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ORIGINAL ARTICLE

THE BDNF GENE PROMOTER METHYLATION IN THE COURSE OF ANTIDEPRESSANT TREATMENT IN ADOLESCENT GIRLS WITH FIRST-LIFETIME DEPRESSIVE EPISODE: A PROSPECTIVE STUDY

METYLACJA PROMOTORA GENU BDNF W PRZEBIEGU LECZENIA PRZECIWDEPRESYJNEGO NASTOLATEK Z PIERWSZORAZOWYM EPIZODEM DEPRESJI: BADANIE PROSPEKTYWNE

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ABSTRACT

Introduction

Epigenetic mechanisms regulating the level of BDNF gene expression correlate with achieving remission in the course of Major Depressive Disorder in adults. Studies in this area may contribute to individualization of antidepressant pharmacotherapy and increasing its effectiveness, but the amount of data on this subject in the pediatric population is limited. To date, no study has prospectively investigated changes in the BDNF gene methylation level following antidepressant treatment in adolescents.

Aim

Therefore, we aimed to examine the BDNF gene exon IV promoter methylation status in the group of adolescents treated for the first-lifetime depressive episode. Moreover, we aimed to verify the usefulness of BDNF methylation status as a predictor of treatment outcome.

Material and methods

Our study included 30 female inpatients diagnosed with depression who underwent antidepressant treatment. Before starting treatment and after a minimum of 6 weeks, the level of methylation of the BDNF gene exon IV promoter was examined. Results. No statistically significant difference in the level of BDNF gene methylation before or after treatment was observed, and the usefulness of BDNF gene methylation as a prognostic factor for treatment response was not proven.

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Conclusions
Further studies on a larger group of patients are necessary to verify whether the dynamics of methylation changes in the BDNF gene mirrors the results obtained in adults. Studies on this subject are essential to increase the effectiveness of depression treatment in adolescent patients.

Keywords: mood disorders, depression, adolescence, brain-derived neurotrophic factor, DNA methylation, antidepressant treatment

STRESZCZENIE
Wstęp
Mechanizmy epigenetyczne regulujące poziom ekspresji genu BDNF korelują z osiągnięciem remisji w przebiegu zaburzeń depresyjnych w populacji osób dorosłych. Badania w tym obszarze mogą przyczynić się do indywidualizacji leczenia przeciwdepresyjnego i zwiększenia jego skuteczności, jednakże ilość danych na ten temat jest ograniczona, szczególnie w odniesieniu do populacji pediatrycznej. Do tej pory nie badano prospektywnie zmian w poziomie metylacji genu BDNF w trakcie leczenia przeciwdepresyjnego u nastolatków.

Cel
Celem niniejszego badania jest ocena metylacji promotora egzonu IV genu BDNF w grupie nastolatków leczonych z powodu pierwszego w życiu epizodu depresyjnego oraz weryfikacja przydatności poziomu metylacji BDNF jako predyktora odpowiedzi na leczenie przeciwdepresyjne.

Materiał i metody
Do badania włączono 30 pacjentek hospitalizowanych z rozpoznaniem epizodu depresji, które poddano leczeniu przeciwdepresyjnemu. Przed rozpoczęciem leczenia i po co najmniej 6 tygodniach, oznaczono poziom metylacji promotora egzonu IV genu BDNF.

Wyniki
Nie zaobserwowano statystycznie istotnej różnicy w poziomie metylacji genu BDNF przed lub po leczeniu, a przydatność metylacji genu BDNF jako czynnika prognostycznego odpowiedzi na leczenie nie została udowodniona.

Wnioski
Konieczne są dalsze badania na większej grupie pacjentów, aby zweryfikować, czy dynamika zmian metylacji genu BDNF odzwierciedla wyniki uzyskiwane w badaniach osób dorosłych. Badania w tym obszarze wydają się niezbędne do zwiększenia skuteczności leczenia przeciwdepresyjnego nastolatków.

