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Early growth response 1 (EGR1) is downregulated in peripheral blood from patients with major psychiatric disorders

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Abstract

Objectives: To evaluate relative expression of genes with the potential to translate environmental stimuli into long-term alterations in the brain - namely Early Growth Response (EGR)1, EGR3, and Cryptochrome Circadian Regulator 2 (CRY2) - in peripheral blood from patients with Bipolar Disorder (BD), Schizophrenia (SZ), Major Depressive Disorder (MDD) and healthy controls (HC).

Methods: Thirty individuals ranging from 18 to 60 years were recruited for each group (BD, SZ, MDD or HC) from a Brazilian public hospital. Therefore, individuals' peripheral blood was collected and EGR1, EGR3 and CRY2 gene expression analyzed by PCR Real Time.

Results: EGR1 mRNA levels are significantly lower in psychiatric patients when compared to HC, but there is no difference for EGR3 and CRY2. Exploring the findings for each diagnosis, there is a significant difference between each diagnosis group only for EGR1, which was lower in BD, MDD and SZ as compared to HC. No significant correlations were found between gene expression and clinical features.

Conclusions: EGR1 is downregulated in psychiatric patients, regardless of the diagnosis and may be a potential common target in major psychiatric disorders. EGR1, as a transcription factor, modulates many other genes and participates in crucial neuronal and synaptic processes, such as plasticity, neurotransmitters metabolism, vesicular transport and signaling pathways. The study of EGR1 and its upstream regulators in psychiatry might lead to potential new therapeutic targets.

Keywords: Psychiatric disorders; bipolar disorder; schizophrenia; major depressive disorder; early growth response 1.

Introduction

Schizophrenia (SZ), Bipolar Disorder (BD) and Major Depressive Disorder (MDD) are among the leading causes of disability worldwide and usually associated with high morbidity and mortality.[1] The pathophysiology underlying these complex disorders includes both genetic and environmental factors,[2] and the existence of a shared genetic predisposition between diagnoses has become increasingly evident, especially considering those reflecting similar clinical symptoms, risk factors, and drug therapies.[3] As environmental factors have been considered as important triggers for psychiatric illnesses, it has increased the interest in genes with the potential to translate environmental stimuli into long-term alterations in the brain, acting as key regulators of the neuronal gene expression and neural plasticity. In this scenario, members of the immediate early genes (IEGs) transcription factors family, such as the Early Growth Response 1 (EGR1) and the Early Growth Response 3 (EGR3), have been strongly suggested as potential mediators of the genetic and environmental influences in major psychiatric disorders.[4]

EGR1 and EGR3 play important roles in the modulation of neuronal activity and neuroplasticity across the brain, participating in pathways related to learning and memory formation.[5–8] They are expressed at basal levels throughout brain regions under normal physiological conditions and rapidly induced by several stimuli. It is well known that EGR1 may be induced by cell stress, injury, neurotransmitters, cytokines, growth and differentiation factors and other extracellular signals.[5] EGR3 expression is also induced at high levels in response to environmental changes, such as stressful events and sleep deprivation.[9,10]

Acting as transcription factors, these genes modulate downstream targets which play crucial roles for the normal functioning of the brain. This means that changes in EGR1 and EGR3 expression could potentially influence the activity of several biological pathways involved in the pathophysiology of psychiatric disorders. Indeed, EGR1 has

been associated with mood disorders and with SZ,[4,11,12] and has been pointed as an important target in the response to treatment.[13,14] In addition, a growing number of studies have suggested that EGR3 is potentially involved with relevant pathways associated with major psychiatric disorders. [15–20]

The day-night cycle represents another important stimulus when referring to environmental factors and its association with psychiatric illness is well described.[21] The circadian system modulates various physiological activities such as metabolism, hormone secretion, cell proliferation and apoptosis. Cryptochrome Circadian Regulator 2 (CRY2) is one of these circadian clock genes, involved in cell-cycle progression and DNA-damage checkpoint control.[22] Psychiatric disorders are frequently associated with deregulation in circadian rhythm responses, such as cortisol secretion and sleep, and affective symptoms can be exacerbated reflecting a disruption of circadian rhythms.[23] For example, a study reported that patients with BD have lower CRY2 expression when compared to controls,[24] and CRY2 has been associated with MDD and SZ,[25–27] and also with rapid cycling in BD.[28]

Considering the extensive and crucial physiological roles of these genes, a dysfunction in their regulation could impair neuronal pathways and cognitive functions, reflecting in poor outcomes seen in neuropsychiatric illness. Therefore, the aim of this study was to evaluate the gene expression of EGR1, EGR3 and CRY2 in three severe mental illnesses using a transdiagnostic approach, to understand whether these genes show modified expression in major psychiatric disorders in comparison to controls, and if it has diagnostic specificity for SZ, BD or MDD.

