

## RESEARCH ARTICLE

# Prenatal Nutritional Deficiency Reprogrammed Postnatal Gene Expression in Mammal Brains: Implications for Schizophrenia

Jiawei Xu, MD\*; Guang He, MD\*; Jingde Zhu, MD; Xinyao Zhou, MD; David St Clair, MD; Teng Wang, MD; Yuqian Xiang, MD; Qingzhu Zhao, MD; Qinghe Xing, MD; Yun Liu, MD; Lei Wang, MD; Qiaoli Li, MD; Lin He, MD; Xinzhi Zhao, MD

\*These authors contributed equally to this work.

Children's Hospital and Institutes of Biomedical Sciences, Fudan University, Shanghai, China (Drs Xu, Zhou, T. Wang, Xiang, Xing, Liu, L. Wang, Li, L. He and X. Zhao); Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai, China (Drs Xu, G. He, Zhou, T. Wang, Xiang, Q. Zhao, Xing, Liu, L. Wang, Li, L. He and X. Zhao); Center for Reproductive Medicine, The First Affiliated Hospital of Zhengzhou University, Shanghai, China (Dr Xu); Cancer Epigenetics and Gene Therapy Program, Shanghai Cancer Institute, Shanghai Jiao Tong University, Shanghai, China (Dr Zhu); Department of Mental Health, University of Aberdeen, Scotland (Dr St Clair).

Xinzhi Zhao, PhD, Children's Hospital and Institutes of Biomedical Sciences, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China ([xzzhao@fudan.edu.cn](mailto:xzzhao@fudan.edu.cn)); or Lin He, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, 1954 Huashan Road, Shanghai 200030, China ([helin@bio-x.cn](mailto:helin@bio-x.cn))

## Abstract

**Background:** Epidemiological studies have identified prenatal exposure to famine as a risk factor for schizophrenia, and animal models of prenatal malnutrition display structural and functional brain abnormalities implicated in schizophrenia.

**Methods:** The offspring of the RLP50 rat, a recently developed animal model of prenatal famine malnutrition exposure, was used to investigate the changes of gene expression and epigenetic modifications in the brain regions. Microarray gene expression analysis was carried out in the prefrontal cortex and the hippocampus from 8 RLP50 offspring rats and 8 controls. MBD-seq was used to test the changes in DNA methylation in hippocampus depending on prenatal malnutrition exposure.

**Results:** In the prefrontal cortex, offspring of RLP50 exhibit differences in neurotransmitters and olfactory-associated gene expression. In the hippocampus, the differentially-expressed genes are related to synaptic function and transcription regulation. DNA methylome profiling of the hippocampus also shows widespread but systematic epigenetic changes; in most cases (87%) this involves hypermethylation. Remarkably, genes encoded for the plasma membrane are significantly enriched for changes in both gene expression and DNA methylome profiling screens ( $p = 2.37 \times 10^{-9}$  and  $5.36 \times 10^{-9}$ , respectively). Interestingly, *Mecp2* and *Slc2a1*, two genes associated with cognitive impairment, show significant down-regulation, and *Slc2a1* is hypermethylated in the hippocampus of the RLP50 offspring.

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**Conclusions:** Collectively, our results indicate that prenatal exposure to malnutrition leads to the reprogramming of postnatal brain gene expression and that the epigenetic modifications contribute to the reprogramming. The process may impair learning and memory ability and result in higher susceptibility to schizophrenia.

**Keywords:** DNA methylation, hippocampus, prefrontal cortex, schizophrenia, transcriptome

## Introduction

Converging evidence suggests that schizophrenia is a neurodevelopmental disease with environmental influences during early brain development (Brandon and Sawa, 2011; Owen et al., 2011). A multitude of epidemiological studies have demonstrated that maternal exposure to various harmful environmental events, such as famine, infections, and nutritional deficits, during critical stages of pregnancy significantly increases the risk of schizophrenia in the offspring (Brown et al., 1996; Malaspina et al., 2008; Brown, 2011). The first direct evidence for the association between prenatal exposure to famine and schizophrenia arose from a study of the Dutch Hunger Winter of 1944–1945, at the end of World War II (Susser and Lin, 1992; Jones, 1994). The offspring of mothers who suffered a nutrition deficiency in the first trimester of gestation showed a two-fold increase in their risk of schizophrenia in adulthood (Roseboom et al., 2001; Painter et al., 2005). We have performed replication studies in two independent birth cohorts exposed to the 1959–1961 famine in China, and in both studies our findings are remarkably similar to the Dutch data (St Clair et al., 2005; Xu et al., 2009). Overall, previous Dutch and Chinese studies provide strong evidence that prenatal famine plays a role in the risk of schizophrenia (for a detailed comparison of the converging evidence, see Susser and St Clair, 2013).

