

# Epigenetic alterations of the promoter region of the POMC gene in adolescent depressive disorder patients with non-suicidal self-injury behaviors

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## Research article

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# Abstract

## Background

The incidence of non-suicidal self-injury (NSSI) behavior among adolescents increases year by year. Patients with a history of both depression and NSSI behaviors tend to have greater risk of suicide. At present, the mechanism of adolescent depressive disorder patients with NSSI behaviors is not clear, epigenetic mechanism may be involved. Proopiomelanocortin (*POMC*) gene may be associated with depressive disorder. The purpose of this study was to investigate deoxyribonucleic acid (DNA) methylation of *POMC* gene promoter region of adolescent depressive disorder patients with NSSI behaviors.

## Methods

Bisulfite Sequencing PCR (BSP) was used to test the methylation level of *POMC* promoter of 15 adolescent depressive disorder patients with NSSI behaviors and 15 healthy controls (HC). The online software Methylation Share Rate (MSR) Calculator was used to draw the lollipop model of the methylation state. Self-made questionnaires were used to collect clinical data of the case group and control group. Hamilton depression scale-24 (HAMD-24), Hamilton anxiety scale (HAMA), Symptom Checklist-90 (SCL-90) were used to evaluate the characteristics and severity of depressive, anxiety and psychotic symptoms. Adolescent self-injury questionnaire was used to assess NSSI behaviors and its severity.

## Results

BSP analysis found that the *POMC* methylation level of cytosine-guanine dinucleotide 1 (CpG1) site was higher in adolescent depressive disorder patients with NSSI behaviors than that of HC ( $P < 0.05$ ). Overall significance in *POMC* methylation at CpG1 sites between adolescent depressive disorder patients with NSSI behaviors and HC was gender-independent, methylation level at CpG1 sites was higher in both male and female patients than that in HC. The CpG1 methylation had correlation with the family history ( $P < 0.05$ ). We also found that *POMC* methylation level at CpG17 sites in female patients was significantly higher than that of the female HC ( $P < 0.05$ ).

## Conclusions

There was abnormal methylation in the *POMC* promoter region of adolescent depressive disorder patients with NSSI behaviors, the methylation of CpG1 sites may act as epigenetic markers for those adolescents.

## Background

Adolescent depressive disorder, which is a group of diseases mainly manifested by low mood, may be accompanied by varying degrees of cognitive and behavior changes, psychotic symptoms and suicidal self-injurious behaviors, etc. [1-5], it was estimated that the prevalence of depressive disorders in adolescents (13-18years) is 3%-9% [6,7]. Adolescent depressive disorder, which is a common social problem, deserves more and more attention.

The pathogenesis of depression is complex, studies have shown that epigenetics, especially DNA methylation, played an important role in the occurrence, development and progression of depressive disorders [8-10], such as the methylation of tachykinin receptors 2 [11], glucocorticoid receptor gene, the mineralocorticoid receptor gene [12], brain-derived neurotrophic factor [13] and catechol-O-methyltransferase [14]. In addition, research on epigenetics proves that environmental factors are also closely related to the occurrence of diseases [15]. Abnormal DNA methylation may be caused by various environmental and lifestyle exposures, these factors may affect the methylation status of CpG in the regulatory region by interfering with the specific binding between transcription factors and recognition sites [16,17]. Adverse life experiences or stress reaction in childhood or adolescence would lead to the functional changes of hypothalamic-pituitary-adrenal (HPA) axis or disorders of adrenocorticotrophic hormone (ACTH) in the body [18]. Depression may be caused by a combination of the above genetic and environmental factors. Studies have shown that ACTH which derived from POMC are closely related to the occurrence of depressive disorders [19]. Some studies that have found a correlation between POMC and depressive disorders, but all these studies focus on the relationship between *POMC* gene polymorphism and depressive disorders [20], however, there are no epigenetic studies of *POMC* in depressive disorder.