Methods

Study design and participants

Four groups of subjects were studied: BD, SZ and MDD patients, and healthy controls. Each group consisted of 30 individuals ranging from 18 to 60 years. Outpatients

were recruited from HCPA ambulatories, and controls from Hemotherapy Service blood bank of the same hospital, in Porto Alegre, Brazil. Inclusion criteria for patients were diagnosis of a major psychiatric disorder (BD, SZ or MDD), with no current substance use disorder, and absence of chronic inflammatory diseases or other severe medical conditions. The diagnosis of psychiatric disorders was established by trained psychiatrists based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), using the Structured Clinical Interview of the DSM-5 for BD and SZ; and based on DSM-IV using Mini-International Neuropsychiatric Interview (MINI) for MDD. Controls were individuals with no history of psychiatric or neurologic illnesses, no use of illicit substances, and absence of chronic inflammatory diseases or other severe medical conditions. One control individual did not answer the questionnaires properly, and for this reason was excluded from the analyses. The Research Ethics Committee of Hospital de Clínicas de Porto Alegre (HCPA) approved the research protocol (2019-0025). All participants provided written informed consent prior to enrollment in the study.

All patients and controls were interviewed to assess socio-demographic variables related to sex, age, and education. Moreover, all patients undergone clinical interviews when clinical variables were assessed, such as age of onset, duration of illness, hospitalizations, number of episodes (when applicable), suicide attempt, comorbidities, smoking and alcohol and other substances use. Patients were also assessed using clinical scales as follows: for BD: (a) HDRS (Hamilton Depression Rating Scale), (b) CGI (Clinical Global Impression), (c) YMRS (Young Mania Rating Scale), (d) MINI (Mini-International Neuropsychiatric Interview); for SZ: (a) Calgary Depression Scale for Schizophrenia, (b) CGI, (c) PANSS (Positive and Negative Syndrome Scale); and for MDD: (a) HDRS, (b) CGI, (c) MINI, (d) BDI (Beck's Depression Inventory), and (e) CTQ (Childhood Trauma Questionnaire).

Total RNA isolation and complementary DNA preparation

Peripheral blood was collected from patients and controls in vacutainer tubes containing EDTA and the whole blood was processed within 2 hours of the collection. 500 µL of anticoagulated blood was added to 1.3 mL of RNA*later* stabilization solution and mixed thoroughly. Samples mixed with RNA*later* were stored at -80 °C until RNA isolation. RNA was extracted using a RiboPure-Blood Kit (Invitrogen - AM1928) according to the manufacturer's protocol. RNA quantification was determined using a Qubit RNA HS Assay Kit (Invitrogen - Q32855) with the Qubit Fluorometer. Total RNA samples were spectrophotometrically scanned (260 and 280 nm; Nanodrop ND-1000, Wilmington, DE, USA). The A260/A280 ratio was > 1.9, excluding relevant protein contamination. 300 ng of each RNA sample was used for complementary DNA (cDNA) synthesis using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems – 4368814) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Five potential reference genes were tested - namely RPLPO (Large Ribosomal Protein), RRN18S (18S ribosomal RNA), B2M (Beta-2-Microglobulin), ACTB (Actin Beta) and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) - to select the most stable gene (the one which presented stable expression levels in RNA isolated from peripheral blood) as the endogenous control. B2M showed the best performance, then this housekeeping gene was run together with each target gene and used in the analyses to perform normalization procedures. Pre-designed TaqMan Gene Expression Assays (Applied Biosystems, Foster City, California) were chosen for the target genes: early growth response 1 (EGR1, Hs00152928_m1), early growth response 3 (EGR3, Hs00231780_m1), and cryptochrome circadian regulator 2 (CRY2, Hs00323654_m1). Approximately 70 ng of the cDNA was combined with Taqman PCR Master Mix (QuatroG, Porto Alegre, RS, BR - 100082), a pre-designed TaqMan Gene Expression

Assay, and B2M. qRT-PCR was performed with a QuantStudio 3 Real-Time PCR System (Applied Biosystems) with a 96-well format for B2M and the three target genes. All PCR measurements were performed in triplicate. Control wells containing no cDNA template showed no amplification. The threshold cycle (Ct) was automatically determined from amplification plots and gene expression was quantified using the relative threshold method (Crt) with B2M as the endogenous control. Delta cycle relative threshold values ($\Delta Crt = Crt_{target\ gene} - Crt_{endogenous\ control}$) were calculated for each sample and $2^{-\Delta Crt}$ values were included in the R software for analysis (version 4.1.1).

Statistical analyses

All data were analyzed using R (version 4.1.1) and the interface Rstudio 1.4. First, demographic and clinical variables were compared between the groups using Kruskal-Wallis rank sum test, Pearson's Chi-squared test, and Fisher's exact test. Second, $2^{-\Delta Crt}$ values were normalized using the R package bestnormalize (version 1.8.2) and the differences in normalized mRNA expression detected by qPCR were tested using Multivariate analysis of variance (MANOVA) post hoc Tukey, followed by analysis of variance (ANOVA) for each gene. Third, linear regression was used to confirm the differences among groups controlling for demographic variables (age, sex and education). $p < 0.05$ was considered significant. Each gene was investigated to compare controls versus patients (regardless the psychiatric diagnosis) and after separately for every psychiatric disorder versus controls, and data were expressed as median ± interquartile interval. The correlation of mRNA levels with clinical parameters and psychiatric medications was tested by Pearson's correlation test. Receiver-operating characteristic (ROC) curves were performed to evaluate the capacity of each gene to predict the groups.