Słowa kluczowe: zaburzenia nastroju, depresja, nastolatki, neurotroficzny czynnik pochodzenia mózgowego, metylacja DNA, leczenie przeciwdepresyjne

Introduction

Major Depressive Disorder (MDD) is a significant phenomenon among adolescents and adults worldwide. According to the World Health Organization, 14% of the 10–19-year-old experience mental health conditions such as MDD, anxiety disorders, and behavioral

disorders, which are the main root of disabilities and sickness in this age group (WHO, 2021). MDD might lead to suicide attempts which constitute one of the leading causes of death among youth (WHO, 2021). Importantly, meta-analyses show that mental health problems in childhood are strong indicators of mental illness in adulthood (Mulraney *et al.,* 2021). Despite the significant socioeconomic implications of MDD, its pathogenesis is still uncertain, and the treatment outcomes remain unsatisfying. Studies point to the role of gene-environment interactions mediated by epigenetic mechanisms as core mechanisms underlying the emergence of psychiatric disorders, including MDD (Tarapati Rana *et al.,* 2021).

Among many hypotheses addressing the etiopathogenesis of depression, the neurotrophic theory of depression is one of the most frequently investigated. It assumes that environmental stress leads to structural changes in the brain through epigenetic alterations in neurotrophic factors' genes, such as the brain-derived neurotrophic factor (BDNF) gene. Physiologically, brain-derived neurotrophic factor (BDNF) plays a part in both developing and mature brains as it stimulates neurogenesis and eliminates unnecessary neurons (Bathina and Das, 2015). BDNF's low levels are associated with decreased neuroplasticity observed in patients with affective disorders and neurodegenerative diseases. BDNF is mainly expressed in the hippocampus, prefrontal cortex, amygdala, and hypothalamus, which are the brain areas responsible for the emotional and cognitive functions (Bruno Perosa Carniel *et al,* 2021). In support of the neurotrophic theory of depression, researchers report MDD to be associated with diminution of relevant limbic structures, with decreased post-mortem BDNF levels in these brain areas (Duman and Li, 2012).

Nowadays, psychiatry is increasingly striving to personalize treatment methods, which would enable a more effective selection of drugs and, consequently, higher remission rates and a shorter recovery process. The analysis of epigenetic modifications related to neuroplastic processes, such as the methylation of the BDNF gene, is a promising direction in the studies examining biomarkers in MDD (Bathina and Das, 2015). For instance, Kang *et al.* (2013) observed increased BDNF promoter methylation to be associated with the history of suicide attempts among MDD patients (Kang *et al.,* 2013). Additionally, higher methylation status was related to a worse prognosis of the disease course (Kang *et al.,* 2013).

The BDNF gene expression is controlled by nine promoters, each regulating the expression of distinct BDNF transcripts contributing to a region-specific BDNF effect in the brain. BDNF exon IV is expressed differently throughout the development, with transcripts gradually increasing during embryonic and postnatal development and slightly reducing in the adult brain (Aid *et al.,* 2007). Studies on adults show that methylation of the BDNF gene exon IV promoter changes significantly with antidepressant treatment and might serve as a potential predictor of antidepressant treatment response (Molendijk *et al.,* 2011) (Maryna Polyakova *et al.,* 2015) (Webb *et al.,* 2020). To date, no study has investigated changes in the BDNF gene methylation level following antidepressant treatment in adolescents.

Aim

We aimed to analyze prospectively the changes in BDNF gene exon IV promoter methylation status in adolescents treated for the first-lifetime depressive episode. Secondly, we aimed to verify the usefulness of BDNF methylation status as a predictor of treatment outcome.

Material and methods

Ethical Declaration

The study was performed in line with the Declaration of Helsinki and was verified and approved by the Bioethics Committee of the Poznań University of Medical Sciences.

We received written consent to participate in the study from the participants' legal guardians and patients over 13 years of age.