Adolescent patients with depressive disorder may have NSSI behaviors, the relationship between depression and NSSI behaviors is complex and is still being explored. Some studies believed that depression was an important risk factor of NSSI behaviors [2,21], and anhedonia may be one of the motivations of NSSI behaviors [22]. Elevated  $\beta$ -endorphin, as a analgesic medium, can reduce pain and increase pain tolerance so that patients cannot feel pain, thus reinforcing NSSI behaviors [23]. Interestingly, both  $\beta$ -endorphin and ACTH come from POMC.

There have been studies showing that *POMC* is associated with mental illness. Primary study showed that the mice exhibited depression-like behavior when POMC neurons in the arcuate nucleus were inhibited [24]. Studies have pointed out that heroin addicts treated with intravenous diacetylmorphine could cause an *POMC* promoter methylation and reduce the level of stress hormone in the patient's body, thus playing a role in emotional regulation of treatment [25]. In alcohol dependence, *POMC* promoter methylation is associated with craving [26]. The degree of *POMC* promoter methylation in smokers is significantly higher than that in non-smokers. Researchers believe that the change of *POMC* methylation in smokers indicates the adaptability of stress signals, thereby possibly serving as a marker related to addiction [27]. Although the causality of adolescent patients with depressive disorder and NSSI behaviors can't be clear, studies have shown that the patients who had a history of depression and NSSI behaviors at the same time may have a greater risk of suicide [28,29]. Therefore, it is important for us to find

biological markers of adolescent depressive disorder patients with NSSI behaviors for early detection, prevention and individualized treatment [30]. We hypothesize that the epigenetics of the promoter region of the *POMC* gene has altered in adolescent depressive disorder patients with NSSI behaviors. The purpose of our study was to investigate whether the methylation of the *POMC* gene promoter is related with the NSSI behaviors of adolescent depression disorders.

## Methods

### Subject recruitment

The study sample included 15 adolescent depressive disorder patients with NSSI behaviors (5 males, 10 females) and 15 HC (6 males, 9 females). For the case group, the inclusion criteria were as follows: each participant suffered from depressive disorder as defined by fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5); the diagnosis was made by at least two experienced psychiatrists according to the Structured Clinical Interview for DSM-5 (SCID); between the ages of 10 and 19 years old. The exclusion criteria were as follows: severe somatic or brain disease, such as cerebral ischemia, cerebral hemorrhage, epilepsy, cardiovascular and renal diseases; depressive disorder due to other psychoactive drug substance abuse (legal or illegal substances); pregnant women or patients with acquired immuno-deficiency syndrome; the score of HAMD-24 third item (suicide)  $\geq 2$  points; bipolar or related disorders, disruptive mood disorders, persistent mood disorders, other and unspecified mood disorders, abuse of alcohol or other psychoactive substances; patients who have received electroconvulsive therapy, transcranial magnetic stimulation or systematic psychotherapy in the last three months; other mental disorders defined in DSM-5. For the control group, the inclusion criteria were as follows: aged from 10 to 19 years old; who were not concomitant psychiatric disorders, such as schizophrenia spectrum, bipolar or related disorders, depression, anxiety, compulsion, separation, somatic symptoms or other related disorders defined in DSM-5. The exclusion criteria were as follows: first-degree relatives have psychiatric and neurological disorders or previous NSSI behaviors.

### Molecular analysis

Participants' peripheral blood (5 mL) was extracted and placed in anticoagulation tubes containing EDTA. Then it was centrifuged to extract the leukocyte layer. The online software CpG Island Searcher was used to analyze the gene promoter region CpG island, ranging from 2000 bp upstream to 500 bp downstream of the transcription start point, to obtain sequence information. Sequenom Epi Designer software was used for analyzing and designing methylated primers at both ends of CpG island. The extraction of DNA was performed using the DNA Kit (GENERAY, GK0122). DNA samples were bisulfite-converted using the Bisulfite Kit (QIAGEN, cat; 59824). DNA amplification was conducted using polymerase chain reaction (PCR). After purification of the PCR product using Kit (Generay, cat; GK2043), each product was visualized on a standard agarose gel. T/A cloning was performed and positive clones were selected from each clone for sequencing. Finally, comparing with the measured sequence and the original sequence to determine the methylation sites and number, and analyze the degree of methylation.