Results

Demographic and clinical data

Demographic and clinical variables were presented in Table 1. There were significant differences in age, sex, and years of education between groups, thus the following analyses were controlled for such variables. There was no significant difference among the groups of patients regarding duration of illness and CGI. On clinical evaluation, our sample showed moderate depressive symptoms in those with BD (HDRS = 17.83 (±5.75); YMRS = 2.47 (±2.47)), moderate to severe depressive symptoms in those with MDD (HDRS = 21.30 (±4.91)) and mildly to moderately ill in those with SZ (PANSS = 61.04 (±16.67)). All patients were taking psychiatric medications (antidepressants, mood stabilizers, antipsychotics and/or benzodiazepines), and mostly more than one psychiatric medication, as detailed in Supplementary Figure 1. However, the small sample size in each medication group precluded the study of medication effects on the outcomes). As expected for a transdiagnostic sample, medications were different in each diagnostic group, with some overlap (Supplementary Table 1). In addition, it is noteworthy that 90% of patients with MDD were taking antidepressants (mostly selective serotonin reuptake inhibitors), 50% of individuals with BD were taking lithium, and 100% of patients with SZ were taking antipsychotics (of these, 83% were using clozapine).

Table 1. Demographic and clinical characteristics of participants.

	Control n = 29	MDD n = 30	BD n = 30	SZ n = 30	p-value
Age, mean (SD)	39.69 (9.52)	47.14 (8.88)	43.73 (12.54)	42.10 (9.36)	0.038 [a]
Sex, n (%)					<0.001 [b]
Female	15 (52%)	21 (70%)	22 (73%)	5 (17%)	
Male	14 (48%)	9 (30%)	8 (27%)	25 (83%)	
Years of education, mean (SD)	11.36 (3.96)	8.41 (4.74)	12.77 (4.16)	9.21 (3.19)	<0.001 [a]

Age at onset in years, mean (SD)	n/a	28.97 (13.00)	21.30 (9.22)	21.10 (6.33)	0.032 [a]
Duration of illness in years, mean (SD)	n/a	18.00 (12.69)	22.43 (12.22)	21.41 (9.20)	0.300 [a]
CGI, median [interquartile range]	n/a	5 [4 - 5]	5 [4 - 5]	4 [3.5 - 5]	0.117 [a]

Columns show Mean (SD) for all categories, except for sex and CGI. Data are presented n (%) for variable sex, and median [median of lower half – median of upper half] for variable CGI.

BD, Bipolar Disorder; MDD, Major Depressive Disorder; SZ, Schizophrenia; CGI, Clinical Global Impression Scale; SD, Standard Deviation.

[a] Kruskal-Wallis rank sum test; [b] Pearson's Chi-squared test.

EGR1, EGR3 and CRY2 gene expression in psychiatric patients versus healthy controls

Using RT-qPCR, we compared the EGR1, EGR3 and CRY2 mRNA levels in whole blood between psychiatric patients and controls. Our first analysis, from a transdiagnostic perspective, we compared EGR1, EGR3 and CRY2 mRNA levels between all psychiatric patients independent of diagnostic group and controls. Figure 1 and Supplementary Table 2 shows that EGR1 mRNA levels are significantly lower in psychiatric patients compared to healthy controls (Bonferroni p-value < 0.001), but there was no difference for EGR3 (Bonferroni p-value = 0.738) and CRY2 (Bonferroni p-value = 1.00) transcripts when comparing these two groups. Linear regression results shown that there were no gender-related differences in EGR1 mRNA levels among all participants (p-value = 0.918).

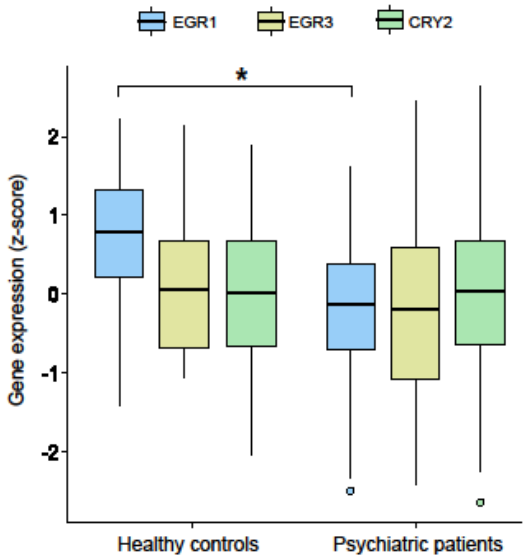

Figure 1.

Table 2. MANOVA results of genes assessed as a function of four groups (healthy controls, MDD, BD, and SZ).

Gene	F value	Num DF	Den DF	p-value	Bonferroni p-value
EGR1	9.74	3	116	< 0.001	< 0.001
EGR3	0.93	3	116	0.429	1.000
CRY2	0.491	3	116	0.689	1.000

Num DF, numerator degrees of freedom; Den DF, denominator degrees of freedom.
EGR1= Early Growth Response 1; EGR3= Early Growth Response 3; CRY2= Cryptochrome Circadian Regulator 2.

EGR1, EGR3 and CRY2 gene expression in the individual diagnoses (BD, MDD and SZ) versus healthy controls

Further we examined if changes in EGR1 mRNA levels or in EGR3 or CRY2 expression were particularly associated with any of the three diagnoses. When we grouped the psychiatric patients by diagnosis (BD, MDD or SZ), there was a significant difference between groups only for EGR1 ($p < 0.001$) (Table 2). EGR1 transcripts were

significantly lower in BD ($p < 0.001$), MDD ($p < 0.001$), and SZ ($p = 0.026$) compared to healthy controls controlling for age, sex, and education (Figure 2 and Supplementary Table 3).

