Research Article

Mechanism of Earthquake Simulation as a Prenatal Stressor Retarding Rat Offspring Development and Chinese Medicine Correcting the Retardation: Hormones and Gene-Expression Alteration

X. G. Zhang,1 H. Zhang,1 R. Tan,2 J. C. Peng,3 X. L. Liang,1 Q. Liu,1 M. Q. Wang,4 and X. P. Yu5

1 The School of Nursing, Chengdu University of T.C.M., Chengdu 610075, China
2 School of Biology and Engineering, Southwest Jiaotong University, Chengdu 610031, China
3 Earthquake Emergency Security Center, Sichuan Provincial Seismological Bureau, Chengdu 610041, China
4 Molecular Laboratory of T.C.M., Chengdu University of T.C.M., Chengdu 610075, China
5 School of Humanity and Information Management, Chengdu Medical University, Chengdu 610075, China

Correspondence should be addressed to X. P. Yu, 18908006961@189.cn

Received 26 August 2012; Revised 3 October 2012; Accepted 5 October 2012

Academic Editor: Jang-Hern Lee

Copyright © 2012 X. G. Zhang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

We aimed to investigate the mechanism of shaking as a prenatal stressor impacting the development of the offspring and Chinese medicines correcting the alterations. Pregnant rats were randomized into earthquake simulation group (ESG), herbal group (HG) which received herbal supplements in feed after shaking, and control group (CG). Findings revealed body weight and open field test (OFT) score of ESG offspring were statistically inferior to the CG and HG offspring. The corticosterone levels of ESG were higher than those of CG but not than HG. The dopamine level of ESG was slightly lower than that of the CG and of HG was higher than that of ESG. The 5-HT of ESG was higher than CG and HG. The growth hormone level of the ESG was significantly lower than ESG but not than CG. Gene expression profile showed 81 genes upregulated and 39 genes downregulated in ESG versus CG, and 60 genes upregulated and 28 genes downregulated in ESG versus HG. Eighty-four genes were found differentially expressed in ESG versus CG comparison and were normalized in ESG versus HG. We conclude that maternal shaking negatively affected physical and nervous system development, with specific alterations in neurohormones and gene expression. Chinese herbal medicine reduced these negative outcomes.

1. Introduction

Maternal effects have been demonstrated as an essential factor for offspring development in many species. Because of the long period of perinatal mother-infant interaction in mammals, the growth and development and variations of offspring are very likely to be influenced by maternal impacts, leaving long-term consequences for both psychological and physiological health [1]. Recent human studies have shown that long-lasting and a wide variety of prenatal stressors, from anxiety and partner relationship problems to natural disasters, increase the risk for a diverse range of adverse neurodevelopmental outcomes in the child, including impaired cognitive development and behavioral problems [2, 3]. Animal experiments have convincingly demonstrated that prenatal maternal stress affects pregnancy outcome and results in early programming of brain functions with permanent changes in neuroendocrine regulation, gene expression, and behavior in offspring [4]. Prenatal restraint stress in rats is a common experimental model of early stress known to have long-term behavioral and neurobiological consequences [5, 6]. PS modifies the plastic responses of the adult brain, including the circuitry of the hippocampus-hypothalamus-pituitary-adrenals (HHPA), that participate in the neuroendocrine control of feeding and metabolism in adult life [7].
As a typical prenatal stress, shaking can significantly impact the psychological and intellectual development of fetus and birth outcomes [8] in human. Naturally, earthquake is a fierce shaking. Tan et al. [9] reported that rates of birth defects after an earthquake were significantly higher than those before earthquake, whose spectrum was dramatically altered after earthquake, with the markedly increased occurrences of ear malformations; meanwhile the ratio of preterm birth after earthquake was significant increased than that of before earthquake. Oyarzo et al. [10] reported that women exposed to the February 27th 2010 Chilean earthquake during her first trimester delivered smaller newborns and they were more likely diagnosed with early preterm delivery, preterm delivery, and PROM but were less likely diagnosed with intraterine growth retardation and late delivery compared to those exposed at third trimester, indicating disasters such as earthquakes are associated to adverse perinatal outcomes that impact negatively the entire maternal-neonatal healthcare system. Like the other alterations induced by PS in behavior those in learning and their direction appears to be dependent on the intensity, duration, and timing of the maternal stress [11].