The primers used in the experiment were as follows: B474-S1-F, TAAATGYGAATTAGGTAGATGT; B474-S1-R, AACACAAAAAACAACACTCCC; the fragment length is 312 bp. BSP-F (control), AGGGAGGTGAAGTAGTGGTG; BSP-R (control), ACTCCTCCACTCTTCTCCTCC; the fragment length is 247 bp.

## Scales assessment

The characteristics and severity of depressive symptoms in patients were assessed with HAMD-24, which consisting of 7 factors (Anxiety/somatization, Weight, Cognitive disorder, [Circadian changes](#), Hysteresis, Dyssomnia, Feeling of despair), each item adopted the 5 degree scoring of 0 to 4 points, representing no, slight, moderate, severe, very severe respectively. The characteristics and severity of anxiety symptoms in patients were assessed with HAMA, which consisting of 2 factors (Somatic anxiety, Psychic anxiety), each item adopted the 5 degree scoring of 0 to 4 points, representing no, slight, moderate, severe, very severe respectively. The psychiatrists (all authors) were simultaneously trained in the use of the HAMD-24, HAMA, SCL-90 before this study was initiated. The mental health of the patients was assessed with SCL-90, which consisting of 10 factors (Somatization, Obsession, Interpersonal sensitivity, Depressed, Anxious, Hostile, Terror, Paranoia, Psychosis, Average), each item adopted the 5 degree scoring of 0 to 4 points, representing no, mild, moderate, a little severe and severe respectively. We separately calculated the factor scores of the above scales for each case group member. All these scales are the Chinese version and have good reliability and validity among the Chinese population [31-33].

## Statistical analysis

SPSS 21.0 software was used to establish the database and all data were expressed as mean  $\pm$  standard deviation. The independent samples t-test and one-way analysis of variance was used to compare the continuous variables between groups. The chi-square test was used to compare the categorical variables between groups. Spearman correlation analysis was used to assess the correlation of CpG1 DNA methylation level of patients and related factor scores of SCL-90, HAMD and HAMA. The online software MSR Calcalate was used to draw the lollipop model of the methylation state. Each line represents the methylated state of a positive clone after sequencing and comparison, white circles represents unmethylated CpG sites and black circles represents the methylated CpG sites. All statistical tests were considered statistically significant at  $P < 0.05$ .

## Results

Data from 15 adolescent depressive disorder patients with NSSI behaviors and 15 HC were analyzed. The results showed that there was no statistical difference in gender ( $t = 0.435$ ,  $P = 0.670$ ) and age ( $t = -2.016$ ,  $P = 0.063$ ) between the two groups.

### Lollipop model of the methylation state

Each line represents the methylated state of a positive clone after sequencing and comparison, white circles represents unmethylated CpG sites and black circles represented the methylated CpG sites (**Figure 1**). In this study, we explored the level of DNA methylation at 24 *POMC* promoter region CpG sites. We could clearly find that the mean level of methylation of case group was higher than that of control group. What's more, four sites (CpG1, CpG2, CpG3, and CpG9) among 24 CpG sites of *POMC* gene were expressed in adolescent depressive disorder patients with NSSI behaviors and HC, methylation level of CpG1 site was higher in adolescent depressive disorder patients with NSSI behaviors than that of HC ( $P < 0.05$ ). (**Table 1a, 1b, 1c, 1d and 1e**).

**Table 1a. Methylation level of different CpG sites between case and control group**

Group	CpG1	CpG2	CpG3	CpG4	CpG5
Case group	6.13 ± 1.36	2.67 ± 0.98	4.80 ± 1.21	0.67 ± 0.90	0.60 ± 0.63
Control group	4.07 ± 1.22	2.13 ± 0.99	4.60 ± 1.56	0.73 ± 1.16	0.93 ± 1.10
t	4.384	1.486	0.387	-0.176	-1.018
<i>P</i>	0.000 <sup>***</sup>	0.149	0.701	0.862	0.320