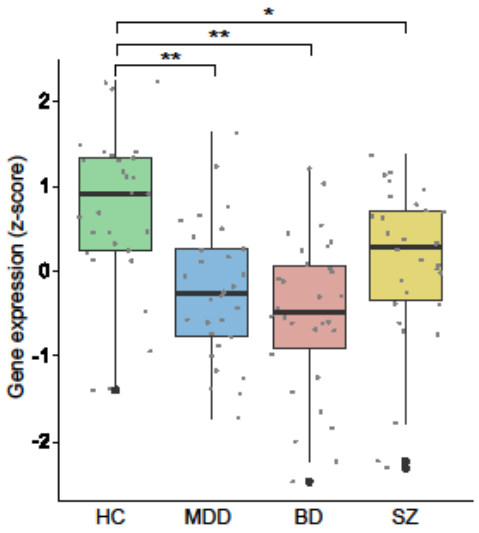

Figure 2.

EGR1, EGR3 and CRY2 gene expression to differentiate patients from healthy controls

In line with the idea of a transdiagnostic approach, we also tested the ability of the three genes to differentiate patients from controls, regardless of the psychiatric diagnosis (BD, MDD or SZ). The results showed a classification that achieved an Area Under the Curve (AUC) of 0.78 (0.67 – 0.88), with 0.91 sensitivity and 0.45 specificity for EGR1; AUC of 0.50 (0.37 – 0.63), with 0.46 sensitivity and 0.54 specificity for EGR3; and AUC of 0.49 (0.37 – 0.62), with 0.5 sensitivity and 0.48 specificity for CRY2. None of these genes presented a good score, nonetheless once again EGR1 showed the best performance to differentiate psychiatric patients from healthy controls (Figure 3).

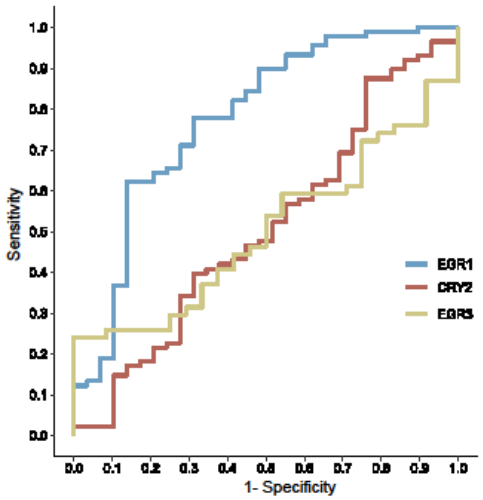

Figure 3.

Moreover, as EGR1 showed the best performance, we decided to explore further each diagnosis separately versus HC to check if AUC would improve. We observed that a better AUC score was obtained only for Controls x BD, with an AUC of 0.84 with 0.70 sensitivity and 0.86 specificity. Controls x MDD shows an AUC of 0.79 with 0.63 sensitivity and 0.86 specificity; and Controls x SZ an AUC of 0.70 with 0.36 sensitivity and 0.86 specificity (Figure 4). We also tried to use EGR1 data to differentiate the disorders (BD, MDD, and SZ), but this approach did not return good results (Supplementary Figure 2).

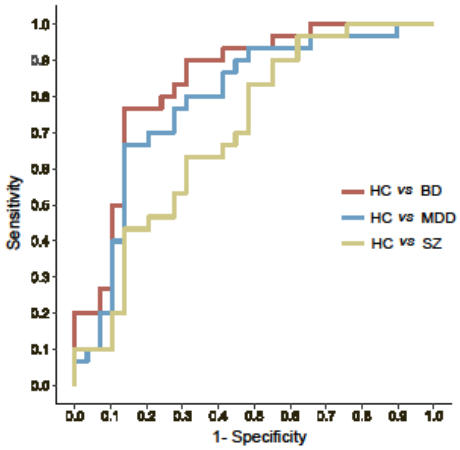

Figure 4.

Effects of medications and clinical characteristics on EGR1, EGR3 and CRY2 mRNA levels

No significant correlations were found between ΔCrt values for the three genes analyzed and clinical parameters, such as specific scales for each disorder. Regarding BD, for example, HDRS, CGI, YMRS and MINI had no correlation with ΔCrt values for EGR1, EGR3 or CRY2. No significant correlations were observed in SZ between Calgary Depression Scale for Schizophrenia, CGI, or PANSS and the ΔCrt values of these three genes; nor in MDD between HDRS, CGI, MINI, BDI, or CTQ and the ΔCrt values for EGR1, EGR3 or CRY2. Furthermore, logistic regression also did not show a significant association between ΔCrt values and duration of illness, age at onset, years of education, or the use of any psychiatric medication.