Participants

We recruited patients of the Child and Adolescent Psychiatry Clinic in Karol Jonscher Clinical Hospital in Poznań between January 2021 and April 2023. The inclusion criteria involved age 11–17, admitted with the initial diagnosis of the first episode of depression, no history of psychiatric disease or treatment, no other ongoing somatic disease or medical treatment, no history of addiction or previous organic causes of depressive symptoms. The exclusion criteria involved mental retardation, substance abuse, consent withdrawal, failure to continue treatment throughout the six weeks, and failure to collect blood samples at any of the time points. To qualify for the study, at least a moderate level of depression symptoms must have been present according to ICD-10 which was still an applicable classification in Poland during the recruitment time. According to these criteria, moderate depression requires the presence of at least two core symptoms and at least 3 of the remaining symptoms (Table 1.) for a minimum of 2 weeks (WHO, 1992). The recruitment process has already been described in detail in our previous publication that was based on the same sample of patients (Zwolinska *et al.* 2024).

The diagnosis was established through the patient's examination by the child psychiatrist, weekly observation, and interview with the parent. Participation in the study did not influence the decision-making process regarding the initiation of pharmacotherapy or the type of SSRI. The additional low-dose sedative was introduced among patients with irritability, severe anxiety, and/or insomnia.

Clinical assessment

Patients were qualified and monitored using the Children's Depression Inventory-2 (CDI-2) short form and Hamilton Depression Rating Scale (HDRS). These scales are used both in diagnosing and controlling remission of MDD. The CDI-2 is a self-report used in clinical assessment of depression symptoms in children and adolescents (age 7–17). Results are standardized by the age and sex of patients (Kovacs, 2015). The HDRS is an objective scale that is performed by the psychiatrist. We used HDRS17, which includes 17 assessments about symptoms of depression in the past week, yielding a minimum total score of 0 (least severe) and a maximum score of 52 (most severe). A final score between 0–7 indicates clinical remission (Hamilton, 1960).

BDNF methylation analysis

5 ml of peripheral blood from each fasting participant was collected into EDTA tubes between 7 and 10 a.m. to assess the BDNF

Table 1. Depression criteria according to ICD-10.

Core symptoms	Remaining symptoms
	Loss of confidence or self-esteem
	Unreasonable feelings of self-reproach or guilt
Depressed mood	Recurrent thoughts of death or suicide
Loss of interest	Diminished ability to think/concentrate or indecisiveness
Reduction in energy	Change in psychomotor activity with agitation or retardation
	Sleep disturbance
	Change in appetite with weight change

Treatment with selective serotonin reuptake inhibitor (SSRI) - sertraline or fluoxetine – was introduced based on clinical indications included in the NICE guidelines (NICE, 2019).

exon IV promoter methylation using quantitative methylation-specific real-time PCR (qMS-PCR). Two versions of the primers were created based on DNA sequence regions that

obtained the promoter region of exon IV (27.722.850 – 27.723.477 Homo sapiens chromosome 11, GRCh37.p13). The primers were designed with Methyl Primer Express™ Software v1.0 (Applied Biosystems, Waltham, MA, USA). We performed the analysis using the primer described in Table 2. Isolated genomic DNA and CpGenome Human Methylated & Non-Methylated DNA Standard Set (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was converted using a sodium bisulfite kit. Chemical modification of 500 ng of genomic DNA and standards was performed using an EZ DNA Methylation Gold Kit™ (Zymo Research, Irvine, CA, USA). After sodium bisulfite conversion, the percentage of methylation index (MI) was assessed by qPCR with two pairs of primers for the methylated and unmethylated promoter region of the BDNF gene with FastStart Essential DNA SYBR Green Master (Roche, Basel, Switzerland). The MI, expressed as a percentage of gene methylation (MI – %), was calculated for each sample using the following formula: $MI = [1/(1 + 2^{-(CtU-CtM)})] \times 100\%$, where CtM and CtU are derived from qMSP with primers for the methylated and unmethylated gene sequences, respectively.

Statistical analysis

Statistical analysis was executed using PQStat Software version 1.8.2.238. The distribution of the variables was studied using the Shapiro-Wilk Test. Since our data did not follow normal distribution, the Wilcoxon Test was performed to compare paired samples, and the Mann-Whitney Test was used for independent groups. The nominal variables were compared through the Chi-Squared Test. The logistic regression was performed to analyze the correlation between initial MI and treatment outcome, including the potential confounding factors (age, BMI, depression severity, time of treatment). The significance level was set at $\alpha < 0.05$ for all analyses.