**Notes:** \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

**Table 1b. Methylation level of different CpG sites between case and control group**

Group	CpG6	CpG7	CpG8	CpG9	CpG10
Case group	0.40 ± 0.74	1.80 ± 1.15	0.27 ± 0.46	2.33 ± 1.11	0.07 ± 0.26
Control group	0.47 ± 0.92	1.47 ± 1.30	0.60 ± 1.06	1.93 ± 0.88	0.20 ± 0.56
t	-0.220	0.744	-1.122	1.090	-0.837
<i>P</i>	0.828	0.463	0.271	0.285	0.410

**Table 1c. Methylation level of different CpG sites between case and control group**

Group	CpG11	CpG12	CpG13	CpG14	CpG15
Case group	1.00 ± 1.31	0.80 ± 0.78	0.60 ± 0.74	0.67 ± 0.72	0.33 ± 0.49
Control group	0.80 ± 0.94	0.87 ± 0.83	0.67 ± 0.72	0.40 ± 0.51	0.33 ± 0.49
t	0.480	-0.227	-0.250	1.169	0.000
<i>P</i>	0.635	0.822	0.804	0.252	1.000

**Table 1d. Methylation level of different CpG sites between case and control group**

Group	CpG16	CpG17	CpG18	CpG19	CpG20
Case group	0.53 ± 0.64	0.47 ± 0.52	0.73 ± 0.80	0.53 ± 0.64	0.20 ± 0.41
Control group	0.47 ± 0.64	0.27 ± 0.46	0.60 ± 0.91	0.27 ± 0.46	0.27 ± 0.59
t	0.285	1.122	0.426	1.313	-0.357
P	0.778	0.271	0.673	0.201	0.724

**Table 1e. Methylation level of different CpG sites between case and control group**

Group	CpG21	CpG22	CpG23	CpG24
Case group	0.07 ± 0.26	0.13 ± 0.35	0.07 ± 0.26	0.13 ± 0.35
Control group	0.07 ± 0.26	0.07 ± 0.26	0.13 ± 0.35	0.07 ± 0.26
t	0.000	0.592	-0.592	0.592
P	1.000	0.559	0.559	0.559

**Comparison of correlation between gender and CpG sites in case and control group**

We found that while overall significance in *POMC* methylation level at CpG1 sites between adolescent depressive disorder patients with NSSI behaviors and HC was gender-independent, this effect was driven by male patients. In addition, we concluded that *POMC* methylation at CpG17 sites in female patients was higher than that of female HC, and the difference was statistically significant ( $P < 0.05$ ). (Table 2a, 2b, 2c, 2d and 2e)

**Table 2a. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG1	CpG2	CpG3	CpG4
Male	Case group	6.80 ± 0.84	3.00 ± 1.00	4.80 ± 1.30	0.60 ± 0.55
	Control group	4.17 ± 1.33	2.17 ± 1.17	4.17 ± 1.47	0.33 ± 0.52
t		3.825	1.254	0.747	0.830
P		0.004**	0.241	0.474	0.428
Female	Case group	5.80 ± 1.48	2.50 ± 0.97	4.80 ± 1.23	0.70 ± 1.06
	Control group	4.00 ± 1.23	2.11 ± 0.93	4.89 ± 1.69	1.00 ± 1.41
t		2.873	0.890	-0.132	-0.527
P		0.011*	0.386	0.896	0.605

Notes: \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 2b. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG5	CpG6	CpG7	CpG8
Male	Case group	0.20 ± 0.45	0.20 ± 0.45	1.40 ± 1.14	0.00 ± 0.00
	Control group	1.00 ± 1.27	0.50 ± 0.84	1.00 ± 0.89	0.33 ± 0.52
t		-1.336	-0.717	0.653	-1.581
<i>P</i>		0.214	0.492	0.530	0.175
Female	Case group	0.80 ± 0.63	0.50 ± 0.85	2.00 ± 1.16	0.40 ± 0.52
	Control group	0.89 ± 1.05	0.44 ± 1.01	1.78 ± 1.48	0.78 ± 1.30
t		-0.220	0.130	0.367	-0.849
<i>P</i>		0.829	0.898	0.718	0.408

