Research report

Stress-induced depressive behaviors are correlated with Par-4 and DRD2 expression in rat striatum

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A B S T R A C T

Depression is a common mental disorder; however, its molecular mechanism has not been fully elucidated. In this study, we investigated the role of maternal deprivation (MD) and chronic mild stress (CMS) in the pathogenesis of depression in rat models. The mRNA levels of prostate apoptosis response-4 (Par-4) and dopamine receptor D2 (DRD2) genes in the striatum were measured by real-time PCR. Methylation level in the promoter of Par-4 gene was detected by bisulfite sequencing. Correlation between gene expression and depression-like behaviors were analyzed. Our results demonstrated that MD and CMS alone or their combination (dual stresses: DS) caused depression-like behaviors in rats. The mRNA levels of Par-4 and DRD2 genes in the striatum were significantly lower in MD-, CMS-, and DS-treated rats than in control rats. Importantly, Par-4 and DRD2 mRNA levels significantly correlated with depression-like behaviors. However, no significant differences in total methylation levels in the promoter of Par-4 gene were found between four groups. Our study suggested that either maternal deprivation or chronic mild stress plays a crucial role in the development of depression-like behaviors in rats. This process is associated with down-regulated Par-4 and DRD2 gene expression in the striatum through a non-methylation mechanism.

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1. Introduction

Depression is a common mental disorder with a tendency toward gradually increased morbidity worldwide [1]; however, the pathogenesis of depression remains unclear. Psychological stress, especially early life stress and chronic psychological stress, is believed to trigger depression. For example, children who experienced early bereavement have a higher risk of suffering from major depression in adulthood [1]. Animals that experienced maternal deprivation (MD) have impaired stress responses and show characteristics of depression-like behaviors in adulthood [2]. Chronic mild stress (CMS) can lead to depressive behaviors such as decreased interest and lack of pleasure [3]. However, the molecules that mediate the onset of depression are not fully identified.

It is generally agreed that psychological stress leads to depression by influencing the metabolism of monoamine neurotransmitter systems [4,5]. However, treatment with 5-HT/NE reuptake inhibitors or monoamine oxidase inhibitors elicited poor therapeutic effects in more than 30% of depressed patients [6]. Even in patients that showed remission after treatment with these antidepressants, a number of residual symptoms associated with dopaminergic malfunction, such as loss of motivation, attention, and pleasure, still affect patients [1]. Moreover, decreased concentration of dopamine metabolites in the cerebrospinal fluid was observed in suicide depressive patients [7] while patients with severe depression showed an obvious reduction in the striatal volume [1,7]. Thus, a dopaminergic dysfunction subtype of depression was proposed in the clinic [2]. Interestingly, decreased dopamine levels in the nucleus accumbens (NAC) were also observed in a variety of depression animal models [2,3,8]. These findings suggested that the dopaminergic system might play a crucial role in the pathogenesis of depression, but the molecular mechanism remains unclear.

The prostate apoptosis response-4 (Par-4) protein is expressed in striatal neurons along with DRD2 and interacts with DRD2 in neural cells [9]. Par-4 normally enhances DRD2 signaling and thereby inhibits dopamine/DRD2-mediated neurotransmission [10]. Interestingly, an autopsy study of patients with major depression revealed a 67% decrease in Par-4 expression in post-mortem temporal cortex [11]; knockout of Par-4 gene led to depression-like behavior in mice. Furthermore, a competitive binding experiment revealed that Par-4 works as a key ligand of DRD2 [10]. These findings suggested that Par-4 might be the key intermediary between DRD2 and depression.

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It has been demonstrated that exogenous stimuli, such as environmental stress, can result in aberrant gene promoter methylation, and subsequent changes in gene transcription [12]. In this study, depression-like behaviors were therefore measured through sucrose consumption, forced swim, and open field tests. The correlation of Par-4 with DRD2 expression in the striatum, their effect on behavioral changes, and CpG methylation of Par-4 promoter was investigated.

