Brain-Derived Neurotrophic Factor Epigenetic Modifications Associated with Schizophrenia-like Phenotype Induced by Prenatal Stress in Mice

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ABSTRACT

BACKGROUND: Prenatal stress (PRS) is considered a risk factor for several neurodevelopmental disorders including schizophrenia (SZ). An animal model involving restraint stress of pregnant mice suggests that PRS induces epigenetic changes in specific GABAergic and glutamatergic genes likely to be implicated in SZ, including the gene for brain-derived neurotrophic factor (BDNF).

METHODS: Studying adult offspring of pregnant mice subjected to PRS, we explored the long-term effects of PRS on behavior and on the expression of key chromatin remodeling factors including DNA methyltransferase 1, ten-eleven-translocation hydroxylases, methyl CpG binding protein 2, histone deacetylases, and histone methyltransferases and demethylase in the frontal cortex and hippocampus. We also measured the expression of BDNF.

RESULTS: Adult PRS offspring demonstrate behavioral abnormalities suggestive of SZ and molecular changes similar to changes seen in postmortem brains of patients with SZ. This includes a significant increase in DNA methyltransferase 1 and ten-eleven-translocation hydroxylase 1 in the frontal cortex and hippocampus but not in cerebellum; no changes in histone deacetylases, histone methyltransferases and demethylases, or methyl CpG binding protein 2, and a significant decrease in *Bdnf* messenger RNA variants. The decrease of the corresponding *Bdnf* transcript level was accompanied by an enrichment of 5-methylcytosine and 5-hydroxymethylcytosine at *Bdnf* gene regulatory regions. In addition, the expression of *Bdnf* transcripts (IV and IX) correlated positively with social approach in both PRS mice and nonstressed mice.

CONCLUSIONS: Because patients with psychosis and PRS mice show similar epigenetic signature, PRS mice may be a suitable model for understanding the behavioral and molecular epigenetic changes observed in patients with SZ.

Keywords: BDNF, DNA methylation, DNMT, Prenatal stress, Schizophrenia, TET

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Evidence from clinical and preclinical studies indicates that exposure to stress during pregnancy exerts profound effects on the neurodevelopment and behavior of offspring (1–3). Prenatal stress (PRS) is considered a risk factor for several neurodevelopmental disorders, including schizophrenia (SZ), bipolar (BP) disorder, depression, and anxiety (4–7). A substantial amount of evidence from our group (3,8) and other researchers (9–11) suggests that epigenetic modifications of DNA and chromatin induced by environmental factors, including stress, may contribute to the complex phenotypes of neuropsychiatric disorders. Patients with psychosis exhibit an increase in brain DNA methyltransferases (DNMTs; DNMT1 and DNMT3a) and ten-eleven-translocation hydroxylase 1 (TET1) (12–15). The DNMTs and TETs are important components of the DNA methylation and demethylation dynamic regulating the expression of key molecules involved in brain function (16). Studying postmortem brains of patients with SZ, we have shown that overexpression of DNMTs and TETs is associated with an enrichment of 5-methylcytosine (5MC) and 5-hydroxymethylcytosine (5HMC) at SZ candidate gene promoters, including reelin (*RELN*), glutamic acid decarboxylase 67 (*GAD1*), and brain-derived neurotrophic factor (BDNF) exon IX, leading to expression down-regulation (14,16–20). Reduced expression of *BDNF* and increased promoter methylation of *BDNF* exons IV and IX have been reported in frontal cortex (FC) and hippocampus (HP) as well as in cerebrospinal fluid and blood of patients with SZ (21–25), BP disorder (26), depression, and anxiety (27–30).

In recent years, we have directed the focus of our research to the study of whether the altered epigenetic signature detected in postmortem brains of patients with SZ is also present in offspring of mice stressed during pregnancy (PRS mice). These PRS mice, similar to patients with SZ, show a delay of inhibitory neuron progenitor migration in the

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developing neocortex (31). Adult PRS mice have behavioral deficits similar to behaviors observed in patients with psychosis (3,32,33). The adult PRS mouse brain, similar to postmortem brains of patients with chronic SZ, is characterized by a significant increase in DNMTs, an enrichment of 5MC and 5HMC at GABAergic (*Gad1, Reln*) and glutamatergic (*Bdnf*) SZ candidate gene promoters, and a decrease in the expression of these genes (3,8). Decreased BDNF and increased DNMT1 expression as well an increase in the methylation of *Bdnf* IV promoter have also been reported recently in HP and amygdala of adult PRS rats (11).