In Chinese medicine, PS from shaking or an analog of earthquake is considered as a factor which impairs kidney Qi (shen qi) [12]. As kidney is the root of earlier heaven (the congenital constitution), it governs reproduction and development and holds orifice of labor, whence agility and emanates. Jin Kui Shen Qi Wan (JKSQW) is a typical herbal formula supplementing kidney Qi, which recovers the physiological functions of kidney [13].

The current study involves shaking as a prenatal stressor. A first goal was to establish that earthquake simulation led to significant delays in development. A second goal was to examine whether Chinese traditional medicine could be used to address these negative effects. Based on the above information, we hypothesized parental kidney is injured from PS derived from earthquake simulation on rats, traits are handed down to offspring, showing development retardation; JKSQW could recover the dysfunctions of kidney whose underlying mechanism could involve development, hormones and gene expression alterations.

2. Materials and Methods

2.1. Grouping. Forty-five Sprague-Dawley (SD) female rats (230 g~270 g) and 45 male rats (225 g~261 g) were involved in this research. The rats were housed in a room with a temperature of 22°C, 12 hour light/dark cycle and fed with food and water ad libitum. After a week of adaption housing, the female rats were mated with the male rats. Pregnancy was confirmed by vaginal plug test. Then the 34 pregnant rats were randomized into three groups, control group (CG) (n = 11), earthquake simulation with conventional chow group (ESG) (n = 11), and earthquake plus herbal group (HG) (n = 12), and they were housed under pregnant rat cages until the delivery. With this procedure, all the groups were transferred with equivalent stress during pregnancy. There was no statistical difference of gestation time detected or body weight of the first day of gestation (CG: 234.87±2.20, ESG: 234.98 ± 1.95, and HG: 235.16 ± 1.96, ANOVA test, P > 0.05 (g)) in the three groups. After delivery, all the litters of the three groups were housed with their mothers until the 25th day after birth.

2.2. Earthquake Simulation. The ESG cages housing pregnant rats were manually shaken up and down 3 times to simulate an initial earthquake and then were shaken for 50 times over the next 15 minutes to modulate an aftershock [14]. The shaking was performed twice a day until delivery. Severity of the shake was measured with a seism velocimeter (DX-6Y2, Cheng Du Mei Huan Tech. Co. Ltd.), showing 9.6~10.5 of seismic intensity, 950 mg~1050 mg of vertical peak ground accelerations (PGA), which was similar to the PGA (1080 mg) of Wenchuan earthquake, May 12, 2008, China.

2.3. Chinese Herbal Formula Feed. The feed of HG rats was supplemented with herbal medicine until delivery, which consisted of (Radix Rehmanniae Preparata (Shu Di Huang), Fructus Corni Officinalis (Shan Zhu Yu), Cortex Moutan Radicis (Mu Dan Pi), Rhizoma Dioscoreae Oppositae (Shan Yao), Sclerotium Poriae Cocos (Fu Ling), Rhizoma Alismatis Orientalis (Ze Xie), Radix Aconiti Lateralis Preparata (Zhi Fu Zi), and Cortex Cinnamomi Cassiae (Rou Gui)) bought from Tong Ren Tang Technologies, Co., Ltd. The pill of JKSQW was ground and added to the conventional feed 0.5~0.6 g/d.

2.4. Body Weight Measurement. Body weight (g) was measured at the 1st (day 0), 5th (day 5), 10th (day 10), 15th (day 15), 20th (day 20), and 25th (day 25) days after delivery in order to evaluate the body development of the offspring.

2.5. Open Field Test (OFT). A square board (90 cm × 90 cm) painted with yellow and white squares (15 cm × 15 cm). The offspring of 25 days old was placed in the center of the board. We counted how many squares the offspring had crawled across in two minutes. One score was given only when the four paws of an offspring were in one square.

2.6. Hormone Assay. Thirty offspring were randomly selected from the groups, ten for each. Blood sample was taken from arteria femoralis. ELISA (R&D Systems China Co., Ltd.) was employed to determine the serum level of corticosterone (DZE 30590), dopamine (DZE 30238), 5-HT (DZE 30326), and growth hormone (DZE 30549).