2. Materials and methods

2.1. Animals

Parent Sprague-Dawley rats at the age of 3 months were provided by the animal center of Shanghai Biological Science Institution and housed under identical conditions. Food and water were provided ad libitum in accordance for the Guide and Use of Laboratory Animals (Chinese Council). Pregnant rats were checked at 9:00 everyday for delivery. Rats born before 9:00 were designated as postnatal day 0 (PND 0), after 9:00 as postnatal day 1 (PND 1). All experiments were conducted in accordance to an approved protocol from Central South University.

2.2. Maternal deprivation and chronic mild stress

Offspring of over 10 pregnant rats were mixed and randomly allocated to four groups: MD, CMS, dual stress (DS), and the control (C) group. In MD and DS groups, offspring were separated from their mothers for 6 h/day between 09:00 and 15:00 from PND 1 to PND 14 [13]. To block communication between mothers and offspring, both the mothers and the offspring were removed from their original cages and housed separately in individual cages. After 21 days, all rats were grouped and housed according to gender until adulthood (10 weeks). Then, offspring in CMS and DS groups were randomly given one stress per day for 21 days: exposure to electric footshock for 20 s (3 mA, 0.2 s duration every 2 s), swimming for 3 min in cold water (4 °C), water deprivation for 24 h, food deprivation for 24 h, elevated open platform (10 cm × 10 cm × 60 cm) for 1 h, or immobilization for 2 h at 20 °C [14,15]. The primary study revealed no significant differences in behaviors between male and female rats. All data presented in this study only included the data from female rats.

2.3. Sucrose consumption test

A sucrose consumption protocol was developed to evaluate the animals’ anhedonic-like response to a palatable fluid as previously described, with small modifications [16,17]. Rats were given access to two bottles containing 1% sucrose solution in their cages for 24 h followed by access to one bottle of tap water and one bottle of 1% sucrose solution for 24 h. After water-deprivation for the next 23 h, the animals were given pre-weighed bottles of tap water and 1% sucrose solution. Fluid consumption in the next hour was recorded. The consumption of sucrose was calculated as the volume of 1% sucrose solution per 100 g body weight.

2.4. Forced swim test

A forced swim test protocol was developed to evaluate the animals’ despair-like behavior as previously described, with small modifications [7,18]. The forced swim barrel was a Plexiglas cylinder (40 cm in height and 20 cm in diameter). After the cylinder was filled with water (25 ± 1 °C) to a depth of 30 cm, rats were placed in the cylinder for 15 min. Rats were then removed from the water and dried in 32 °C before being returned to their home cages. Twenty-four hours later, the rats were placed in the water-filled cylinder again and the total 5-min test session was recorded by a camera. The float time was calculated by an observer blinded to the experimental design. The float time was defined as the time a rat spent passively floating without struggling, in a slightly bunched but upright position with the head slightly immersed. The water was changed and the cylinder was cleaned after each test to ensure that rat’s behaviors were not affected by detecting another rat’s scent.

2.5. Open field test

An open field arena (100 cm × 100 cm × 30 cm) was constructed from an open rectangular wood box with 25 squares (20 cm × 20 cm) painted on the floor. In the 5 × 5 array, 16 squares along the wall of the box were designated as the “side squares” and the 9 squares in the middle were designated as the “center squares.” At the time of the test, one rat was placed at the center of the field and allowed to explore the area freely for 5 min [2]. The total distance of crawling, frequency of the central area entry, number of excrement, and the number of vertical movement were recorded. The arena was cleaned with 75% alcohol between tests to ensure that each rat’s behaviors were not affected by the detection of another rat’s scent.