Results

Recruitment and clinical characteristics

Forty-nine patients met the inclusion criteria. Fifteen patients were excluded due to lack of consent to participate in the study/lack of compliance in taking the medication/lack of follow-up assessment after treatment. Finally, thirty-four patients were included in the study: thirty girls and four boys. Given the significant gender disproportion, only female patients were finally accepted in the analysis as a studied group.

Table 2. Primers sequence (the 5' to 3' direction).

Forward Methylated DNA (MF)	Reverse Methylated DNA (MR)	Forward Unmethylated DNA (UF)	Reverse Unmethylated DNA (UR)
AGCGAGAGTAGTTTTTTCGC	CATATAACAACGCACGTCAAA	GGTAGTGAGAGTAGTTTTTTTTGT	TCATATAACAACACACATCAAAAC

Study protocol

The clinical (CDI-2 and HDRS) and molecular (BDNF exon IV promoter MI) analyses were conducted twice: before introducing antidepressant treatment (t0) and after a minimum of six weeks after initiation (t1). Based on the CDI and HDRS results at t1, the patients were classified as, responders' or, non-responders'. To be classified as a, responder,' a minimum 50% reduction in symptoms in both HDRS and CDI-2 must have been present or HDRS result < 7.

The characteristics of the patients are presented in Table 3. All of them were females, aged 11–16, of Caucasian origin. The period of antidepressant treatment ranged from six to eleven weeks. 57% of participants received sertraline, and 43% fluoxetine. 17 patients required additional sedative treatment with quetiapine/chlorprotixene/trazodone/hydroxyzine /risperidone/melatonin. 13 individuals responded to the introduced treatment and were classified as 'responders'. There was no statistical difference between

responders and non-responders regarding age, BMI, initial CDI-2/HDRS, treatment time, type of SSRI chosen, and additional sedative use. As expected, responders had significantly lower levels of HDRS and CDI-2 at t1 when compared with non-responders.

Table 3. Studied group characteristics.

	Whole Group (t0) n = 30	**Responders t(1) n = 13**	**Non-responders t(1) n = 17**	**Responders vs Non-responders**
Age [years]	13.07 (±1.26)	12.85 (±0.99)	13.24 (±1.44)	p = 0.4105
Sex	females	females	females	–
BMI [kg/m²]	20.89 (±3.47)	21.51 (±2.44)	20.41 (±4.10)	p = 0.1487
HDRS				
• t(0)	20 [13–30]	20 [14–30]	20 [13–28]	p=0.4445
• t(1)	8.5 [1–24]	4 [1–9]	12 [8–24]	p<0.0001*
CDI-2				
• t(0)	74.5 [60–79]	74 [66–79]	75 [60–79]	p = 0.9830
• t(1)	68 [47–79]	54 [47–68]	74 [60–79]	p < 0.0001*
Time of treatment [weeks]	7.27 (±1.41)	7.46 (±1.39)	7.12 (±1.45)	p = 0.5125
SSRI type				p = 0.6377
• Fluoxetine [n]	13	5	8	–
• Sertraline [n]	17	8	9	–
Additional sedative [n]	17	6	11	p = 0.3095
BDNF promoter MI [%]				
• t(0)	0.21 (±0.06)	0.23 (±0.08)	0.20 (±0.05)	p = 0.3900
• t(1)	0.22 (±0.07)	0.22 (±0.06)	0.21 (±0.07)	p = 0.6293

Continuous variables are presented as the mean and standard deviation; ordinal variables are presented as the median and range, t0 – on admission to the hospital before treatment, t1 – after minimum six weeks of antidepressant therapy, BMI – body mass index, HDRS – Hamilton Depression Rating Scale, CDI-2 – Children's Depression Inventory, BDNF – brain-derived neurotrophic factor, MI – methylation index, SSRI – selective serotonin reuptake inhibitor, * – statistically significant.

Molecular results

The analysis did not demonstrate any significant changes in MI between t0 and t1 in the whole studied group. No significant differences in MI were observed after stratifying the group according to the SSRI chosen (Figure 1a). Regardless of whether the patients clinically responded to treatment, there were no significant shifts in MI (Figure 1b).