Discussion

The field in search of biological markers for psychopathology have used different approaches, from investigating a common pathway in a transdiagnostic approach to identifying molecular signatures of major diagnosis, such as SZ, BD and MDD. The investigation of biomarkers in psychiatry is strongly relevant since they represent biological features of the diseases that can be objectively assessed and might be useful in the clinical practice to support both the diagnosis and the prediction of clinical outcomes related to the course of the illness. Biomarkers could also potentially suggest therapies most likely to be effective to a particular patient, facilitating a personalized treatment. [29] In this study, EGR1, EGR3 and CRY2 were selected based on their potential to mediate gene-environment effects on psychopathology and based on previously reported association with psychiatric disorders,[5,11,12,24,28,30–33] including studies from our group.[15,20]

Our major finding is the downregulation in EGR1 expression in major psychiatric disorders as compared to healthy controls when applying a transdiagnostic approach, indicating that EGR1 may be part of a putative pathway underlying the common physiopathology of such illnesses. When considering the disorders individually, the decrease in EGR1 expression was consistent in BD, MDD and SZ. *Egr1* is a transcription factor involved in neuron maturation[34] *via* N-methyl-D-aspartate (NMDA) dependent hippocampal synaptic plasticity,[35] essential for memory consolidation and reconsolidation.[36] *Egr1* is expressed throughout the brain, maintaining baseline expression levels in areas connected to the control of social and emotion-driven behavior, sensitivity to reward, long-term memory, and cognition.[31] *Egr1*'s transcription is indirectly regulated by stress or learning tasks, leading to changes in neuronal activity, hormones secretion and growth factors release.[31] Furthermore, *Egr1* has the potential to regulate an array of target genes implicated in biological functions linked to synaptic

plasticity, such as neurotransmitters metabolism, vesicular transport and signaling pathways.[31,37]

Therefore, impairments in the functioning of EGR1 may be critical to the cognitive symptoms often described in psychiatric conditions, especially considering that EGR1 acts in the integration of environmental signals at the synaptic plasticity level to modulate central processes such as learning and memory.[31] Sun and colleagues recently predicted that *Egr1* plays a crucial role in brain epigenetic programming,[38] which refers to the process of how life events can leave sustainable marks in the brain.

Our findings showing reduced EGR1 mRNA levels in the blood of SZ individuals compared to controls corroborate with other studies.[39,40] On the other hand, a study investigating transcriptional signatures in major psychiatry disorders reported EGR1 upregulation in fibroblasts and whole blood from SZ patients experiencing elevated psychotic states compared to controls, and this altered EGR1 expression was not observed in MDD and BD.[41] Although conflicting findings, this evidence suggests an involvement of the EGR1 gene in the pathophysiology of SZ.

In addition, it has been shown that EGR1 is significantly downregulated in brain regions, especially the prefrontal cortex of SZ patients.[12,42,43] In MDD, similar data were reported by Covington and colleagues, who found reduced EGR1 expression in the medial prefrontal cortex in non-medicated subjects as well as in depressed patients refractory to treatment, consistent with a deficit in neuronal activity in this brain region reported in MDD.[44] In preclinical studies, *Egr1* mRNA and protein levels were reported to be reduced in the frontal cortex of mice submitted to social isolation stress,[45] and chronic stress caused a reduced expression of *Egr1* in the hippocampus[46] and medial prefrontal cortex,[44] besides an increase of *Egr1* mRNA in the lateral amygdala of rodents.[47] Interestingly, a recent study highlighted the downregulation of *Egr1* as the top dysregulated gene among a thousand of genes involved in depression in a combined portrait of depression, suggesting an impaired neuronal activity.[48] Furthermore, in a

recent study using systems biology approach we found that EGR1 was consistently repressed in the dorsolateral prefrontal cortex of BD, MDD, and SZ patients.[15]

Egr1 has an important role in synaptic plasticity and may participate in the augmentation of apical and total dendritic spine density, together with activity-regulated cytoskeleton-associated protein (*Arc)*, which is another IEG and considered a synaptic plasticity gene.[49] Studies have pointed that synaptic plasticity is reduced in BD, MDD and SZ, what has been evidenced by loss of dendritic spines and dendrites or decreased synapse number.[50–52] In this sense, a reduced expression of *Egr1*, considering it controls several other genes and, ultimately, entire biological pathways, may contribute to the impaired synaptic plasticity reported in psychiatric disorders.

It is well known that SZ, BD and MDD have a relevant genetic component and that environmental factors also play crucial roles in the development and pathophysiology of these illnesses.[31] Alterations in the expression of *Egr1* can change across the central nervous system depending on the intensity, duration, and nature of the stress.[5] Therefore, *Egr1* may represent a connection between early environmental stimuli (such as stressful experiences) and predisposition to psychiatric disorders in adolescence and adulthood, acting through pathways involving neuronal response to stress via dendritic spine arrangement. We know that adverse life events are associated with mood episodes and psychopathology age of onset, as well as with severity of symptoms and comorbidities.

Differently from EGR1, we did not find any difference in EGR3 expression levels between controls and psychiatric patients. EGR family includes four members, the discrepancies in the gene expression pattern of EGR3 and EGR1 between patients and healthy controls in our study could be related to their distinct upstream regulatory pathways, activators, and targets.[31] We also did not find any significant changes between groups regarding CRY2 expression. It is possible that medications could have influenced their differential expression between groups. It is essential to highlight that our sample is