There are several mechanisms, none of which are mutually exclusive, by which prenatal exposure to malnutrition could increase the risk of schizophrenia in adulthood (Xu et al., 2009). Although they are difficult to study retrospectively in humans, some of these mechanisms can potentially be explored using well-designed animal models (Brown and Susser, 2008). We hypothesized that maternal protein deficiency, along with exposure to prenatal nutrition deflection, may play an important role in the association between prenatal famine exposure and risk of schizophrenia. Maternal protein deficiency leads to low birth weight, which is common among people exposed prenatally to famine; there is also a well-established association between low birth weight and increased risk of schizophrenia. Using the prenatal protein deprivation (PPD) model, rats were placed on protein-deficient diets during pregnancy, and the offspring demonstrated neurotransmitter, cellular, electrophysiological, and behavioral disruptions that were associated with schizophrenia, which has been reviewed previously (Brown and Susser, 2008). These include abnormal hippocampal structures and functions, deficits in sensorimotor gating, enhanced behavioral sensitivity to acute treatment with dopamine receptor agonists and N-methyl-D-aspartic acid receptor antagonists, working memory impairments, and reduced pre-pulse inhibition (Palmer et al., 2004 2008).

We have established a prenatal malnutrition (famine) rat model, named RLP50, which was induced by prenatal exposure to a diet restricted to 50% of a low-protein (6%) diet. We observed higher levels of tumor necrosis factor alpha (TNFA) and Interleukin 6 (IL6) in placentas and fetal livers and lower levels in brains. This suggests TNFA and IL6 mediate dysfunction in a common pathway that plays an important role in brain development; in humans these abnormalities may increase the risk of

schizophrenia and other psychiatric diseases (Shen et al.). What is more, using metabonomic and metallomic profiling strategies, we have observed significantly different patterns of metabolites and trace elements in pregnant rats of the RLP50 group. This broadens still further our understanding of the complex biochemical perturbations that prenatal exposure to famine can induce, which may eventually lead to impairment of fetal neurodevelopment (Shen et al., 2008). However, we didn't explore whether prenatal malnutrition could affect gene expression in brains prior to adulthood. If yes, we would conclude that epigenetic modification would play an important role.

Epigenetic modifications regulate long-term gene expression and are reprogrammed genome-wide during early embryo development. Previous studies reported that prenatal protein deficiency resulted in changes in methylation and gene expression in the liver, suggesting the fetal reprogramming contributes to adult metabolism abnormality (Gong et al., 2010; Mortensen et al., 2010). However, few studies have investigated the changes of gene expression and epigenetic modifications in the brain regions of the animals after prenatal malnutrition. The prefrontal cortex (PFC) and hippocampus are two of the brain regions most implicated in schizophrenia (Berman et al., 1986; Mirmics et al., 2000; Harrison, 2004). Are gene expression and DNA methylation patterns altered by prenatal malnutrition in RLP50 rats in those brain regions? This was the next question we wished to explore. Since gene expression in brains shows major spatial as well as temporal differences, it was essential to detect whole-genome gene expression in the PFC and hippocampus, as well as DNA methylation in these two regions (Maniatis et al., 1987; Stein et al., 1990). In the present study, we set out to investigate the PFC and hippocampus in adult offspring of control and RLP50 groups using a combined transcriptomic and epigenetic strategy.

## Methods

### Animal Model

Sprague-Dawley rats (Shanghai Slack Laboratory Animal Co. Ltd.), were used in all experiments under the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academic Press; <http://www.nap.edu/readingroom/books/labrats>). All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee at the Institutes of Biomedical Sciences (School of Life Science, Fudan University).

Seventeen Sprague-Dawley female rats were separated into three groups on the first day after mating, and were fed their respective diets until they were put down at E18 (embryo, 18 days), two days before parturition (control: 5 dams; malnourished group [LP]: 5 dams; famine group [RLP50]: 7 dams). The control group was given a standard rodent diet (20% protein, Research Diets, Inc. D12450B; see [Supplementary Table 1](#)) and water *ad libitum* and the LP group was given a low-protein (6% protein, Research Diets, Inc. D06022301; see [Supplementary Table 1](#)) diet and water *ad libitum*. The RLP50 group were given 50% of the LP group's low-protein diet, reflecting both the protein malnutrition

and food-deficiency likely to prevail during famine. The treatment of the pregnant rats in RLP50 was according to our previous model (Shen et al. 2008). All litters were culled to 8 pups, which were fostered by their own mothers. Rats were raised to be 10 weeks old and were anesthetized before being killed. The PFC and hippocampus from the adult offspring of both control (n = 40) and RLP50 (n = 56) rats were then isolated and stored in RNAlater® Solution (Lifetechnologies).

