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Short communication

The role of genes involved in stress, neural plasticity, and brain circuitry in depressive phenotypes: Convergent findings in a mouse model of neglect

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HIGHLIGHTS

- mPFC gene expression examined in mouse model of maternal neglect. (Characters with spaces: 67).
- Stress (ID3), synaptic plasticity (GRIN1) and myelin related (TPPP) genes explored. (Characters with spaces: 85).
- Genes selected to validate results obtained from peripheral samples in children. (Characters with spaces: 82).
- ID3, GRIN1 and TPPP expression predict depression and anxiety behaviors in mice. (Characters with spaces: 82).

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ABSTRACT

Early life neglect increases risk for the development of psychopathologies during childhood and adulthood, including depression and anxiety disorders. We recently reported epigenetic changes in DNA derived from saliva in three genes predicted depression in a cohort of maltreated children: DNA-binding protein inhibitor ID-3 (*ID3*), Glutamate NMDA Receptor (*GRIN1*), and Tubulin Polymerization Promoting Protein (*TPPP*). To validate the role of these genes in depression risk, secondary analyses were conducted of gene expression data obtained from medial prefrontal cortex (mPFC) tissue of mice subjected to a model of maternal neglect which included maternal separation and early weaning (MSEW). Anxiety and depression-like phenotype data derived using the elevated plus maze (EPM) and forced swimming test (FST), respectively, were also available for secondary analyses. Behavioral tests were conducted in MSEW and control adult male mice when they were between 65 and 80 days old. *ID3*, *GRIN1* and *TPPP* gene expression in the mPFC were found to significantly predict behavioral differences in the EPM and FST. These results further support the role of these genes in the etiology of depressive and anxiety phenotypes following early life stress.

1. Introduction

Child maltreatment is highly prevalent worldwide; maternal neglect is the most common form of maltreatment reported in the United States [1]. Children who are victims of neglect often develop depression and anxiety disorders [2,3]. Epigenetic mechanisms, particularly DNA methylation in several candidate genes, have been associated with increased vulnerability for the development of these problems [4]. Specifically, methylation in the serotonin transporter (*SLC6A4*), brain derived neurotrophic factor (*BDNF*), glucocorticoid receptor (*NR3C1*), and FK506 binding protein (*FKBP5*) genes have been found to predict depression and anxiety in clinical samples [5], with the role of these genes validated in preclinical studies [6,7].

In a genome-wide methylation study, we recently showed that methylation in three genes (DNA Binding Protein Inhibitor ID-3 (*ID3*); Tubulin Polymerization Promoting Protein (*TPPP*); and

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Glutamate Receptor, Ionotropic N-methyl-D-aspartate (NMDA) 1 (*GRIN1*) [8]) were significant predictors of depression in a sample of maltreated children:. These genes are biologically relevant, involved in stress response, synaptic pasticity, and the development of white matter tracts [8]. While the role of *GRIN1* in depression has been shown previously [9], the role of the other two genes implicated, *ID3* and *TPPP*, needs to be further elucidated.

The child study findings were derived using DNA extracted from saliva samples, which may not be the best source to study epigenetic phenomenon in psychiatric disorders. There are two main things to consider: 1) whether changes observed in saliva-derived DNA are relevant in the brain, and 2) whether these epigenetic changes subsequently alter gene expression. Brain tissue is not accessible in studies with child populations, so interest in the utility of peripheral tissue specimens has increased. DNA methylation and gene expression patterns are tissue-specific [10–12], but nonetheless several studies have found that DNA methylation changes correlate between the brain and peripheral tissues, including saliva [13,14].

While human brain tissue is not available, animal models could be used to validate findings obtained from peripheral human samples. Towards this purpose, secondary analyses were conducted on data collected in a study of a mouse model of maternal neglect. In this model, mice are subjected to maternal separation with early weaning (MSEW) [15]. It was previously reported that MSEW mice display mild anxiety-like and depression phenotypes, as characterized by behavior examined using the elevated plus maze and forced swim test, respectively [15]. This paper reports the results of secondary analyses examining the association between anxiety and depression phenotype data and gene expression of *ID3*, *GRIN1*, and *TPPP* in the medial prefrontal cortex (mPFC) of mice subjected to MSEW.