2.6. Real-time quantitative PCR

Rats were sacrificed by rapid decapitation. The striatum tissues were dissected, snap frozen on dry ice, and stored at −80 °C until use. Total RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the user manual. Reverse transcription was performed using ReverTra Aid First Strand cDNA synthesis kit (MBI Fermentas Life Science, Burlington, Canada) according to the manufacturer’s instructions. Real-time quantitative PCR was conducted using a Bio-Rad iCycler iQ (Bio-Rad, California, America) as previously described [3]. Par-4 gene was amplified by the forward primer: 5'-GATACGATGCCCCGCTAAC-3’ and reverse primer: 5'-CAGCCTCCAAGAAGGTTT-3’. DRD2 gene was amplified by the forward primer: 5'-GGCACTACCTGTAATGCTGTTG-3’ and reverse primer: 5'-TGCTGATGCAAGACCTTGGC-3’. As an internal control, β-actin was amplified by the forward primer: 5'-CACCAGGAGGGGGCAGCTAC-3’ and reverse primer: 5'-TAAAGACCTCTATGCAACAGT-3’. The specificity of all amplified fragments was confirmed by melting curve analysis. The mRNA levels of DRD2 and Par-4 were normalized with β-actin before statistical analysis.

2.7. Western blot

The anti-Par-4 mouse monoclonal antibody was purchased from Santa Cruz Biotechnology (San Diego, CA, USA). The anti-β-actin mouse monoclonal antibody and the horseradish peroxidase-conjugated anti-mouse IgG were purchased from Sigma-Aldrich (St. Louis, MI, USA.). The striatum tissue was homogenized in ice-cold buffer and Western blot was conducted as previously described [19]. Thirty micrograms of total protein was loaded on a 10% SDS-PAGE gel. The same blot was re-probed for β-actin to serve as a loading control. The intensity of each band was quantified with Bio-Rad Quantity One software (Bio-Rad, Hercules, CA, USA).

2.8. Sodium bisulfite mapping

Genomic DNA was isolated from striatal tissue using proteinase K/phenol-chloroform extraction method. The DNA pellet was dissolved in 200 μl of TE buffer and the concentration was measured. For bisulfite treatment, the EpiTect Bisulfite Kit (QIAGEN, Hilden, Germany), which can convert over 99% of non-methylated C residues to U, was adopted. Briefly, a total of 1 μg genomic DNA (in 20 μl) was added to 140 μl of conversion reagent followed by incubation in a thermal cycler at 99 °C, 5 min, 60 °C, 25 min, 99 °C, 5 min, 60 °C, 15 min, 99 °C, 5 min, 60 °C, 15 min according to the manufacturer’s manual. After purification, methylated genomic DNA was subjected to PCR amplification. The promoter fragment of Par-4 (414 bp, NM_033485) was amplified by a nest-PCR using the outside primers: 5'-TTATTTTTTTAGGGTTTACAGGT-3’ and 5'-CTACACCTATATACACACTCCC-3’, and inside primers: 5'-GTCTCTTCTTGTGGTTTACAGGT-3’ and 5'-CTACACCTATATACACACTCCC-3’. The amplified fragments were cloned into TA cloning vector (Invitrogen, Carlsbad, CA, USA). Five clones per amplified fragment were sequenced. The methylation ratio of a CpG site was calculated with the following formula: Methylation ratio = total methylation score/clone number/sample number. The total methylation score is the total number of sequenced methylated C residue at one CpG site.

2.9. Statistical analysis

Data was presented as mean ± standard error of the mean, and analyzed using the statistical package for the Social Science Version 17.0. Data was analyzed using one way ANOVA. Each experimental group was compared with control group using the least significant difference (LSD) method. Correlations between Par-4, DRD2 mRNA expression, and behavior indexes were analyzed using Pearson correlation. A p < 0.05 was considered statistically significant.

3. Results

3.1. Effect of MD and CMS on depression-like behavior of adult rats

A significant portion of the experimental animal died during stress and behavioral test with highest mortality rate in DS group (44.4%), followed by MD group (19.0%), CMS group (10.5%), and control (C) group (5.6%). The sample size for final analysis of both the behavioral and molecular data was 17 for C, 17 for CMS, 17 for MD, and 10 for DS group, respectively.