Responders and non-responders did not differ regarding initial/final MI within BDNF exon IV promoter at t0 and t1 (Table 3). The univariate logistic regression analysis revealed no significant association between initial MI and remission status at t1 (Table 4). There were no visible associations between age, BMI, time of treatment, initial level of depressive symptoms, and treatment outcome (Table 4).

Discussion

We found no significant difference between the pre- and post-treatment methylation index in the BDNF gene exon IV promoter and no correlation between initial methylation and the antidepressant treatment outcome in our studied group of adolescents treated for the first-lifetime depressive episode. According to the research performed on adults by Tadić *et al.* (2014), hypomethylation in the BDNF gene was a predictor of worse response to antidepressant treatment regardless of antidepressant class. Still, similarly to our results, methylation levels did not change significantly following the 6-week therapy (Tadić *et al.*, 2014). On the other hand, Chen *et al.* (2011) reported an increase in BDNF exon IV promoter methylation during treatment while also confirming the predictive

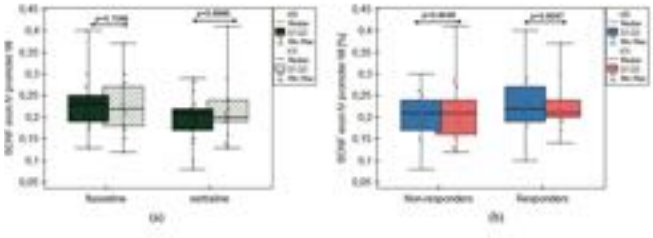

Figure 1. The results of the Wilcoxon test of the BDNF gene exon IV promoter Methylation Index (MI) before (t0) and after (t1) the treatment in the studied group stratified according to the type of SSRI (a) and treatment response (b)

Table 4. Results of the association between analyzed variables and positive treatment outcome in univariate logistic regression models for the whole sample.

Analyzed variable	b	95% CI	p-value
Age	−0.0101	−0.0621–0.0420	0.7035
BMI	0.1790	−0.3484–0.7064	0.5060
Time of treatment	0.0276	−0.0475–0.1027	0.4711
CDI (t0)	0.0112	−0.1552–0.1777	0.8948
HDRS (t0)	−0.0313	−0.2145–0.1518	0.7371
BDNF MI (t0)	6.5499	−5.9334–19.0332	0.3038

value between the level of the methylation and the occurrence of remission (Chen, Ernst and Turecki, 2011). Similar conclusions were reached by Lopez *et al.* (2013) and Wang *et al.* (2018), who in their prospective studies analyzing eight weeks of SSRI therapy, noticed an increase in responders' methylation results (Wang *et al.,* 2018) (Lopez *et al.,* 2013). Therefore, our results do not reflect the outcomes obtained in most of the studies performed on the adult population. Attempts to explain the above discrepancies should be sought in many aspects.

BDNF, as a factor directly related to neurogenesis, is an essential element in the development of the nervous system. Therefore, its gene expression might differ in the developmental population (Lee *et al.,* 2022) (Rodríguez-Carrillo *et al.,* 2023). It has been proven that its level in the blood decreases with age, which may be associated with increased neurodegeneration processes (Erickson *et al.,* 2010) (Molinari *et al.,* 2020) (Ted Kheng Siang Ng *et al.,* 2019). These processes have also been observed in adults with MDD (Webb *et al.,* 2020) (Bakusic *et al.,* 2021). BDNF values are more challenging to interpret in children and adolescents due to the physiologically increased secretion of this neurotrophin in the natural process of nervous system development, typical for this age group. A significant increase in peripheral BDNF and a decrease in methylation of the BDNF gene are observed particularly in early adolescence (Dincheva, Lynch and Lee, 2016) (Miguez *et al.,* 2020) (Zwolińska *et al.,* 2024).