2.7. Gene Expression Profile Chip Experiments

2.7.1. RNA Extraction and Purification. Total RNA was extracted using TRIZOL Reagent (Cat no. 15596-018, technologies, Carlsbad, CA, US) following the manufacturer’s instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Qualified total RNA was further purified by RNeasy mini kit (Cat no. 74106, QIAGEN, GmBH, Germany) and RNeasy micro kit (Cat no.
74004, QIAGEN, GmBH, Germany) and RNase-Free DNase Set (Cat no. 79254, QIAGEN, GmBH, Germany) (Table 1).

2.7.2. RNA Amplification and Labeling. Total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit, One-Color (Cat no. 5190-2305, Agilent technologies, Santa Clara, CA, US), following the manufacturer’s instructions. Labeled cRNA were purified by RNeasy mini kit (Cat no. 74106, QIAGEN, GmBH, Germany).

2.7.3. Hybridization. Each slide was hybridized with 1.65 μg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Cat no. 5188-5245, Agilent technologies, Santa Clara, CA, US) in Hybridization Oven (Cat no. G2545A, Agilent technologies, Santa Clara, CA, US), according to the manufacturer’s instructions. After 17 hours hybridization, slides were washed in staining dishes (Cat no. 121, Thermo Shandon, Waltham, MA, US) with Gene Expression Wash Buffer Kit (Cat no. 5188-5327, Agilent technologies, Santa Clara, CA, US), following the manufacturer’s instructions.

2.7.4. Data Acquisition. Slides were scanned by Agilent Microarray Scanner (Cat no. G2565CA, Agilent technologies, Santa Clara, CA, US) with default settings: dye channel: Green, Scan resolution = 5 μm, PMT 100%, 10%, 16 bit. Feature Extraction software 10.7 (Agilent technologies, Santa Clara, CA, US) Raw data were normalized by Quantile algorithm, Gene Spring Software 11.0 (Agilent technologies, Santa Clara, CA, US) (Table 1).

2.7.5. Real-Time PCR. Primers of the four genes were designed with Primer Express 2.0 (Oebiotec, Shanghai, China) (Table 2). Reverse transcription was performed on PrimerScript RT reagent Kit (Takara, DRR037A, Takara Biotechnology (Dalian) Co., Ltd. China). Total RNA (0.5 μg) was denatured at room temperature then mixed with the reagent in a final volume of 10 μL containing 50 μM oligo dT, 100 μM random primer, 0.5 mM dNTP and the manufacturer’s buffer and Enzyme Mix. The RT reaction was conducted for 15 min at 37°C, and 85°C for 5 s in ABI 9700. First-strand cDNA product was diluted in 100 μL distilled water in preparation for real-time PCR. qPCR was performed using SuperReal PreMix (SYBR Green) kit (Tiangen, FP204, Tiangen Biotech (Beijing) Co., Ltd. Beijing, China). Briefly, 1 μL of diluted cDNA product was used for 40-cycle three-step PCR in a Roche HOLD CYCLE LightCycler 480 II.

2.8. Statistical Analysis. The body development, behavioral test, and hormone level data were analyzed using a Statistical Package for the Social Sciences (SPSS) version 19.0. ANOVA for Repeated Measurement with Greenhouse-Geisser Adjustment was performed to analyze group differences in body weight. A nonparametric Mann-Whitney test was performed to analyze group differences on the OFT. Student’s t-test was performed to analyze group differences in corticosterone, dopamine, 5-HT, and growth hormone. Alpha was set to 0.05 for all analyses.
Table 3: Differentially expressed genes in ESG versus CG, among which 39 genes were upregulated and 81 genes downregulated.