### Microarray Gene Expression Analysis of the PFC and Hippocampus

RNA was extracted from the total PFCs and hippocampi of 8 controls and 8 RLP50 rats each from 5 control and 7 RLP50 dams using mirVana™ miRNA Isolation Ki. An RNA integrity number (RIN, Agilent) > 7.5 was selected for microarray gene expression analysis using NimbleGen Rat Gene Expression 12 x 135K Arrays. NimbleGen probe-set data were normalized using the robust multi-array average method. Seven genes from the PFC and 12 genes from the hippocampus were selected for validation using quantitative real-time polymerase chain reaction (qRT-PCR) in the different samples, as used for the microarray experiments. GO and annotation analysis was conducted using DAVID Tools (Huang da et al., 2009). We also mapped differentially-expressed genes at  $p < 0.05$  and fold change > 1.25 to KEGG (Ng et al., 2010). The molecular networks, consisting of differentially-expressed genes, were generated through Ingenuity pathways analysis.

### MBD-Seq and Data Analysis

Briefly, we pooled equal quantities of DNA from the hippocampi of 5 samples of the same gender but from different dams. A total of 5 µg of genomic DNA was sheared randomly between 100 and 500bp and the DNA fragments were then ligated with ligator. DNA fragments were incubated with recombinant his-tagged methyl-CpG binding domain proteins (MBD), conjugated to magnetic beads. The bound DNA fragments were eluted using Proteinase K digestion, and purified and precipitated using phenol:chloroform:isopropanol. We then prepared GAI libraries from the isolated DNA according to the manufacturer's protocol to generate 36bp single-end reads.

Sequencing reads were mapped to the reference rat genome (Baylor 3.4/rn4) using Bowtie (Langmead et al., 2009). Only uniquely-mapped reads were retained for downstream analysis. We assembled the individual DNA methylomes as previously described (Serre et al., 2010); the peaks were called using MACS (Zhang et al., 2008) and the peaks were annotated using PeakAnalyzer (Salmon-Divon et al., 2010).

### Sequenom EpiTYPER™ validation of the difference in methylation regions

We used Sequenom EpiTYPER™ to amplify the target sequence. The mass spectra were collected and spectra methylation proportions were generated using the EpiTYPER™ software v1.0 under the user's manual. The significance of single loci methylation differences was examined using a two-tailed student's t-test.

### Luminometric Methylation Assay

The principle of the luminometric methylation assay (LUMA) has been described in detail elsewhere (Muthayya et al., 2009). Briefly, 200–500ng genomic DNA was digested for 4h by HpaII + EcoRI

or MspI + EcoRI (New England Biolabs) in 2 separate reactions. Then, 15 µl annealing buffer was added to the digestion product, and samples were analyzed with a PyroMark Q24 system. The LUMA methylation level was expressed as a percentage obtained from the following equation:  $\text{methylation (\%)} = [1 - (\text{HpaII } \Sigma G/\Sigma T) / (\text{MspI } \Sigma G/\Sigma T)] \times 100$ . Eight RLP50 cases and eight controls were assayed at the same time under the manual (Salmon, 1994).

## Results

### Phenotype of Famine Pregnant Rats and Neonatal Rats

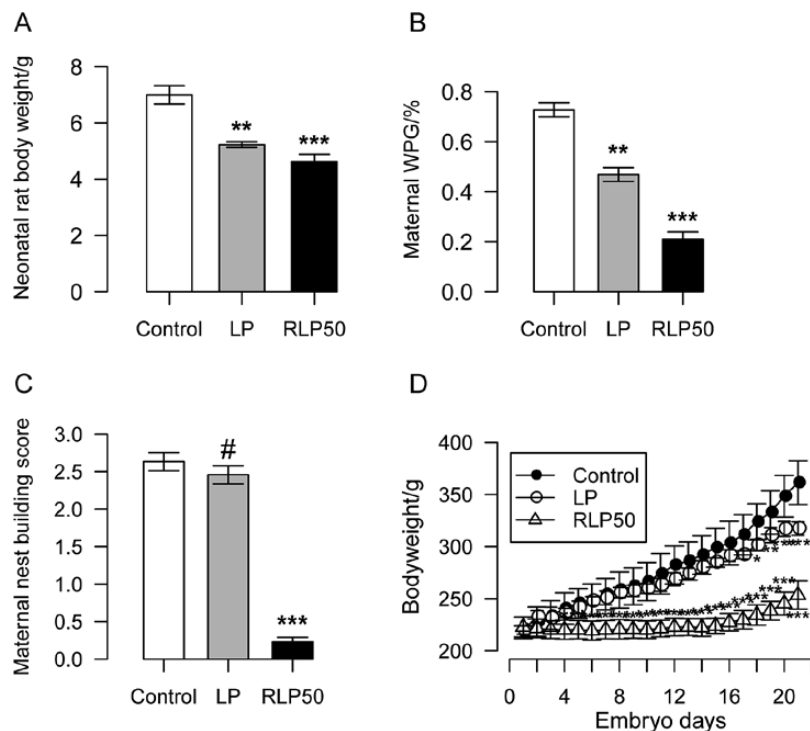
Prenatal malnutrition did not significantly alter birth numbers (see Supplementary Table 2). However, the neonatal birth weights of both the LP and RLP50 groups were lower than those of the control group, with the RLP50 group having the lowest birth weights ( $p < 0.001$ ; Figure 1A). Meanwhile, the RLP50 group showed significant maternal weight percent gain compared to the other two groups ( $p < 0.001$ ; Figure 1B). The maternal weight trend during gestation also supported this (Figure 1D). As previously reported in our lab, we observed a tendency for the LP group to build a smaller nest and a complete disappearance of nest building in the RLP50 group after gestational exposure to simulated famine ( $p = 0.00055$ ; Figure 1C), indicating that gestational exposure to malnutrition and the stress of starvation resulted in maternal behavior disruption.