**Table 2c. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG9	CpG10	CpG11	CpG12
Male	Case group	2.00 ± 0.71	0.20 ± 0.45	0.40 ± 0.55	0.60 ± 0.55
	Control group	1.83 ± 0.75	0.00 ± 0.00	0.50 ± 0.84	1.00 ± 1.10
t		0.376	1.000	-0.229	-0.739
<i>P</i>		0.716	0.374	0.824	0.479
Female	Case group	2.50 ± 1.27	0.00 ± 0.00	1.30 ± 1.49	0.90 ± 0.88
	Control group	2.00 ± 1.00	0.33 ± 0.71	1.00 ± 1.00	0.78 ± 0.67
t		0.946	-1.414	0.519	0.339
<i>P</i>		0.357	0.195	0.611	0.739

**Table 2d. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG13	CpG14	CpG15	CpG16
Male	Case group	0.20 ± 0.45	0.40 ± 0.55	0.20 ± 0.45	0.80 ± 0.84
	Control group	0.83 ± 0.98	0.33 ± 0.52	0.17 ± 0.41	0.67 ± 0.82
t		-1.412	0.208	0.129	0.267
P		0.199	0.840	0.900	0.796
Female	Case group	0.80 ± 0.79	0.80 ± 0.79	0.40 ± 0.52	0.40 ± 0.52
	Control group	0.56 ± 0.53	0.44 ± 0.53	0.44 ± 0.53	0.33 ± 0.50
t		0.784	1.141	-0.186	0.285
P		0.444	0.270	0.855	0.779

**Table 2e. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG17	CpG18	CpG19	CpG20
Male	Case group	0.40±0.55	0.80±0.84	0.80±0.84	0.00±0.00
	Control group	0.67±0.52	0.67±0.82	0.17±0.41	0.17±0.41
t		-0.830	0.267	1.646	-1.000
P		0.428	0.796	0.134	0.363
Female	Case group	0.50±0.53	0.70±0.82	0.40±0.52	0.30±0.48
	Control group	0.00±0.00	0.56±1.01	0.33±0.50	0.33±0.71
t		2.838	0.343	0.285	-0.121
P		0.011*	0.736	0.779	0.905

Notes: \* $P < 0.05$ .

**Table 2f. Comparison of methylation level in male and female patients at different sites in case and control groups**

Gender	Group	CpG21	CpG22	CpG23	CpG24
Male	Case group	0.20 ± 0.45	0.20 ± 0.45	0.20 ± 0.45	0.20 ± 0.45
	Control group	0.00 ± 0.00	0.17 ± 0.41	0.00 ± 0.00	0.17 ± 0.41
t		1.000	0.129	1.000	0.129
P		0.374	0.900	0.374	0.900
Female	Case group	0.00 ± 0.00	0.10 ± 0.32	0.00 ± 0.00	0.10 ± 0.32
	Control group	0.11 ± 0.33	0.00 ± 0.00	0.22 ± 0.44	0.00 ± 0.00
t		-1.058	1.000	-1.512	1.000
P		0.305	0.343	0.169	0.343

### Correlation between methylation level of CpG1 site and family history

The independent samples t-test was used to analyse the correlation between methylation level of CpG1 site and family history. The methylation level of CpG1 site of patients with positive family history was  $5.73 \pm 1.19$ , and that of patients with negative family history was  $7.25 \pm 1.26$ , the results showed that the CpG1 methylation level had statistical correlation with the family history ( $P < 0.05$ ). **Correlation between methylation level of CpG1 site and SCL-90, HAMD, HAMA scales**

Spearman correlation analysis was used to analyse the correlation methylation level of CpG1 site and the factor scores of SCL-90, HAMD and HAMA scales, and the results showed that the CpG1 methylation level had no correlation with these scales.

## Discussion

At present, studies have shown that the *POMC* gene may be related to the pathological process of depression. However, there are no epigenetic studies to investigate whether this gene is related to the NSSI behavior of adolescent depression patients. As we know, this is the first case-control study investigating the epigenetic alterations of *POMC* promoter methylation in adolescent depressive disorder patients with NSSI behaviors.