| Gene ID   | P values | Fold change | Gene symbol | Regulation |
|-----------|----------|-------------|-------------|------------|
| 63847     | 0.007006 | 0.096204    | Fxyd6       | Downregulated |
| 498145    | 0.003225 | 0.17368     | LOC498145   | Downregulated |
| 316628    | 0.004414 | 0.274831    | Ash1        | Downregulated |
| 360547    | 0.005836 | 0.320844    | Sat2        | Downregulated |
| 301245    | 0.007067 | 0.331729    | Yip3        | Downregulated |
| 293023    | 0.009502 | 0.335662    | Klhl25      | Downregulated |
| 288240    | 0.002174 | 0.34925     | Hlcs        | Downregulated |
| 293180    | 0.007695 | 0.352823    | Micalcl     | Downregulated |
| 316426    | 0.003961 | 0.363248    | Spats2l     | Downregulated |
| 293624    | 0.008043 | 0.364195    | Itf7        | Downregulated |
| 683788    | 0.007907 | 0.382175    | LOC683788   | Downregulated |
| 293156    | 0.009012 | 0.413953    | Lrtomt      | Downregulated |
| 25646     | 0.004102 | 0.429726    | Otx1        | Downregulated |
| 290232    | 0.009311 | 0.430944    | Tin2        | Downregulated |
| 498353    | 0.002896 | 0.440115    | Scfd2       | Downregulated |
| 362873    | 0.006203 | 0.440433    | Plix1c      | Downregulated |
| 309415    | 0.009479 | 0.458925    | Fam189a2    | Downregulated |
| 113894    | 0.007725 | 0.463149    | Sqstm1      | Downregulated |
| 305538    | 0.003261 | 0.465171    | Dhs58       | Downregulated |
| 406196    | 0.001118 | 0.467157    | Hcr         | Downregulated |
| 313917    | 0.005676 | 0.482298    | Abhd1       | Downregulated |
| 292811    | 0.009904 | 0.48439     | Ccdc123     | Downregulated |
| 290985    | 0.007918 | 0.491881    | Isca1       | Downregulated |
| 405152    | 0.008771 | 0.516648    | Olr1192     | Downregulated |
| 171355    | 0.005274 | 0.519609    | Pou4f2      | Downregulated |
| 362943    | 0.000172 | 0.526926    | Acd5        | Downregulated |
| 309161    | 0.001612 | 0.543788    | Ccdc85b     | Downregulated |
| 361327    | 0.003693 | 0.596748    | Prr16       | Downregulated |
| 24640     | 0.008865 | 0.602226    | Plkfb2      | Downregulated |
| 619573    | 0.006811 | 0.603084    | Fam104a     | Downregulated |
| 11625     | 0.007447 | 0.653258    | Ube2n       | Downregulated |
| 304342    | 0.005141 | 0.662423    | Zscan21     | Downregulated |
| 192252    | 0.009069 | 0.671766    | Dtcp1p      | Downregulated |
| 114205    | 0.00295  | 0.677239    | Crcp        | Downregulated |
| 311430    | 0.007769 | 0.689602    | Mavs        | Downregulated |
| 287840    | 0.003671 | 0.716317    | Faml100b    | Downregulated |
| 297109    | 0.006823 | 0.764608    | MGC95152    | Downregulated |
| 295037    | 0.000491 | 0.788096    | Mgst2       | Downregulated |
| 100360990 | 0.007759 | 0.815928    | LOC100360990 | Downregulated |
| 501083    | 0.00564  | 1.179002    | Pdc6ip      | Upregulated  |
| 299195    | 0.000513 | 1.189394    | Coq6        | Upregulated  |
| 81716     | 0.007768 | 1.20684     | Ggcx        | Upregulated  |
| 315023    | 0.008157 | 1.265746    | Slc25a32    | Upregulated  |
| 296753    | 0.009238 | 1.284846    | Srp2        | Upregulated  |
| 299147    | 0.005455 | 1.304917    | Ppp2r5e     | Upregulated  |
| 361932    | 0.009554 | 1.307515    | RGD1561393  | Upregulated  |
| 288259    | 0.009614 | 1.31293     | Gart        | Upregulated  |
| 289522    | 0.002341 | 1.325268    | Cox18       | Upregulated  |
| 50688     | 0.002132 | 1.334825    | Cacnb1      | Upregulated  |
| 363171    | 0.000593 | 1.337206    | Tmem42      | Upregulated  |