### Prenatal Exposure to Famine-Disturbed Gene Expression in PFC of RLP50 Adult Offspring

PFC has been implicated in both memory dysfunction and the pathophysiology of schizophrenia (Perlstein et al., 2001). In order to test the hypothesis that prenatal exposure to famine will disturb the gene expression in the PFC in offspring, we identified 415 genes showing modest to significant expression differences ( $p < 0.05$ ), of which 48 genes showed high significance ( $p < 0.01$ ): 37 genes showed up-regulation, while 11 genes showed down-regulation using NimbleGen Rat microarray. Seven genes were selected for technical validation using qRT-PCR, and the results are shown in Table 1.

In particular, *Comt* was up-regulated ( $p = 0.0024$ , fold change = 1.6) in the RLP50 group. *Comt* has been considered especially relevant in the control of the dopamine signal in the PFC in both humans and rats (Karoum et al., 1994; Matsumoto et al., 2003; Williams et al., 2007). Up-regulation of *Comt* suggests that the dopamine transmission may be disturbed, leading to deficits in the modulation of synaptic inputs to prefrontal neurons, considered to be a prominent component of cognitive dysfunction in schizophrenia. The neurotransmitter receptors *Htr1d* (5-hydroxytryptamine receptor 1D) and *Gabrg3* (gamma-aminobutyric acid [GABA] A receptor, gamma 3) were also significantly down-regulated; 5-hydroxytryptamine and GABA are important neurotransmitters in modulating mental activity, and reduced expression of their receptors could result in impaired PFC cognition. Hierarchical clustering analysis based on the top 330 differentially-expressed genes showed distinct clustering of the majority of PFC samples in both groups (Figure 2A): this suggests that gene expression may be reprogrammed in the PFC of adult offspring after prenatal exposure to famine.

We used 330 genes ( $p < 0.05$ , fold change > 1.25) for Gene Ontology (GO) analysis. All gene-associated biological annotation categories are shown in Supplementary Table 2. The highly significant GO categories were membrane, development, signal transduction, and



**Figure 1.** Maternal exposure to famine alters behavior of pregnant rats. (A) Body weight of neonatal rats; (B) maternal weight percent gain (WPG); (C) maternal nest-building behavior; (D) maternal body weight during gestation. Data are expressed as mean  $\pm$  standard error of the mean. Two-tailed student's t-tests and Kruskal-Wallis H tests were used to calculate the statistical significance. Asterisks indicate a particular level of significance (\* $p < 0.01$ , \*\* $p < 0.001$ , # $p > 0.05$ ). All statistical analyses and data manipulations were carried out using the R package.