from a tertiary care hospital, reference for the treatment of severe psychiatric illness in the public health care system, thus most patients are at an advanced stage of the illness progression and are taking multiple drugs, which can interact impacting in the gene expression from numerous biological pathways, and this may have influenced the results. For instance, EGR3 can be upregulated by antipsychotic medications[53] and CRY2 by lithium.[54] It is not possible to rule out the potential medication effects on mRNA levels of the three studied genes. Regarding the effects of medication on EGR1, we believe that its gene expression levels could have been found even more reduced in a drug-free scenario since the medications potentially increase EGR1 mRNA levels. For instance, studies pointed that *Egr1* transcripts were upregulated by lithium in mouse frontal cortex[13] and by valproic acid in neural stem cells,[55] which indicates this pathway is modulated by these drugs considering that *Egr1* stimulates synaptic plasticity and such mood stabilizers reverse synaptic plasticity deficits.[56–58] Moreover, antipsychotics have been suggested as inducers of *Egr1* expression. Increased expression levels of *Egr1* in the striatum and nucleus accumbens of rodents were observed after acute clozapine or haloperidol,[59] or after acute olanzapine, asenapine or haloperidol administration,[60] as well as with acute and chronic low dose of lurasidone.[61]

Our study has some limitations which merit comment. First, we measured EGR1, EGR3 and CRY2 expression in the whole blood, and it is not possible to correlate with the expression pattern in the brain. Second, it is not possible to control nor evaluate the influence of medications and licit drugs (including alcohol) taken by the patients on the gene expression levels. Third, our patient sample is composed of individuals from a tertiary care hospital, which means they are usually critically ill patients, frequently with comorbidities. Fourth, as a cross-sectional study, we cannot suggest if observed molecular changes in EGR1 are present before illness onset or if these changes are a consequence of chronic course of the psychiatric disorders. Fifth, the small sample size may restrict some analysis. Therefore, studies with larger sample sizes are warranted

aiming to replicate these results and shed light through the influence of these genes and related pathways on major psychiatric disorders.

Finally, the results from our study together with the current literature suggest that EGR1 pathway has a key role in mediating the environmental influences and the gene expression regulation associated with major psychiatric disorders. EGR1 and its pathway could be a potential novel target for therapeutic interventions and deserves further investigation. Noteworthy, EGR1 expression is regulated by complex mechanisms, and future research may include the investigation of its direct or indirect regulators in the psychiatry field.

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References

1 Bonadiman CSC, Passos VM de A, Mooney M, Naghavi M, Melo APS, Bonadiman CSC, et al. The Burden of disease attributable to mental and substance use disorders in Brazil: Global Burden of Disease Study, 1990 and 2015. Revista Brasileira de Epidemiologia. 2017;20:191–204.
2 Darby MM, Yolken RH, Sabunciyan S. Consistently altered expression of gene sets in postmortem brains of individuals with major psychiatric disorders. Transl Psychiatry. 2016;6:e890.
3 Pinto JV, Moulin TC, Amaral OB. On the transdiagnostic nature of peripheral biomarkers in major psychiatric disorders: A systematic review. Neurosci Biobehav Rev. 2017;83:97–108.
4 Marballi KK, Gallitano AL. Immediate Early Genes Anchor a Biological Pathway of Proteins Required for Memory Formation, Long-Term Depression and Risk for Schizophrenia. Front Behav Neurosci. 2018;12.
5 Gallo FT, Katche C, Morici JF, Medina JH, Weisstaub NV. Immediate Early Genes, Memory and Psychiatric Disorders: Focus on c-Fos, Egr1 and Arc. Front Behav Neurosci. 2018;12:79.
6 Lamprecht R, LeDoux J. Structural plasticity and memory. Nat Rev Neurosci. 2004;5:45–54.