*POMC* methylation level of CpG1 site was higher in adolescent depressive disorder patients with NSSI behaviors than that of HC. One possibility is that adolescent depressive disorder patients carried an elevated *POMC* promoter methylation before doing NSSI behaviors. The alteration of methylation level could be the onset of a multi-generational effect established by environmental alterations regarding a NSSI behaviors' mother during pregnancy [34], and our research showed that abnormal methylation level at the CpG1 site was correlated with family history. In this assumption, NSSI behaviors might affect the epigenetic regulation of *POMC* in early life and possibly helps to initiate the occurrence and development of NSSI behaviors addition. We presume that high methylation level in adolescent depressive disorder

patients with NSSI behaviors, compared to HC, leads to a lowered level of POMC and its derivatives. That condition may be restored through the epigenetic feedback regulation mechanism of HPA axis. But because of lacking some related data (NSSI behaviors conditions of patients' parents) and biological material from their ancestors, we were not able to deliver further evidence for this assumption. So we need to do more animal studies under controlled prerequisites to provide evidence for the existence of a multi-generational effect caused by some negative environmental conditions in early life on epigenetic patterns of adolescent depression patients.

Another possibility is that abnormal *POMC* methylation is the result of NSSI behaviors and the adaptation of the HPA axis to maintain the steady state of POMC and its derivatives. Studies have shown that adolescent patients with depressive disorder suffer from anhedonia due to disorders of the HPA axis or certain substances in the body [18]. Anhedonia may be one of the motivations of NSSI behaviors, which promotes NSSI behaviors in adolescents with depressive disorder. NSSI behaviors, as a stress or pain mediator, activated POMC neurons to interfere with the HPA axis by stimulating the production of  $\beta$ -endorphin and ACTH in body of adolescent patients with depressive disorder, resulting in the dysfunction of HPA. On one hand, adolescent patients with depressive disorder stimulate the body to produce  $\beta$ -endorphin [35], ACTH and other substances through NSSI behaviors to activate POMC neurons to interfere with the HPA axis, so that the body reaches a steady state. On the other hand, some studies also suggested that NSSI individuals may have emotional management disorders with inability to express emotions, and NSSI behaviors can be an emotional regulation strategy for patients with depressive disorder to involve in regulating emotions [36-38]. What's more, while overall significance in *POMC* methylation at CpG1 sites between adolescent depressive disorder patients with NSSI behaviors and HC was gender-independent from our research, this effect was more pronounced in male patients. Studies have found that male students were earlier and more prone to NSSI behaviors than female student. Relatively speaking, when dealing with adverse events, most male students were more likely to adopt a negative coping style than female, preferring to compare themselves with peers who were not doing well in everything, and reluctant to talk and confide [39].

There are limitations of our work. Although we concluded that there was abnormal methylation in the *POMC* promoter region, considering the sample size of the preliminary experiment was small, the results may be biased, so whether this anomaly could be used as a possible marker needs to be further verified by expanding the sample size. In addition, the missing some data of these patients' parents and their ancestors, it is difficult for us to further investigate whether the abnormal methylation of CpG1 site is related to the mother's NSSI or other adverse stimuli during pregnancy. For future studies, we will take ample samples and more clinical data to verify the possible epigenetic feedback regulation mechanism and strive for the early detection of biological markers applied in clinical work.

## Conclusion

We concluded that there was abnormal methylation in the *POMC* promoter region, *POMC* CpG1 methylation level in adolescent depressive disorder patients with NSSI behaviors was higher than that of

HC, which may be a possible epigenetic marker of *POMC* gene. Meanwhile, the abnormality of CpG1 methylation may also be a feedback regulatory mechanism. Our research shows that in adolescent depressive disorder patients with NSSI behaviors, the alteration of the promoter region methylation of the *POMC* gene may have possible potential pathophysiological.