2. Material and methods

2.1. Animals

As detailed elsewhere [15,16] experimentally naive C57 and DBA mice were obtained from Jackson Laboratories (Bar Harbor, ME), and subsequently bred in-house. Entire litters were randomized at birth to control or Maternal Separation with Early Weaning (MSEW) conditions, but only male mice were included in the research. Behavioral data were available from 38 C57 control animals, 35 C57 MSEW animals, 24 DBA control animals, and 29 DBA MSEW animals. From these behaviorally characterized animals, a total of 11 C57 control animals, 11 C57 MSEW animals, 10 DBA control animals, and 10 DBA MSEW animals were included in gene expression microarray analysis. Control animals were left undisturbed and weaned at postnatal day (P) 23. MSEW animals were separated from the dams for 4 h per day on P2–P5, and 8 h per day on P6–P16, and weaned on P17. The Yale University Institutional Animal Care and Use Committee approved all experimental procedures.

2.2. Behavioral assessments

Male mice were between 65 and 80 days of age during behavioral testing, and scoring of all behavior was completed blind to rearing status. As described previously [15], all mice were administered the elevated plus maze task followed by the forced swim test.

2.2.1. Elevated plus maze (EPM)

Elevated plus maze testing was performed as previously described [15,17]. Mice were given 15 min to explore a plus shaped maze, constructed of white Plexiglass according to the dimensions of most commercially available mouse mazes (e.g., San Diego Instruments, Panlab Harvard Apparatus). The maze, positioned 31.5 cm above the floor, contained two open and two closed arms, all 30 cm in length, connected by a 6 cm center square. Closed arms were encased in 28 cm high black walls. The maze was placed inside an opaque testing box (100 cm × 100 cm × 30 cm), which was positioned in the center of a dimly lit room devoid of any obvious visual cues. A video camera placed 3 ft above the maze acquired digital video, which was later viewed by an observer blind to experimental condition. Additionally, video tracking was performed offline using software written by the authors to determine time spent in each arm and speed at each time point. Behavior was scored for total open and closed arm entries (defined as all four limbs entering the arm), and total time spent in the open and closed arms.

2.2.2. Forced swim test

The forced swim test was performed according to published procedures [18,19] with minor modifications. Mice were placed in 4 l glass cylinders (16 cm diameter) filled to a depth of 10 cm with 25 °C water. Each mouse was tested for 15 min and the cylinder was cleaned and filled with fresh water following each test. At completion, mice were removed from the water, dried, placed in holding cages, and put under heat lamps for 30 min before being returned to their home cages. Digital video was acquired from above and later scored in 5-min intervals for 15 min by an observer blind to experimental condition. Behavior was classified as immobile (defined as the absence of movement with exception of what is necessary to keep the animal's head above water), or mobile (defined as active swim if there was movement of all four limbs, or paddling if engaged in low frequency movements involving only one or two limbs). Primary outcome measures included total time mobile and total time immobile in each of the three time periods assessed (i.e., 0–5, 5–10, 10–15 min).

2.3. Tissue collection and RNA extraction

As described previously [20], mice were anesthetized with chloral hydrate (1500 mg/kg in sterile saline, IP) and rapidly decapitated. Whole brain was collected and placed in RNAlater (Qiagen) for 24 h, and then stored at −20 °C. Brains were removed from RNAlater, sectioned at 300 μm on a vibratory microtome (Vibratome), and sections were stored in 0.025% methylene blue in RNAlater at −20 °C for 48 h. Medial prefrontal cortex (mPFC), defined as anterior cingulate, pre-limbic cortex, and infra-limbic cortex, was dissected under a stereomicroscope, placed in RNAlater at 4 °C, and processed within 24 h. RNA and DNA were extracted using the Qiagen AllPrep mini kit. RNAlater was removed from the samples and 600 μL of buffer RLT (Qiagen) containing β-mercaptoethanol was added. Tissue was sonicated for 5 s at 10% power on ice, prior to RNA extraction. Protein was precipitated from the RNA column flow-through using the 2D Clean-Up Kit (GE) and pellets frozen at −80 °C.

2.4. Microarray analysis

As detailed elsewhere [20], total RNA was collected and analyzed on a Bioanalyzer (Agilent). Samples with RNA integrity (RIN) >9 were used for the array analysis. Five hundred ng of RNA from each sample were hybridized to Illumina Mouse WG-6 arrays, whole genome expression arrays that profile more than 45,500 biologically relevant transcripts. The Lumi package in R/Bioconductor was used to perform variance stabilization and quantile normalization. The gene expression analyses examined in this manuscript were limited to *ID3*, *TPPP*, and *GRIN1*.