Depressive mood, interest decline, and psychomotor retardation are the main symptoms of depression [20]. In animal models, the decrease in sucrose consumption reflects the interest decline in human [7]. As shown in Table 1, sucrose consumption test revealed a significant difference in percent consumption of sucrose between groups (F = 3.35, p = 0.026). LSD multiple comparison revealed that
MD and CMS rats consumed less sucrose than that control rats did (p < 0.05), but there is no significant difference between DS and control rats (p > 0.05).

Forced swim test was widely used to evaluate “behavioral despair” and “suicide” [7,18]. In this study, a significant difference in float time in forced swim test was observed between groups (F = 5.971, p = 0.002), while MD, CMS, and DS rats exhibited a significantly longer float time compared to control rats (p < 0.05).

In the open field test, the distance crawled reflects whether animals have “psychomotor retardation” symptom; while the decrease in vertical movement reflects the animal’s loss of curiosity in a novel environment [7]. As shown in Table 2, open field test exhibited a significant difference in distance crawled (F = 3.586, p = 0.019) and the number of vertical movement (F = 12.253, p = 0.0001), but no significant differences in the frequency of central area entry (F = 0.594, p = 0.622) and the number of excrement (F = 1.959, p = 0.130) were observed between groups. LSD multiple comparisons showed that MD rats crawled less distance than control rats did (p < 0.05), whereas there are no significant differences in distance crawled between CMS-, DS-treated rats and control rats (p > 0.05); MD, CMS, and DS rats showed significant decreases in the number of vertical movement compared to control rats (p < 0.05), while CMS rats had less vertical movement than MD rats (p < 0.05).

### Table 3

<table>
<thead>
<tr>
<th>Par-4 mRNA</th>
<th>DRD2 mRNA</th>
<th>Par-4 protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal deprivation</td>
<td>0.0225 ± 0.0015</td>
<td>0.0990 ± 0.0602</td>
</tr>
<tr>
<td>Chronic mild stress</td>
<td>0.0138 ± 0.0072</td>
<td>0.1069 ± 0.0947</td>
</tr>
<tr>
<td>Dual stress</td>
<td>0.0259 ± 0.0113</td>
<td>0.1724 ± 0.1323</td>
</tr>
<tr>
<td>Control</td>
<td>0.0424 ± 0.0200</td>
<td>0.3210 ± 0.2052</td>
</tr>
<tr>
<td>F value</td>
<td>4.829</td>
<td>4.103</td>
</tr>
<tr>
<td>p value</td>
<td>0.009</td>
<td>0.017</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to control group.

^ a p < 0.05 compared to maternal deprivation group.

3.2. MD and CMS decreased DRD2 mRNA, Par-4 mRNA and protein levels in the striatum

Rats were sacrificed on PND 92 to examine mRNA level of Par-4 and DRD2 in the striatum by real-time PCR. As shown in Table 3, significant differences in Par-4 (F = 4.829, p = 0.009) and DRD2 (F = 4.103, p = 0.017) mRNA levels were observed between groups. LSD multiple comparisons revealed that Par-4 mRNA expressions in MD, CMS and DS rats were significantly lower than in control rats (p < 0.05, p < 0.01, p < 0.01, respectively). Similarly, DRD2 mRNA expression in MD, CMS, and DS rats was significantly lower than in control rats (p < 0.01, p < 0.01, p < 0.05, respectively). There was a significant difference in Par-4 protein expression between groups (F = 14.597, p < 0.0001). LSD multiple comparisons revealed that Par-4 protein expressions in MD, CMS, and DS rats were lower than in control rats (p < 0.05); CMS and DS rats expressed even lower Par-4 protein levels than MD rats (p < 0.05) (Table 3, Fig. 1).