Another aspect that may explain the lack of differences in MI before and after the treatment is the course of the disease among adolescents, which is known to differ from the presentation of the symptoms observed in adults. For instance, anhedonia and concentration disorders in MDD are more specific to older patients, while vegetative symptoms (appetite and weight change, loss of energy, and insomnia) are typical among youth (Rice *et al.,* 2019). Moreover, early depression is often characterized by acts of self-aggression, irritability or rebellious attitude (Gijzen *et al.,* 2021) (Richard G. Wight *et al.,* 2004)

(Tatsiopoulou *et al.,* 2020). It might be possible that different manifestations of symptoms in child depression result from different neurobiological underpinnings when compared with adults.

Remarkably, MDD among youth is significantly influenced by environmental factors (Stefanie Nelemans *et al.,* 2021). Yet, the number of studies in the adolescent population investigating epigenetic changes occurring as a response to environmental stressors is limited (Ochi and Dwivedi, 2023). Animal models have proven that severe stress in the early stages of life is associated with an increase in peripheral BDNF and the occurrence of epigenetic changes in its gene (Suri *et al.,* 2013). Interestingly, in the study performed on adults by Unternaehrer *et al.* (2015), there was a significant association between the history of low maternal care in childhood and greater DNA methylation in the BDNF gene (Unternaehrer *et al.,* 2015).

For the present research, the predictive value of the methylation level as a prognostic indicator of treatment response was not proven. In this context, it is worth considering the research of Lieb *et al.* (2018), which showed a correlation between the occurrence of remission in adults with MDD and the hypermethylation in the specific region of exon IV of the BDNF gene. However, that correlation characterized specifically the group of patients who suffered from severe depression before the treatment (Lieb *et al.,* 2018). It is assumed that younger patients are also more likely to respond to therapy when they present with severe depressive symptoms. Still, the relationship with epigenetic changes in the BDNF gene in this group of patients remains unknown (Kirsch *et al.,* 2008). Our study found no significant correlation between initial depressive symptoms severity and the treatment outcome.

Although the results of our work require an extended analysis, possibly on larger groups of patients, they demonstrate the importance of addressing depression in adults and youth separately. Further prospective studies should be designed to evaluate the association between antidepressant treatment and BDNF methylation status in order to expand our knowledge of depression pathophysiology across the lifespan and develop personalized treatment strategies. Further attempts to understand the relationship between epigenetic changes and MDD remission should combine molecular and clinical markers.

When interpreting the results of the foregoing study, certain limitations should be considered. Firstly, our research had a moderate study group size, which may have resulted in the insufficient statistical power for some tests. Therefore, potential statistical errors should be taken into account due to unsatisfactory analytical sensitivity. Secondly, the study group was limited to female patients, which may be considered both an advantage and disadvantage since the research on BDNF suggests sex-specific differences in BDNF gene expression, particularly during puberty (Ochi and Dwivedi, 2023) (Bath, Schilit and Lee, 2013) (Carbone and Handa, 2013).

Furthermore, we did not analyze the history of trauma, while studies emphasize a significant relationship between BDNF gene methylation and environmental stress (Ochi and Dwivedi, 2023) (Jo Wrigglesworth *et al.,* 2019). Another factors that could considerably impact our results include the family history of the disease, perinatal and intergenerational factors (Braithwaite *et al.,* 2015) (Provenzi *et al.,* 2022) (Labaut *et al.,* 2024). Moreover, certain environmental factors, such as diet or exposure to pollution, constitute significant limitations in methylation research (Kageyama *et al.,* 2022) (Rodríguez-Carrillo *et al.,* 2022) (Vicente Mustieles *et al.,* 2022).

Although the results of our work require an extended analysis, possibly on larger groups of patients, they demonstrate the importance of addressing depression in adults and youth separately. Further prospective studies should be designed to evaluate the association between antidepressant treatment and BDNF methylation status in order to expand our knowledge of depression pathophysiology

across the lifespan and develop personalized treatment strategies. Further attempts to understand the relationship between epigenetic changes and MDD remission should combine molecular and clinical markers.

Conclusions

We found no significant difference between the pre- and post-treatment methylation index in the BDNF gene exon IV promoter and no correlation between initial methylation and the antidepressant treatment outcome in our studied group of adolescents treated for the first-lifetime depressive episode. Continuation of research on larger study groups seems essential to verify the dynamics of epigenetic changes in the BDNF gene during antidepressant treatment in adolescence.

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