7 Li L, Yun SH, Keblesh J, Trommer BL, Xiong H, Radulovic J, et al. Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. Mol Cell Neurosci. 2007;35:76–88.
8 Minatohara K, Akiyoshi M, Okuno H. Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. Frontiers in Molecular Neuroscience. 2016;8:78.
9 Honkaniemi J, Zhang JS, Longo FM, Sharp FR. Stress induces zinc finger immediate early genes in the rat adrenal gland. Brain Res. 2000;877:203–8.
10 Thompson CL, Wisor JP, Lee C-K, Pathak SD, Gerashchenko D, Smith KA, et al. Molecular and anatomical signatures of sleep deprivation in the mouse brain. Front Neurosci. 2010;4:165.
11 Etemadikhah M, Niazi A, Wetterberg L, Feuk L. Transcriptome analysis of fibroblasts from schizophrenia patients reveals differential expression of schizophrenia-related genes. Sci Rep. 2020;10:630.
12 Ramaker RC, Bowling KM, Lasseigne BN, Hagenauer MH, Hardigan AA, Davis NS, et al. Post-mortem molecular profiling of three psychiatric disorders. Genome Med. 2017;9:72.
13 Kim SH, Yu HS, Park HG, Ahn YM, Kim YS, Lee YH, et al. Egr1 regulates lithium-induced transcription of the Period 2 (PER2) gene. Biochim Biophys Acta. 2013;1832:1969–79.
14 Liu S, Zhang F, Shugart YY, Yang L, Li X, Liu Z, et al. The early growth response protein 1-miR-30a-5p-neurogenic differentiation factor 1 axis as a novel biomarker for schizophrenia diagnosis and treatment monitoring. Transl Psychiatry. 2017;7:e998.
15 Bristot G, De Bastiani MA, Pfaffenseller B, Kapczinski F, Kauer-Sant'Anna M. Gene Regulatory Network of Dorsolateral Prefrontal Cortex: a Master Regulator Analysis of Major Psychiatric Disorders. Mol Neurobiol. 2020;57:1305–16.
16 Gallitano AL, Tillman R, Dinu V, Geller B. Family-based association study of early growth response gene 3 with child bipolar I disorder. J Affect Disord. 2012;138:387–96.
17 Maple AM, Rowe RK, Lifshitz J, Fernandez F, Gallitano AL. Influence of Schizophrenia-Associated Gene Egr3 on Sleep Behavior and Circadian Rhythms in Mice. J Biol Rhythms. 2018;33:662–70.
18 Nie F, Zhang Q, Ma J, Wang P, Gu R, Han J, et al. Schizophrenia risk candidate EGR3 is a novel transcriptional regulator of RELN and regulates neurite outgrowth via the Reelin signal pathway in vitro. J Neurochem. 2021;157:1745–58.
19 Pfaffenseller B, Kapczinski F, Gallitano AL, Klamt F. EGR3 Immediate Early Gene and the Brain-Derived Neurotrophic Factor in Bipolar Disorder. Front Behav Neurosci. 2018;12:15.
20 Pfaffenseller B, da Silva Magalhães PV, De Bastiani MA, Castro M a. A, Gallitano AL, Kapczinski F, et al. Differential expression of transcriptional regulatory units in the prefrontal cortex of patients with bipolar disorder: potential role of early growth response gene 3. Transl Psychiatry. 2016;6:e805.
21 Boland EM, Alloy LB. Sleep disturbance and cognitive deficits in bipolar disorder: toward an integrated examination of disorder maintenance and functional impairment. Clin Psychol Rev. 2013;33:33–44.
22 Tamanini F, Chaves I, Bajek MI, van der Horst GTJ. Structure function analysis of mammalian cryptochromes. Cold Spring Harb Symp Quant Biol. 2007;72:133–9.
23 Walker WH, Walton JC, DeVries AC, Nelson RJ. Circadian rhythm disruption and mental health. Transl Psychiatry. 2020;10:28.
24 Lavebratt C, Sjöholm LK, Soronen P, Paunio T, Vawter MP, Bunney WE, et al. CRY2 Is Associated with Depression. PLoS One. 2010;5.
25 Johansson A-S, Owe-Larsson B, Hetta J, Lundkvist GB. Altered circadian clock gene expression in patients with schizophrenia. Schizophr Res. 2016;174:17–23.
26 Kovanen L, Donner K, Kaunisto M, Partonen T. PRKCDBP (CAVIN3) and CRY2 associate with major depressive disorder. J Affect Disord. 2017;207:136–40.

27 Kovanen L, Kaunisto M, Donner K, Saarikoski ST, Partonen T. CRY2 Genetic Variants Associate with Dysthymia. PLoS One. 2013;8:e71450.
28 Sjöholm LK, Backlund L, Cheteh EH, Ek IR, Frisén L, Schalling M, et al. CRY2 is associated with rapid cycling in bipolar disorder patients. PLoS ONE. 2010;5:e12632.
29 García-Gutiérrez MS, Navarrete F, Sala F, Gasparyan A, Austrich-Olivares A, Manzanares J. Biomarkers in Psychiatry: Concept, Definition, Types and Relevance to the Clinical Reality. Frontiers in Psychiatry. 2020;11.
30 Charrier A, Olliac B, Roubertoux P, Tordjman S. Clock Genes and Altered Sleep–Wake Rhythms: Their Role in the Development of Psychiatric Disorders. Int J Mol Sci. 2017;18:938.
31 Duclot F, Kabbaj M. The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. Front Behav Neurosci. 2017;11.
32 Fiedorowicz JG, Coryell WH, Akhter A, Ellingrod VL. Chryptochrome 2 Variants, Chronicity, and Seasonality of Mood Disorders. Psychiatr Genet. 2012;22:305–6.
33 Huentelman MJ, Muppana L, Corneveaux JJ, Dinu V, Pruzin JJ, Reiman R, et al. Association of SNPs in EGR3 and ARC with Schizophrenia Supports a Biological Pathway for Schizophrenia Risk. PLoS One. 2015;10:e0135076.
34 Veyrac A, Gros A, Bruel-Jungerman E, Rochefort C, Kleine Borgmann FB, Jessberger S, et al. Zif268/egr1 gene controls the selection, maturation and functional integration of adult hippocampal newborn neurons by learning. Proc Natl Acad Sci U S A. 2013;110:7062–7.
35 Mokin M, Keifer J. Expression of the immediate-early gene-encoded protein Egr-1 (zif268) during in vitro classical conditioning. Learn Mem. 2005;12:144–9.
36 Jones MW, Errington ML, French PJ, Fine A, Bliss TV, Garel S, et al. A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. Nat Neurosci. 2001;4:289–96.
37 Koldamova R, Schug J, Lefterova M, Cronican AA, Fitz NF, Davenport FA, et al. Genome-wide approaches reveal EGR1-controlled regulatory networks associated with neurodegeneration. Neurobiol Dis. 2014;63:107–14.
38 Sun Z, Xu X, He J, Murray A, Sun M, Wei X, et al. EGR1 recruits TET1 to shape the brain methylome during development and upon neuronal activity. Nat Commun. 2019;10:3892.
39 Liu S, Zhang F, Shugart YY, Yang L, Li X, Liu Z, et al. The early growth response protein 1-miR-30a-5p-neurogenic differentiation factor 1 axis as a novel biomarker for schizophrenia diagnosis and treatment monitoring. Transl Psychiatry. 2017;7:e998.
40 Xu Y, Yue W, Yao Shugart Y, Li S, Cai L, Li Q, et al. Exploring Transcription Factors-microRNAs Co-regulation Networks in Schizophrenia. Schizophr Bull. 2016;42:1037–45.
41 Cattane N, Minelli A, Milanesi E, Maj C, Bignotti S, Bortolomasi M, et al. Altered Gene Expression in Schizophrenia: Findings from Transcriptional Signatures in Fibroblasts and Blood. PLOS ONE. 2015;10:e0116686.
42 Pérez-Santiago J, Diez-Alarcia R, Callado LF, Zhang JX, Chana G, White CH, et al. A combined analysis of microarray gene expression studies of the human prefrontal cortex identifies genes implicated in schizophrenia. J Psychiatr Res. 2012;46:1464–74.
43 Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang J-J, Griffin JL, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry. 2004;9:684–97.
44 Covington HE, Lobo MK, Maze I, Vialou V, Hyman JM, Zaman S, et al. Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. J Neurosci. 2010;30:16082–90.
45 Matsumoto K, Ono K, Ouchi H, Tsushima R, Murakami Y. Social isolation stress down-regulates cortical early growth response 1 (Egr-1) expression in mice. Neuroscience Research. 2012;73:257–62.
46 Xu Y, Pan J, Sun J, Ding L, Ruan L, Reed M, et al. Inhibition of phosphodiesterase 2 reverses impaired cognition and neuronal remodeling caused by chronic stress. Neurobiol Aging. 2015;36:955–70.