### Table 2

<table>
<thead>
<tr>
<th>Result of open field test.</th>
<th>Distance crawled (cm)</th>
<th>Central area rate</th>
<th>Excrement number</th>
<th>Number of vertical movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal deprivation</td>
<td>1309.76 ± 226.55</td>
<td>0.07 ± 0.07</td>
<td>1.59 ± 2.27</td>
<td>13.47 ± 10.08</td>
</tr>
<tr>
<td>Chronic mild stress</td>
<td>484.81 ± 342.58</td>
<td>0.19 ± 0.30</td>
<td>3.00 ± 2.15</td>
<td>7.41 ± 5.86</td>
</tr>
<tr>
<td>Dual stress</td>
<td>610.39 ± 267.32</td>
<td>0.17 ± 0.30</td>
<td>1.90 ± 1.79</td>
<td>8.00 ± 3.97</td>
</tr>
<tr>
<td>Control</td>
<td>679.67 ± 464.75</td>
<td>0.28 ± 0.79</td>
<td>1.59 ± 1.50</td>
<td>20.76 ± 5.52</td>
</tr>
<tr>
<td>F value</td>
<td>2.856</td>
<td>5.954</td>
<td>1.959</td>
<td>12.253</td>
</tr>
<tr>
<td>p value</td>
<td>0.019</td>
<td>0.122</td>
<td>0.130</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to control group.

^ a p < 0.05 compared to maternal deprivation group.

3.3. Par-4 and DRD2 mRNA expression correlated with the depressive-like behaviors

Par-4 mRNA level showed a significant correlation with percent preference for sucrose (r = 0.408, p < 0.05) in sucrose consumption test and float time (r = −0.537, p < 0.01) in forced swim test. In addition, Par-4 mRNA level also showed a significant correlation with distance crawled (r = 0.504, p < 0.01), the amount of excrement (r = −0.478, p < 0.05), and vertical movement (r = 0.464, p < 0.05), but showed no significant correlation with the frequency of central area entry (r = −0.155, p > 0.05) in open field test (Fig. 2). DRD2 mRNA expression had no significant correlation with percent preference for sucrose (r = 0.133, p > 0.05) in sucrose consumption test. However, DRD2 mRNA expression showed a significant correlation with the length of float time (r = −0.390, p < 0.05) in forced swim test.
Fig. 2. Correlation between Par-4 mRNA expression and depression-like behaviors. (A) Correlation between Par-4 mRNA expression and percent of preference for sucrose. (B) Correlation between Par-4 mRNA expression and float time. (C) Correlation between Par-4 mRNA expression and distance crawled. (D) Correlation between Par-4 mRNA expression and frequency of the central area entry. (E) Correlation between Par-4 mRNA expression and the number of excrement. (F) Correlation between Par-4 mRNA expression and frequency of the central area entry.

Fig. 3. Correlation between DRD2 mRNA expression and depression-like behaviors. (A) Correlation between DRD2 mRNA expression and preference percent for sucrose. (B) Correlation between DRD2 mRNA expression and float time. (C) Correlation between DRD2 mRNA expression and frequency of the central area entry. (D) Correlation between DRD2 mRNA expression and the frequency of the central area entry. (E) Correlation between DRD2 mRNA expression and the number of excrement. (F) Correlation between DRD2 mRNA expression and the frequency of the central area entry.

test. In addition, DRD2 mRNA expression also showed a significant correlation with the distance crawled \( (r = 0.389, p < 0.05) \) and the number of vertical movement \( (r = 0.400, p < 0.05) \), but not with the frequency of the central area entry \( (r = 0.070, p > 0.05) \) and the amount of excrement \( (r = 0.094, p > 0.05) \) in open field test (Fig. 3).