**Table 1.** Microarray and qRT-PCR Results in PFCs and Hippocampi of Both Control and RLP50 Offspring

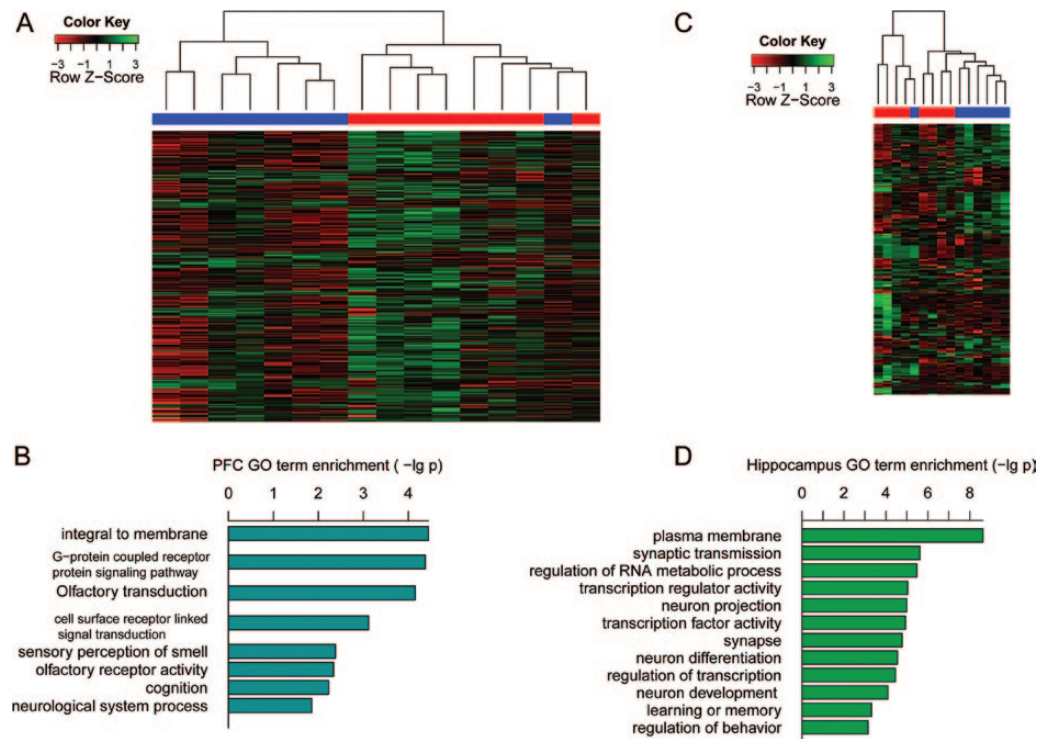
Roch probe #	SEQ_ID	Gene name	Fold change RLP50/C (Microarray)	p-value (Microarray)	Fold change RLP50/C (qPCR)	P-value
<b>PFC</b>						
40	NM_012531	<i>Comt</i>	1.53	0.0016	2.05	0.001
77	NM_012852	<i>Htr1d</i>	0.51	0.048	0.39	0.031
9	NM_012972	<i>Kcna5</i>	0.5	0.01	0.43	0.027
1	NM_013125	<i>SCn5a</i>	0.41	0.028	0.23	0.035
82	NM_024370	<i>Gabrg3</i>	0.63	0.021	0.55	0.017
38	NM_080693	<i>Cacng5</i>	0.59	0.027	0.51	0.029
113	XM_001071808	<i>Cplx3</i>	1.82	0.011	1.75	0.0048
<b>Hippocampus</b>						
129	NM_024483	<i>Adra1d</i>	0.6	0.04	0.41	0.000088
78	NM_153735	<i>Nptx1</i>	0.67	0.048	0.65	0.0063
75	NM_133381	<i>Crebbp</i>	0.56	0.0024	0.87	0.015
2	NM_012832	<i>Chrna7</i>	0.58	0.0052	0.58	0.0056
55	NM_080773	<i>Chrm1</i>	0.58	0.034	0.66	0.0031
69	NM_021679	<i>Nxph3</i>	0.41	0.029	0.3	0.037
29	NM_012574	<i>Grin2b</i>	0.61	0.0094	0.64	0.0024
58	NM_012768	<i>Drd5</i>	0.55	0.005	0.54	0.014
117	NM_017078	<i>Chrna5</i>	0.59	0.042	0.32	0.001
122	NM_012706	<i>Grpr</i>	0.64	0.013	0.27	0.00064
130	NM_012524	<i>Cebpa</i>	0.67	0.0039	0.63	0.03
80	NM_022673	<i>Mecp2</i>	0.76	0.007	0.61	0.0042
20	NM_053870.2	<i>Kcnj4</i>	0.67	0.031	0.57	0.00075

PFC: pre-frontal cortex; qPCR: quantitative polymerase chain reaction; qRT-PCR: quantitative real-time polymerase chain reaction; RLP50: mice fed a famine diet

cognition associated (Figure 2B). Interestingly, the olfactory function associated GO categories was also highly significant. Recent studies have provided evidence for olfactory physiological impairment in schizophrenia patients and their first-degree relatives

(Turetsky et al., 2003, 2009; Turetsky and Moberg, 2009), suggesting that olfactory dysfunction might be an endophenotype relevant to schizophrenia. Molecular networks were also identified using IPA: the top network is shown in Supplementary Figure 1A.





**Figure 2.** Prenatal exposure to famine reprogrammed gene expression in RLP50 offspring's PFCs and hippocampi. (A) Heat map of top 330 genes differentially expressed between RLP50 and control PFC samples. The top bar indicates the groups: blue = RLP50; red = control. (B) PFC-relevant gene ontology categories enriched. (C) Heat map of top 300 genes differentially expressed between RLP50 and control hippocampus samples. The top bar indicates the groups: blue = RLP50; red = control. (D) Hippocampus-relevant gene ontology categories enriched.

### Gene Expression was Large-Scale Reprogrammed in Hippocampus of RLP50 Offspring Due to Prenatal Malnutrition

The hippocampus has long been implicated in the pathophysiology of schizophrenia. We observed a large-scale reprogramming of gene expression in the hippocampi of RLP50 adult offspring. We identified 2987 genes with significant ( $p < 0.05$ ) expression differences, and 841 genes with highly significant ( $p < 0.01$ ) expression differences, of which 356 genes were up-regulated and 485 genes were down-regulated. Hierarchical clustering analysis based on the top 300 differentially-expressed genes also supported the view that prenatal exposure to famine reprograms gene expression in the hippocampus (Figure 2C). We selected 13 genes which were significantly differently expressed for validation of the microarray (Table 1).