| 114215    | 0.005997 | 1.352079    | Insl3       | Upregulated  |
| Gene ID  | P values | Fold change | Gene symbol | Regulation |
|----------|----------|-------------|-------------|------------|
| 315771   | 0.008317 | 1.369011    | Herc1       | Upregulated |
| 360389   | 0.009442 | 1.375028    | Zfp422      | Upregulated |
| 305923   | 0.008185 | 1.393988    | Zdhhc20     | Upregulated |
| 24803    | 0.005163 | 1.396617    | Vamp2       | Upregulated |
| 363210   | 0.001697 | 1.411325    | Phf3        | Upregulated |
| 50561    | 0.001722 | 1.425023    | Respl8      | Upregulated |
| 362367   | 0.005441 | 1.43527     | Znrf2       | Upregulated |
| 170841   | 0.009557 | 1.458549    | Mutyh       | Upregulated |
| 81678    | 0.003588 | 1.467406    | Itpr2       | Upregulated |
| 502886   | 0.009395 | 1.466283    | Foxj2       | Upregulated |
| 360868   | 0.009274 | 1.471063    | Sftd2       | Upregulated |
| 313757   | 0.005281 | 1.485264    | RGD1565591  | Upregulated |
| 361109   | 0.006669 | 1.486251    | Dcp1a       | Upregulated |
| 192210   | 0.008713 | 1.487999    | Dnajc21     | Upregulated |
| 25262    | 0.008127 | 1.49478     | Itpr1       | Upregulated |
| 311112   | 0.00906  | 1.533447    | Fastkd1     | Upregulated |
| 64086    | 0.004012 | 1.55121     | Cnk1g1      | Upregulated |
| 366693   | 0.007515 | 1.567923    | Rbm25       | Upregulated |
| 690961   | 0.006894 | 1.577038    | Cog2        | Upregulated |
| 292148   | 0.004257 | 1.589999    | Eif3a       | Upregulated |
| 691918   | 0.002531 | 1.596744    | LOC691918   | Upregulated |
| 362317   | 0.001503 | 1.599092    | Krist1      | Upregulated |
| 54323    | 0.001154 | 1.610286    | Arc         | Upregulated |
| 304813   | 0.005676 | 1.614358    | Ppp1r12b    | Upregulated |
| 58983    | 0.00216  | 1.617294    | Rabggtga    | Upregulated |
| 361944   | 0.004739 | 1.617335    | Elf2        | Upregulated |
| 314862   | 0.000215 | 1.618023    | Dyrk2       | Upregulated |
| 29642    | 0.003006 | 1.62079     | Slc38a2     | Upregulated |
| 291409   | 0.00357  | 1.622726    | Zfp236      | Upregulated |
| 246282   | 0.001061 | 1.623318    | Zfp91       | Upregulated |
| 362132   | 0.00226  | 1.626565    | Epc2        | Upregulated |
| 303963   | 0.002236 | 1.631518    | Dzip3       | Upregulated |
| 116670   | 0.006773 | 1.634179    | Ppp1r12a    | Upregulated |
| 302670   | 0.004529 | 1.63737     | Zrsr2       | Upregulated |
| 360993   | 0.006601 | 1.637448    | Smek2       | Upregulated |
| 59319    | 0.001208 | 1.6438      | Nyw1        | Upregulated |
| 287249   | 0.009286 | 1.659325    | Cnot6       | Upregulated |
| 362132   | 0.007917 | 1.663529    | Epc2        | Upregulated |
| 303511   | 0.004368 | 1.665157    | Ikkf3       | Upregulated |
| 363210   | 0.008478 | 1.665263    | Phf3        | Upregulated |
| 362096   | 0.00268  | 1.668933    | Setx        | Upregulated |
| 316583   | 0.001117 | 1.700923    | B3gnt7      | Upregulated |
| 362817   | 0.008175 | 1.701909    | Cdk2        | Upregulated |
| 304157   | 0.009185 | 1.708222    | Nrip1       | Upregulated |
| 314169   | 0.009008 | 1.729076    | Fam179b     | Upregulated |
| 303919   | 0.007784 | 1.731828    | Lrcc58      | Upregulated |
| 309523   | 0.003447 | 1.734164    | Kif20b      | Upregulated |
| 291773   | 0.003136 | 1.741424    | RGD1562997  | Upregulated |
| 314423   | 0.003545 | 1.743689    | Bcl11b      | Upregulated |
| 362622   | 0.007916 | 1.756522    | Ccld21      | Upregulated |
| 497198   | 0.005781 | 1.770803    | Impact      | Upregulated |
Table 3: Continued.