3.4. Down-regulation of Par-4 expression is not associated with DNA methylation of Par-4 promoter

In the genomic DNA of mammalian cells, the CpG dinucleotides-rich DNA region is usually located at the 0.5–5 kb region of the first
Fig. 4. Methylation level of Par-4 gene promoter. The transcription initiation site was represented by an arrow. Five cloned fragments were then sequenced from each rat and methylation for each CpG pair was averaged. (A) Methylation rate at each detected CpG site in control group. (B) Methylation rate at each detected CpG site in maternal deprivation (MD) group. (C) Methylation rate at each detected CpG site in chronic mild stress (CMS) group. (D) Methylation rate at each detected CpG site in dual stress (MD + CMS) group. (E) The total methylation level. There are no significant differences observed between groups.
exon and promoter of numerous genes [21]. In the Par-4 promoter, the −760 to −300 bp upstream region of the transcription start site contains one CpG island with 51 CG sites (Gene Bank: NM_033485). Generally, the cytosine of CpG dinucleotides is the preferred base for DNA methylation [22]. We measured the cytosine methylation of CpG dinucleotides ranging from −760 bp to −300 bp of the Par-4 promoter using sodium bisulfite mapping. Results of DNA sequencing showed no significant differences between groups in total Par-4 promoter methylation level (F = 0.314, p = 0.815) (Fig. 4).

4. Discussion

In the clinic, major depression and anxiety disorders are frequently observed in subjects with a history of traumatic childhood experiences [23,24]. Depressive mood and interest decline are the main symptoms of depression. In MD rats, the decrease in sucrose consumption rate, decrease in vertical activity during open field test and increase in float time in forced swim test reflect decreases in interest decline-, despair-, and exploratory-like behaviors. In contrast, no changes in the frequency of the central area entry and the number of excrement indicated that MD did not induce an anxiety-like behavior. However, there are conflicting reports on whether MD can lead to anxiety-like behavior. Rentesi et al. reported that Wistar rats treated with MD for 24 h showed an increase in anxiety-like behaviors in rats at 60 days [25]. Conversely, Esquivel et al. reported that rats treated with MD for 4.5 h/day for 21 days did not show significant increase in anxiety-like behavior [26]. These conflicting outcomes may be a result of differences in MD paradigms. Long-lasting MD may lead to depressive behavior, whereas short term MD may lead to anxiety-like behavior in animals. In the present study, rats were treated with MD for 6 h/day for 14 days, which is a long-lasting MD paradigm. It is therefore not surprising that only depression-like behaviors were observed. Previous studies had demonstrated that CMS can lead to a variety of depression-like behavior in animals, including lack of pleasure and behavioral despair [2,27]. In this study, rats treated with CMS exhibited a reduction in sucrose consumption rate, decreased vertical activity in open field test, and extension in float time in forced swim test. These outcomes suggested that CMS also induced depression-like behaviors. No changes in distance crawled, frequency of the central area entry, and the amount of excrement in open field test suggested that CMS did not affect the anxiety level. At present, no study identified the effect of DS on depression-like behavior in animals. In the present study, DS extended float time and decreased vertical movement, but did not affect the distance crawled, frequency of the central area entry, or the amount of excrement. These results indicated that DS produced despair-like behavior and reduced exploratory behavior, but had no influence on anxiety-like behaviors in rats. In theory, animals that experienced early MD should be more prone to develop depression-like behavior upon exposure to stress in adulthood. However, DS rats showed less depression-like behavior than MD and CMS rats. We hypothesized that the sample bias caused by a higher mortality rate in DS rats might be one of the reasons.