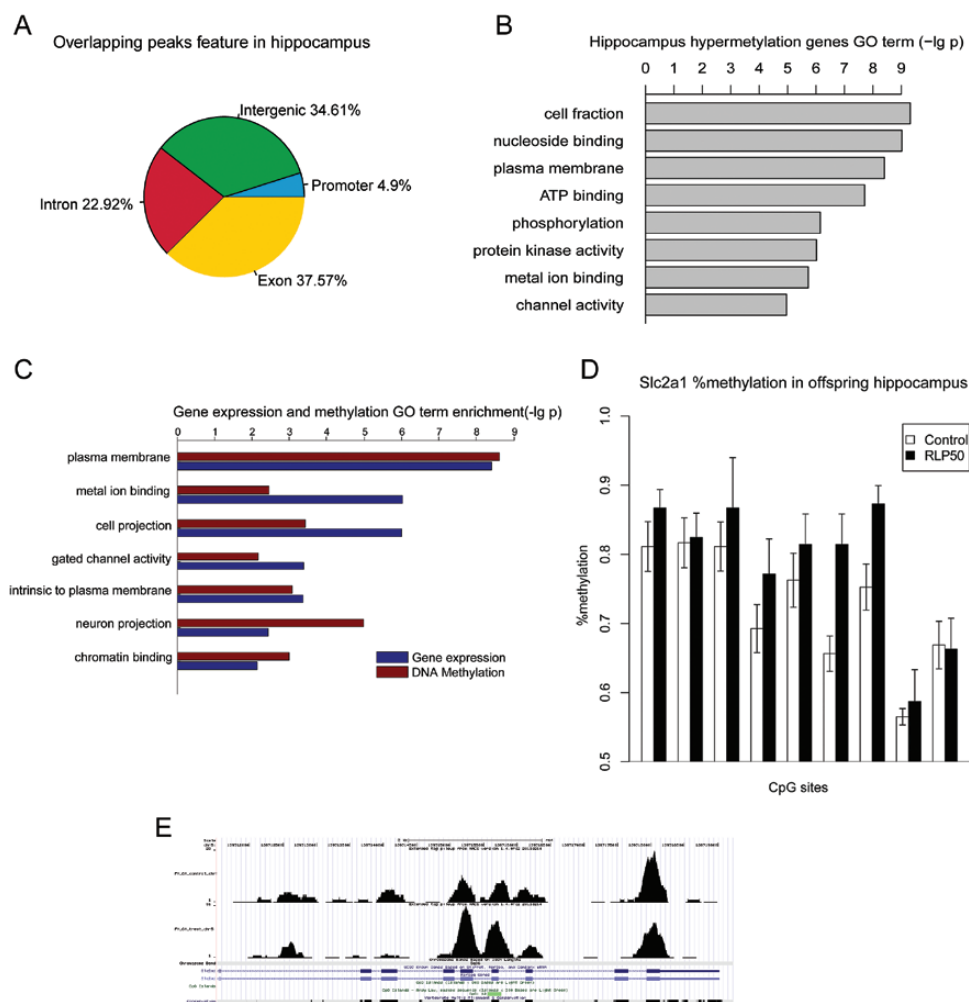
We used 1372 genes with  $p < 0.05$  and fold change  $> 1.25$  for GO analysis, and the results are shown in Supplementary Table 3. The differential-expression genes related to synaptic function and neurodevelopment were highly enriched, suggesting that prenatal exposure to famine severely disturbs the function of the hippocampus. Interestingly, the genes implicated in transcription regulation were also significantly enriched (Figure 2D). Hierarchical clustering of the 95 genes in the transcription regulator activity term is displayed in Figure 3A. Transcription regulators may bind a promoter or enhancer DNA sequence or interact with a DNA-binding transcription factor. Most of the genes in the transcription regulator activity category showed modest down-regulation, including the *Mecp2* gene ( $p = 0.0070$ , fold change =  $-1.32$ ), which encodes an important transcriptional regulator for RNA polymerase II promoter. *Mecp2* has been shown to activate many genes, binding to DNA in the

hypothalamus of mice, and the transcriptional activator *Creb1* is involved in the process (Chahrouh et al., 2008). We also detected that the expression of *Creb1* ( $p = 0.029$ , fold change =  $-1.18$ ) and its co-activator *Crebbp* ( $p = 0.0072$ , fold change =  $-1.79$ ) were down-regulated.

Molecular networks were also identified using IPA, with the top five networks being shown in Supplementary Figure 1B-F. The top molecular networks and the top molecules are involved in neurological disease, psychological disorders, and development disorders.