| Gene ID  | P values | Fold change | Gene symbol | Regulation |
|---------|----------|-------------|-------------|------------|
| 315804  | 0.00029  | 1.773739    | Rfx7        | Upregulated|
| 363287  | 0.002339 | 1.775948    | Hdac4       | Upregulated|
| 361688  | 0.00606  | 1.778637    | Suv420h1    | Upregulated|
| 363555  | 0.002239 | 1.787221    | Wfikkn1     | Upregulated|
| 304809  | 0.001337 | 1.791911    | Kdm5b       | Upregulated|
| 498803  | 0.003675 | 1.797804    | Otud1       | Upregulated|
| 64624   | 0.005484 | 1.803225    | Cul5        | Upregulated|
| 304817  | 0.00381  | 1.807047    | Ip09        | Upregulated|
| 54311   | 0.008729 | 1.82334     | Timm17a     | Upregulated|
| 25486   | 0.006615 | 1.978189    | Tspyl2      | Upregulated|
| 293765  | 0.003013 | 2.076238    | Olr327      | Upregulated|
| 171347  | 0.00179  | 2.47901     | Mat2a       | Upregulated|
| 363083  | 0.007379 | 2.521284    | Fbxl22      | Upregulated|

3. Results

3.1. Body Development and Behavior Test. ANOVA for Repeated Measurement with Greenhouse-Geisser Adjustment (Mauchly's W = 0.085, Approx. Chi-square = 214.490, df = 14, P ≤ 0.001, Greenhouse-Geisser = 0.541) showed a statistically significant difference of the body weight of the observation time spots of offspring among CG, ESG, and HG offspring (body weight: df = 2.705, mean square = 39791.256, F = 1923.553, P ≤ 0.001; body weight * group df = 5.410, mean square = 415.400, F = 20.081, P ≤ 0.001). Generally, HG offspring was heavier than CG, which is heavier than ESG (Figure 1).

A Mann-Whitney test showed significant difference between the three groups on the OFT (Mann-Whitney U = 1448.500, Wilcoxon W = 2529.500, Z = -3.189, P = 0.000) (Figure 2): the OFT scores of HG and CG were both significantly higher than those observed in the ESG.

3.2. Hormone Levels. The corticosterone levels of CG was statistically lower than ESG and slightly than HG (Figure 3(a)). The dopamine level of ESG was slightly lower than the CG and of HG was significantly higher than the ESG (Figure 3(b)). The 5-HT of ESG showed a highest level and the CG lowest (Figure 3(c)). The growth hormone level of the HG was statistically higher than the CG and ESG (Figure 3(d)).

3.3. Gene Expression Profile

3.3.1. ESG versus CG. Gene expression profile showed 81 genes upregulated and 39 genes downregulated (P < 0.01) in ESG versus CG comparison (Table 3 (see Supporting Information 1), Figure 4), among which 14 GO annotations were obtained including, ligase activity, regulation of metabolic process, positive regulation of metabolic process, cellular component assembly, membrane bounded organelle, biosynthetic process, cellular component biogenesis, and cellular response to stimulus. (Table 4 (Supporting Information 2)), and among which 12 KEGG pathways were annotated, including oocyte meiosis, vascular smooth muscle contraction, RIG-I-like receptor signaling pathway, long-term potentiation, ubiquitin mediated proteolysis, and long-term depression (Table 5).

3.3.2. ESG versus HG. Gene expression profile showed 60 genes upregulated and 28 genes downregulated (P < 0.01) in ESG versus CG (Table 6 (Supporting Information 3), Figure 5), among which five GO annotations were obtained including protein complex localization, cellular component assembly, cellular component biogenesis, anatomical structure formation, and organelle lumen (Table 7), and among which 5 KEGG pathways were annotated, including cell cycle, Jak-STAT signaling pathway, Type II diabetes mellitus, One carbon pool by folate, and insulin signaling pathway (Table 8).