47 Monsey MS, Boyle LM, Zhang ML, Nguyen CP, Kronman HG, Ota KT, et al. Chronic Corticosterone Exposure Persistently Elevates the Expression of Memory-Related Genes in the Lateral Amygdala and Enhances the Consolidation of a Pavlovian Fear Memory. PLOS ONE. 2014;9:e91530.
48 Gammie SC. Creation of a gene expression portrait of depression and its application for identifying potential treatments. Sci Rep. 2021;11:3829.
49 Puang SJ, Elanggovan B, Ching T, Sng JCG. MEF2C and HDAC5 regulate Egr1 and Arc genes to increase dendritic spine density and complexity in early enriched environment. Neuronal Signal. 2020;4:NS20190147.
50 Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch Gen Psychiatry. 2000;57:65–73.
51 Kang HJ, Voleti B, Hajszan T, Rajkowska G, Stockmeier CA, Licznerski P, et al. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat Med. 2012;18:1413–7.
52 Konopaske GT, Lange N, Coyle JT, Benes FM. Prefrontal cortical dendritic spine pathology in schizophrenia and bipolar disorder. JAMA Psychiatry. 2014;71:1323–31.
53 Yamagata K, Kaufmann WE, Lanahan A, Papapavlou M, Barnes CA, Andreasson KI, et al. Egr3/Pilot, a zinc finger transcription factor, is rapidly regulated by activity in brain neurons and colocalizes with Egr1/zif268. Learn Mem. 1994;1:140–52.
54 Geoffroy PA, Curis E, Courtin C, Moreira J, Morvillers T, Etain B, et al. Lithium response in bipolar disorders and core clock genes expression. The World Journal of Biological Psychiatry. 2018;19:619–32.
55 Almutawaa W, Kang NH, Pan Y, Niles LP. Induction of neurotrophic and differentiation factors in neural stem cells by valproic acid. Basic Clin Pharmacol Toxicol. 2014;115:216–21.
56 Choi CH, Schoenfeld BP, Bell AJ, Hinchey P, Kollaros M, Gertner MJ, et al. Pharmacological reversal of synaptic plasticity deficits in the mouse model of Fragile X syndrome by group II mGluR antagonist or lithium treatment. Brain Research. 2011;1380:106–19.
57 Gray NA, Zhou R, Du J, Moore GJ, Manji HK. The use of mood stabilizers as plasticity enhancers in the treatment of neuropsychiatric disorders. J Clin Psychiatry. 2003;64 Suppl 5:3–17.
58 Nanavati D, Austin DR, Catapano LA, Luckenbaugh DA, Dosemeci A, Manji HK, et al. The effects of chronic treatment with mood stabilizers on the rat hippocampal post-synaptic density proteome. Journal of Neurochemistry. 2011;119:617–29.
59 Nguyen TV, Kosofsky BE, Birnbaum R, Cohen BM, Hyman SE. Differential expression of c-fos and zif268 in rat striatum after haloperidol, clozapine, and amphetamine. Proc Natl Acad Sci U S A. 1992;89:4270–4.
60 de Bartolomeis A, Iasevoli F, Marmo F, Buonaguro EF, Eramo A, Rossi R, et al. Progressive recruitment of cortical and striatal regions by inducible postsynaptic density transcripts after increasing doses of antipsychotics with different receptor profiles: insights for psychosis treatment. Eur Neuropsychopharmacol. 2015;25:566–82.
61 Luoni A, Rocha FF, Riva MA. Anatomical specificity in the modulation of activity-regulated genes after acute or chronic lurasidone treatment. Prog Neuropsychopharmacol Biol Psychiatry. 2014;50:94–101.