### Region-Specific CpGs Methylation Altered in the Hippocampus of RLP50 Adult Offspring

Our gene-expression microarray results suggested there might be gene- or region-specific DNA methylation differences in the hippocampus between adult offspring of the control and RLP50 groups. We therefore performed genome-wide methylation studies to search for differentially-methylated loci between adult offspring of the RLP50 and control groups, using MBD following next-generation sequencing. We identified 6627 significant peaks with fold enrichment  $> 3$ , among which 5762 (86.9%) peaks suggested hypermethylation in the hippocampi of RLP50 offspring, and the remaining 865 (13.1%) peaks suggested potential hypomethylation. After annotating 1754 peaks, the false discovery rate is lower than 0.05, with PeakAnalyzer detecting the genes most probably affected. We observed that most peaks were located in the gene body, and only a small portion were located in the promoter region (Figure 3A). Then the annotated genes were subjected to GO analysis; the most significant GO terms are shown in Figure 3B. As expected, several function terms were enriched in the methylome profiling,

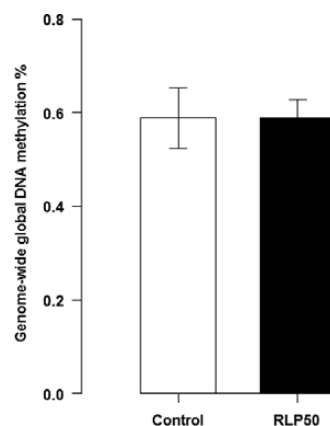


**Figure 3.** Prenatal exposure to malnutrition induces cytosine methylation alterations in the hippocampi of adult RLP50 offspring. (A) Distribution of significant peaks location; (B) potential affected genes Gene Ontology analysis; (C) overlapping Gene Ontology terms in genome-wide genes expression and methylation of the hippocampus; (D) intragenic methylation status of *Slc2a1*; (E) view of *Slc2a1* reads peaks in the hippocampi of both group using UCSC genome browser.

as well as in the gene-expression profiling analysis (Figure 3C), including the plasma membrane, which was highly significant ( $p = 2.37 \times 10^{-9}$  in gene expression, and  $p = 5.36 \times 10^{-9}$  in DNA methylome). The plasma membrane controls the movement of ions and organic molecules in and out of cells. Therefore, the abnormality of the plasma membrane will lead to dysfunction in the transporting of materials needed for functioning of the hippocampus. The intragenic peaks in *Slc2a1* showed high significance for down-regulation ( $p = 0.0022$ , fold change = -1.61) in adult offspring of RLP50; we validated this using the MassARRAY EpiTYPER (Sequenom), showing hypermethylation in adult offspring of the RLP50 group (Figure 3C and D). Our results suggested that epigenetic alteration contributed partly to the regulation of large-scale gene-expression reprogramming in the hippocampi of adult offspring of the RLP50 group.

#### Estimation of Genome-Wide Global DNA Methylation Between the Hippocampi of Adult Offspring of Control and RLP50 Groups

Changes in genomic DNA methylation are crucial for tissue-specific gene expression, and global DNA methylation may partly contribute to gene activity (Li, 2002). To explore whether the large-scale gene-expression reprogramming in the hippocampus



**Figure 4.** Genome-wide global DNA methylation between the hippocampi of adult offspring of the control and RLP50 groups.

is associated with global genomic DNA methylation, we used LUMA to detect the global genomic DNA methylation in the hippocampus. However, we didn't observe global genomic methylation differences between the adult offspring of the control and RLP50 groups (Figure 4). Prenatal exposure to famine represents

a kind of environmental change in gestation, which [McClellan et al. \(2006\)](#) conjectured would lead to genome-wide epigenetic alterations in offspring. We concluded that there might be gene- or region-specific DNA methylation to regulate gene expression in the hippocampus.

## Discussion

In the present study, we performed the first genome-wide screen of transcriptome and DNA methylome in mammals' brain after exposure to prenatal nutrition deficiency. We demonstrate that prenatal nutrition deficiency affects gene expression in the PFC and hippocampus regions of adult brains. There was larger-scale gene-expression reprogramming in the hippocampus than in the PFC. The differentially-expressed genes in the hippocampus were enriched in several gene ontology categories related to synaptic function and neurodevelopment, consistent with previous finding in PPD models that prenatal nutrition deficiency leads to hippocampus abnormality associated with schizophrenia ([Bronzino et al., 1997](#); [Morgane et al., 2002](#)). The hippocampus is believed to be crucially involved in the neuropathology and pathophysiology of schizophrenia, and many reports have found that hippocampus dysfunction is caused by deficits of some gene expression in synapses in schizophrenic subjects, and that the changes probably result from altered development rather than tissue damage ([Harrison, 2004](#)). Therefore, the impairment of hippocampal development and functions may be important for the increased risk to schizophrenia after prenatal exposure to malnutrition.