Although dual stressed rats exhibited the highest mortality rate (44.4%), a higher mortality rate was observed in the MD rats (19%) compared to the CMS rats (10.5%). This suggested that MD is a stronger stressor than CMS. Particularly, by considering that the control group had 5.6% death, CMS actually caused less than 5% death. Therefore, stressors given to rats in the CMS group are mild. We also observed that the high mortality rate in DS group caused an imbalance in sex ratio (male:female = 7:3) and much smaller sample size (n = 4) in male rats (data not shown). This is why we only analyzed the data for female rats in the present study. The small sample size in DS group due to the high mortality rate may be influenced by sample bias. This might be one of the reasons why DS-treated rats did not exhibit more depression-like behavior and molecular alterations compared to the MD- or CMS-treated rats. Another explanation is that early life stress may have no effect on the development of depression upon exposure to stress in adulthood, but this is unlikely and is contrary to current theory. Even so, the sample bias does not seem to overturn the primary conclusion that maternal deprivation, chronic mild stress, and double stress can induce depression-like behavior in the adult rats.

Par-4 is a key ligand of DRD2. The binding of Par-4 to DRD2 regulates cAMP signaling, and subsequently affects function of the DA-cAMP-CREB pathway [1,10]. For instance, Par-4 mutant mice exhibited an increased cAMP activity and enhanced DA-cAMP-CREB signaling pathway [10]. Importantly, these mice showed depression-like behaviors. Thus, Par-4 was proposed to be a key intermediary between DRD2 and depression. Our previous study demonstrated that MD reduces DAT, DRD1 and DRD2, but not DRD3 gene expression in the NAc, however, only the changes in DRD2 mRNA level correlated with the behavioral abnormality in both spatial learning and memory in rats [13]. In the current study, MD, CMS, and DS led to a decrease in expressions of Par-4 mRNA and protein as well as DRD2 mRNA in the striatum. These results further support the relationship between Par-4 and DRD2 and their crucial roles in the pathogenesis of depression [28,29]. Indeed, both the Par-4 and DRD2 mRNA expression in the striatum strongly correlated with the depression-like behaviors, evident in the sucrose consumption rate, float time, distance crawled, and vertical movement. We therefore hypothesize that Par-4 might be a leading factor in the Par-4/DRD2 signaling.

DNA methylation was involved in the regulation of individual responses to environmental stress [30]. For instance, individual housing altered mammary transcripts of genes associated with DNA methylation in mice [31]. Early life stress leads to high sensitivity of the HPA axis in adulthood while methylation plays a key role in the development of sensitivity [32,33]. Murgatroyd et al. also found that early life stress leads to depression through induction of the hypomethylation of arginine vasopressin (AVP) [34]. Pruitt et al. study in rat ovarian cells demonstrated that Ras regulates the expression of Par-4 through methylation [27]. Our previous study demonstrated that MD decreased DRD2 mRNA expression in the NAc via a non-methylation mechanism [13]. However, Par-4 and DRD2 are both expressed in striatal neurons [9]. We therefore hypothesized that the decreased Par-4 mRNA expression in the striatum of MD, CMS, and DS-treated rats might be a result of hyper-methylation. However, our study demonstrated no significant differences in total methylation level in the Par-4 promoter between groups. This may suggest several possibilities. First, downregulation of Par-4 mRNA expression is not caused by the hyper-methylation. Second, methylation changes are located out of the −760 to −300 bp region of Par-4 promoter. Methylation of the 200–300 bp upstream region of the transcription start site might be more consistently associated with repressed transcription [35,36], while methylation of a far upstream region and exon 1 is also associated with decreased transcription [37]. In addition, methylation at the gene body of some genes has been reported to be associated with gene expression [38,39]. Third, gene expression is regulated by a variety of mechanisms. Besides DNA methylation, histone modification, chromatin remodeling and regulation of noncoding RNA also regulate gene expression [40,41]. Whether any other regulatory mechanism mediates the reduction of Par-4 in individuals with depression needs further studies.

In conclusion, our study suggested that early life stress and chronic psychological stress play a crucial role in the pathogenesis of depression. Occurrence of depression correlates with the stresses-induced down-regulation of Par-4 and DRD2 gene expression in the striatum through a non-methylation mechanism. Our
study provides new insights for the pathogenesis of depression and new targets in treating depression in the clinic.

Conflicts of interest

The authors declare no conflict of interest.

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