## Abbreviations

**ACTH**: adrenocorticotrophic hormone; **BSP**: Bisulfite Sequencing PCR; **CpG1**: cytosine-guanine dinucleotide 1; **DNA**: deoxyribonucleic acid; **DSM-5**: fifth edition of the Diagnostic and Statistical Manual of Mental Disorders; **HAMA**: Hamilton anxiety scale; **HAMD-24**: Hamilton depression scale-24; **HC**: healthy controls; **HPA axis**: hypothalamic-pituitary-adrenal axis; **MSR**: Methylation Share Rate; **NSSI**: non-suicidal self-injury; **PCR**: polymerase chain reaction; **POMC**: Proopiomelanocortin; **SCL-90**: Symptom Checklist-90; **SCID**: Structured Clinical Interview for DSM-5;

## Declarations

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shandong mental health center, reference number: (2020) Ethics Review (R11). All participants and their legal guardians signed the informed consent before participating in the study.

### Consent for publication

Not applicable.

### Availability of data and materials

Researchers interested in the study may contact corresponding author to obtain relevant data via email: liulf521@163.com.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

LFL supervised the quality control of the subject. XJB designed the research; DDZ and XJB conducted sample collection and contributed reagents/materials tools; TLZ, LNW, XJB, MMS and CH were responsible for clinical diagnosis and clinical evaluation; DDZ analyzed the data; DDZ was responsible for manuscript writing. All authors read and approved the final manuscript.

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## Notes

Doudou Zheng and Xiaojiao Bi contributed equally to this study.

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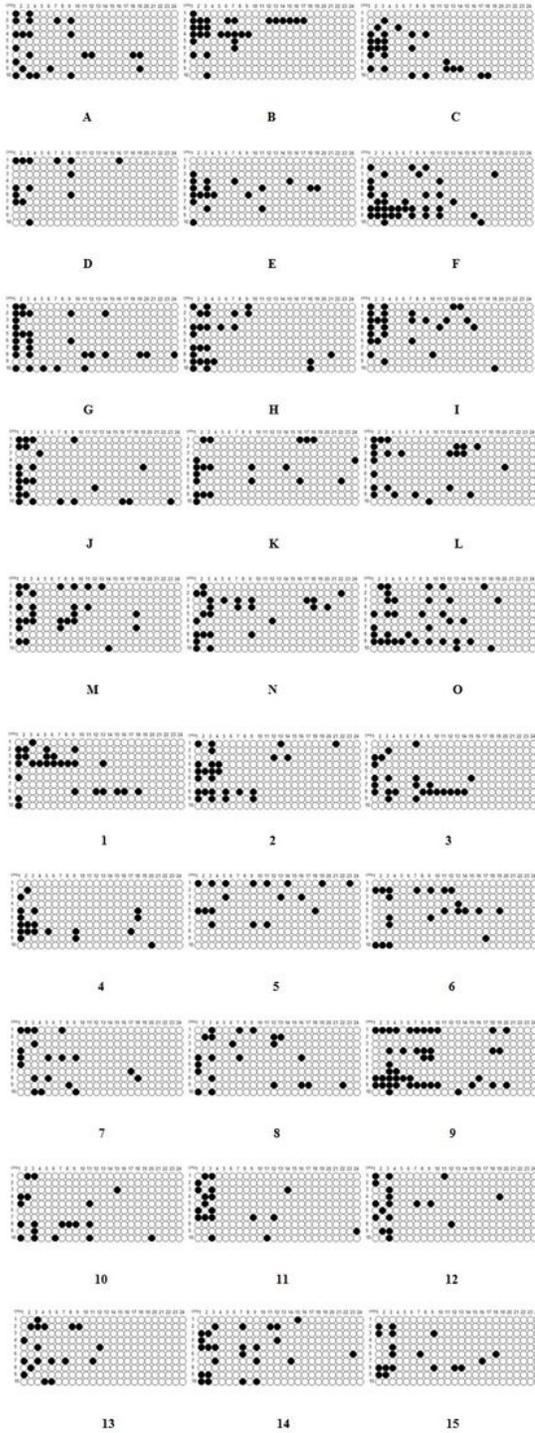
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## Figures



Notes: A-O represented the level of methylation of 15 adolescent depressive disorder patients with NSSI behaviors; 1-15 represented the level of methylation of 15 HC.

**Figure 1**

The level of methylation of POMC gene in different sites