Changes in epigenetic modification may contribute to the gene-expression reprogramming in the hippocampus caused by prenatal nutrition deficiency. We observed that the differentially-expressed genes in the hippocampus were enriched in the gene ontology categories implicated in transcription regulation, which are tightly associated with epigenetic modifications. The important epigenetic regulator *Mecp2* was down-regulated. *Mecp2* plays a critical role in neurodevelopment, and loss of function as well as increased dosage of its human homologue cause a number of neuropsychiatric disorders, including schizophrenia ([Chahrour et al., 2008](#)). The DNA methylome analyses in the hippocampus identified numerous region-specific changes of DNA methylation. Moreover, we demonstrated hypermethylation of *Slc2a1*, which showed down-regulated expression in the hippocampus of the RLP50 offspring. Epigenetic modifications have been considered to be involved in the pathophysiology of schizophrenia. Changes of DNA methylation were identified in the brain tissues of schizophrenia patients in several post-mortem studies. However, none of these studies were performed in the hippocampus, and therefore it is difficult to compare our results with published studies on DNA methylation and schizophrenia. Our results are intriguing and suggest that epigenetic profiling in the hippocampi of schizophrenia patients may be partly responsible for their disease development.

DNA methylation and gene expression were poorly correlated in our study. This is consistent with recent reports ([Kulis et al., 2012](#)). Nevertheless, several gene ontology categories were functionally enriched in both gene expression and DNA methylome-profiling screens. This suggests an indirect association between DNA methylation and expression. Notably, the most differentially methylated regions we identified were hypermethylated in the gene body region in RLP50 offspring. This is consistent with well-established data that show an association between the methylated promoter and repressed transcription start sites, while gene

body methylation is not associated with transcriptional repression. DNA methylation in the gene body has been proposed to function in the regulation of alternative splicing via repressed transcriptional regulator protein CTCF binding that will pause on RNA polymerase II ([Shukla et al., 2011](#)). It is possible that changes of intragenic DNA methylation affect gene splicing, and influence the expression of their functionally-related genes. Further transcriptome assay, such as RNA-seq, may prove or clarify this hypothesis. Another explanation would be cell-specificity: the gene expression and methylation patterns are highly different between distinct cell types such as neurons and glia cells. In the present study, cell type specificity was not accounted for and might thus have contributed to the weak correlation between DNA methylation and gene expression. Moreover, the Methyl-CpG binding domain proteins followed by next generation sequencing used in this study were sensitive to highly-methylated and high-CpG density regions, so there may be some methodological bias in the DNA methylome profiling. Finally, DNA methylation is only a part of epigenetic regulations: the role of histone modifications and DNA hydroxymethylation in prenatal nutrition deficiency should be investigated in further studies.

The prefrontal cortex is responsible for complex cognitive behavior and its dysfunction is associated with schizophrenia. However, there were few studies that have reported changes of gene expression after prenatal exposure to nutrition deficiency. In our study, the gene-expression reprogramming was less significant in the PFC of RLP50 offspring compared with that of the hippocampus. At the same time, we did not observe significant changes in cytosine (data not shown). Nevertheless, we observed that maternal nutrition deficiency disturbed the expression of neurotransmitter receptors, cognition, and neurological system-associated genes in offspring PFCs, suggesting that cognitive impairments result from prenatal malnutrition.

There were some limitations in our study. On account of the limited number of animals on which gene-expression profiling was performed, we did not try to identify sex-specific changes of gene expression. We thought this was appropriate since prenatal exposure to famine increases the risk of schizophrenia in both males and females. However, females are more vulnerable than the males to development of some schizophrenia-related functional abnormalities, such as a disruption of sensorimotor gating and enhanced sensitivity to dopaminergic drugs in the PPD model. There may therefore be some specific changes of gene expression in the female offspring of RLP50 that our results have failed to detect. Also, in our RLP50 model we mainly focused on maternal protein deficiency that would result in low birth weights. However, some micronutrients, such as folate, Vitamin D, and iron, may also be involved in the association between famine and schizophrenia. It will be of great interest to compare our results to those where the reprogramming of gene expression and epigenetic modifications occur in models in which only specific micronutrients are restricted. Finally, prenatal exposure to famine alone is unlikely to cause increased risk of schizophrenia independent of other as-yet-unidentified factors. Schizophrenia is a highly-heritable disorder and risk/protective alleles for schizophrenia may also influence the reprogramming process induced by prenatal malnutrition. Our results are interesting and merit follow-up studies; it will be interesting to determine whether field studies examining prenatally famine-exposed schizophrenia cases and unexposed siblings and other control groups can detect any of the differences we have observed in our animal studies. In the meantime, testing the expression

reprogramming due to prenatal malnutrition in transgenic animals promises to be a valuable next step in investigating and understanding gene and environment interactions in schizophrenia, and how epigenetic modifications may play a crucial role in these processes.

In summary, we have shown that maternal exposure to nutrition deficiency results in genome-wide reprogramming of gene expression in the brains of adult offspring, and causes dysfunction of the prefrontal cortices and hippocampi. Our study represents an initial step toward characterizing the molecular mechanisms of increased risk for schizophrenia after prenatal exposure to famine.

## Supplementary Material

For supplementary material accompanying this paper, visit <http://www.ijnp.oxfordjournals.org>

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## Statement of Interest

None

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