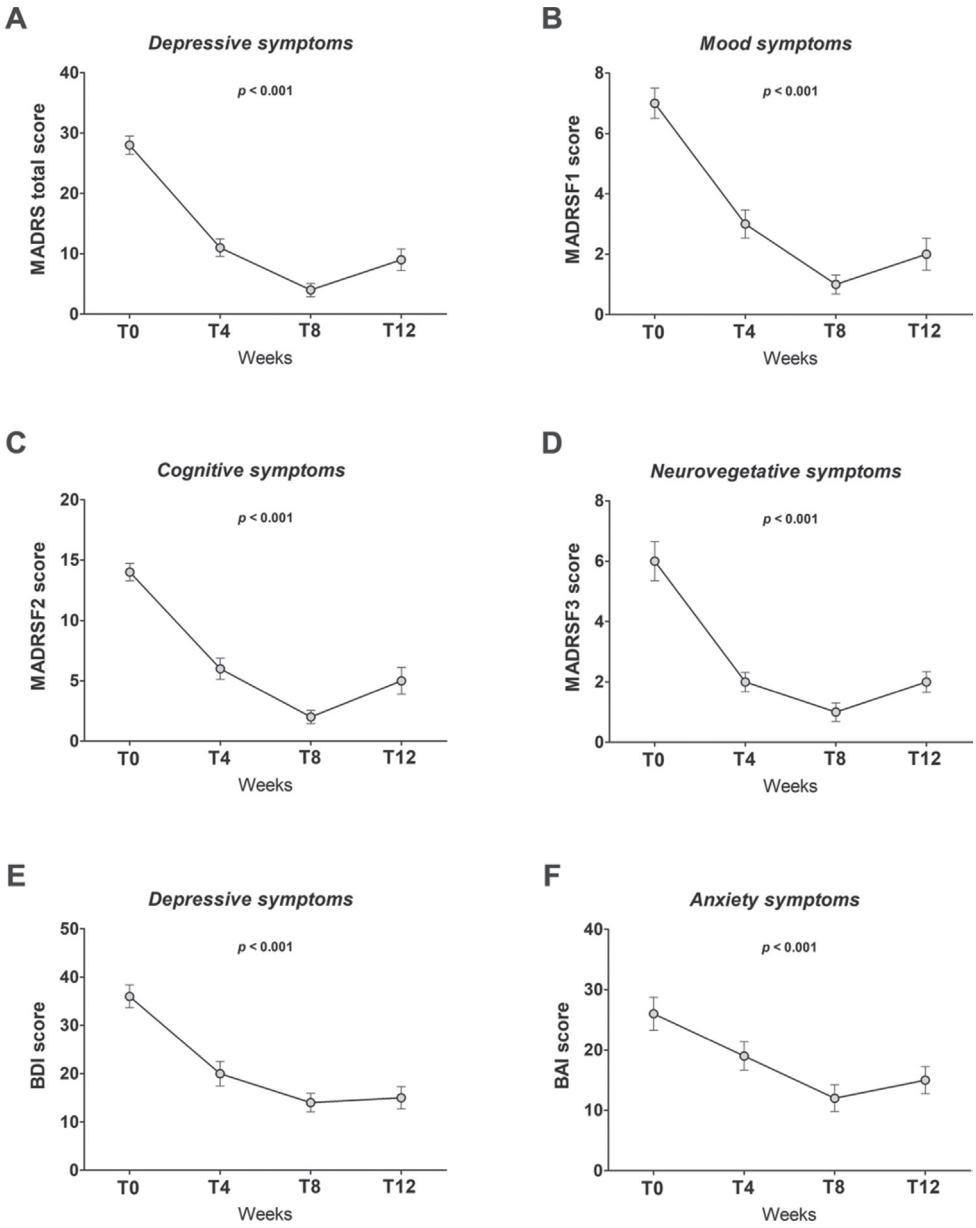
Supplementary Figures



Supplementary Figure 1. Representation of the number and the relative percentage of patients identified as responders or non-responders to psychotherapy at T12 (left column) and as remitters or non-remitters at T24 (right column).



Supplementary Figure 2. Longitudinal analyses of transcriptional changes in stress response-related genes during the 8-weeks of psychotherapy program and at the follow-up (T12) in responder (A) and non-responder patients (B). For each timepoint, the results are represented as relative (to baseline) mRNA levels ± s.e.m.



Supplementary Figure 3. Symptomatological changes over time, in different timepoints, in patients undergoing trauma-focused psychotherapy, assessed by different scales. Symptom's assessments were made using the Montgomery–Åsberg Depression Rating Scale (MADRS) total score (A) and relative dimensions according to the three-factor model, comprising MADRSF1 to evaluate mood symptoms (B), MADRSF2 to depict cognitive symptoms (C), and MADRSF3 to assess neurovegetative symptoms (D). Depressive and anxiety symptoms were evaluated using Beck Depression Inventory II (BDI-II; E) and Beck Anxiety Inventory (BAI; F), respectively.