In the present study, we tested whether 1) in addition to DNMT1, the expression of TETs is modified in the brain of PRS mice in a manner similar to brains of patients with SZ, and whether 2) such an increase in TETs participates in the regulation of the DNA-methylation and hydroxymethylation dynamics of SZ susceptibility genes focusing on BDNF as a target gene. The goal of our study was to establish, using a mouse model of prenatal exposure to stress, whether the decrease of *Bdnf* expression related to PRS is associated with changes in methylation and demethylation processes brought about by increased expression of DNMT and TET, modeling the epigenetic changes detected in the brains of patients with SZ and BP disorder.

METHODS AND MATERIALS

Animals and PRS Procedure

All procedures were performed according to National Institutes of Health guidelines for animal research (34) and were approved by the Animal Care Committee of the University of Illinois at Chicago. Pregnant mice (Swiss albino ND4, Harlan Laboratories, Indianapolis, Indiana) were individually housed with a 12-hour light-dark cycle and food and water ad libitum. Control dams were left undisturbed throughout gestation, whereas stressed dams were subjected to repeated episodes of restraint stress, as described previously (3) with slight modification. The stress procedure consisted of restraining the pregnant dam in a transparent tube (12 cm × 3 cm) under a bright light for 45 min three times per day from the seventh day of pregnancy until delivery. After weaning (postnatal day 21), male mice were selected for the study and housed four to five per cage separately by condition.

Behavioral Tests

Behavioral characteristics, first for locomotor activity and then for social interaction, were examined in PRS male mice and nonstressed (NS) control mice on consecutive days at postnatal day 75. We selected postnatal day 75 for behavioral testing because at this postnatal time the performance of the offspring was more reproducible and stable than the performance measured at earlier developmental time points.

Locomotor Activity. A computerized Animal Activity Monitoring System with VersaMax software (AccuScan Instruments, Columbus, Ohio) was used for the quantification and tracking of locomotor activity in mice as described previously (35). Each activity cage consisted of a Perspex box (20 cm × 20 cm × 20 cm divided into quadrants; AccuScan Instruments, Columbus, Ohio) surrounded by horizontal and vertical infrared sensor beams. The total number of interruptions of the horizontal sensors was taken as a measure of horizontal activity, whereas the number of interruptions of the vertical sensors was used as a measure of vertical activity. Activity was recorded for 15 min between 1 and 3 PM.

Social Interaction. Social approach of PRS mice and NS control mice was measured using a three-chambered apparatus (36), which is a rectangular, transparent three-chambered box with each chamber measuring 20 cm long × 40.5 cm wide × 22 cm high. Small openings in the clear Plexiglas walls (10 cm wide × 5 cm high) divide the center compartment from the two side compartments. Two identical wire cups were placed in left and right chambers, one for enclosing a stranger (novel) mouse and one as a control object. Between tests, the apparatus was thoroughly washed with 70% ethanol and then distilled water. Tests were performed under dim and even lighting between 10 AM and 3 PM. Sessions were recorded on videotape for data analysis.

The test mouse was first placed in the center chamber and habituated by allowing it to explore the entire apparatus freely for 5 min. Then the mouse was gently coerced into the center chamber and confined by shutting the openings for the side chambers. The stranger mouse was placed in the wire cup of one of the side chambers, and the test was initiated by opening both doorways allowing the test mouse to explore all three chambers freely for 10 min. Social approach or interaction was defined as the ratio of the sniffing time for the empty wire cup versus the cup enclosing the stranger mouse. Reliability of measurements was assessed by correlating the scores of two raters.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction was performed using the Applied Biosystems Real-Time PCR System (Stratagene MX3005P; Agilent Technologies, Santa Clara, California) with a SYBR green master mix (Fermentas, Glen Burnie, Maryland). Total RNA from FC and HP was isolated using TRIzol reagent (Life Technologies, Grand Island, New York) and was further purified using the QIAGEN RNeasy kit (Qiagen, Valencia, California). The RNA integrity number was measured with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California). The primer sequences for the genes analyzed are summarized in Table S1 in Supplement 1. Each sample was run in duplicate and repeated twice. For normalizing messenger RNA expression, several housekeeping genes (*Nse, NeuN,* and *β-actin*) were chosen as the internal control. For each housekeeping gene, we measured the gene stability ranking using the NormFinder algorithm (37). This procedure allows for the identification of the housekeeping gene best suited for normalization. Because each of the genes studied yielded similar results when normalized to *Nse, NeuN,* or *β-actin* and because *β-actin* had the highest housekeeping gene stability (NormFinder), we normalized our data to *β-actin*.

Western Blot Analysis

For protein quantification, we conducted measurements as described in detail elsewhere (14). Anti-DNMT1 monoclonal antibodies (.5 μg/mL; Imagenex, San Diego, California), anti-TET1 polyclonal antibody (Millipore Corp, Billerica, Massachusetts), and

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