No genes were found, which were significantly differently expressed simultaneously in ESG versus CG and ESG versus HG. However, 8,426 genes were found no statistical difference in HG versus CG (P > 0.05) among which 84 were found also presented in the differently expressed genes in ESG versus HG (Table 9 (Supporting Information 4)).

3.3.3. RT-PCR Validation. Irf7, Ninj2, Plxnc1, and Isca1 were filtered to validate with RT-PCR according to the set that the flag value of the expression profile chip ≠ A, FC > 2 or FC < 0.5, expression value ≥6 from the GO and
| GO Id       | Name                                | Symbol                                                                                   | Hits | Total | Percent | Enrichment test P value |
|------------|-------------------------------------|------------------------------------------------------------------------------------------|------|-------|---------|-------------------------|
| GO: 0016874 | Ligase activity                     | Ube2n, Hlcs                                                                               |      |       |         |                         |
|            |                                     | Gart, Herc1, Cul5, Rnf168, Ggcx                                                          | 7    | 308   | 2.27%   | 0.0083                  |
| GO: 0019222 | Regulation of metabolic process     | Sqstm1, Ins3, Ube2n, Pou4f2, Otx1, Cnot6, TinF2, RGD1562997, Irf7, Tspyl2, Nrip1, Zscan21, Jarid1b, Bcl11b, Dyrk2, Mll1, Rfx7, Zfp422, Smek2, Suv420h1, Elf2, Cdk2, Hdac4, Impact, Foxj2, Rasd1, Rnf168, Pfn2 | 28   | 2415  | 1.16%   | 0.0089                  |
| GO: 0009893 | Positive regulation of metabolic process | Sqstm1, Ins3, Ube2n, Pou4f2, TinF2, Nrip1, Zscan21, Bcl11b, Dyrk2, Mll1, Cdk2, Hdac4, Rnf168 | 13   | 846   | 1.54%   | 0.0098                  |
| GO: 002607 | Cellular component assembly         | Sqstm1, Xtp3tpa, Vamp2, Cox18, TinF2, Eif3s10, RGD1562997, Srpk2, Mll1, Enth, Pfn2 | 12   | 786   | 1.53%   | 0.0135                  |
| GO: 0043227 | Membrane-bounded organelle          | Sqstm1, Crp, Ube2n, Mutyh, Pou4f2, Vamp2, Itpr1, Otx1, Cnot6, Hlcs, Cox18, TinF2, Isca1, Eif3s10, RGD1562997, Irf7, Srpk2, 1kzf3, Ppp2r5e, Yipf3, Tspyl2, Zsr2, Nrip1, Zscan21, Kif20b, Visa, RGD1565591, Bcl11b, Dyrk2, Scl25a32, Mll1, Enth, B3gnt7, Zfp422, Setx, Suv420h1, Elf2, Phf3 | 55   | 5982  | 0.92%   | 0.025                   |
Table 4: Continued.

| GO Id    | Name                          | Symbol                      | Hits | Total | Percent | Enrichment test P value |
|----------|-------------------------------|-----------------------------|------|-------|---------|-------------------------|
|          |                               | Cdk2, Adck5, Hdac4 Hcr, LOC498145 |      |       |         |                        |
|          |                               | Pdcd6ip, Foxj2, Rasd1, Resp18, Cul5 |      |       |         |                        |
|          |                               | Cacnb1, Timm17a, Arc, Rnf168, Cog2, Itp2, Ggcx |      |       |         |                        |
| GO: 0014854 | Response to inactivity      | Hdac4                        | 1    | 3     | 33.33%  | 0.0288                  |
|          |                               | Crp, Ins3, Ube2n            |      |       |         |                        |
|          |                               | Mat2a, Pou4f2, Ot2x          |      |       |         |                        |
|          |                               | Cnot6, Gart, Tinf2          |      |       |         |                        |
|          |                               | Isca1, RGD1562997           |      |       |         |                        |
|          |                               | Eif3s10, Irf7, Coq6         |      |       |         |                        |
|          |                               | Tspyl2, Nrip1, Mll1        | 34   | 3379  | 1.01%   | 0.0291                  |
|          |                               | Zscan21, Jarid1b           |      |       |         |                        |
|          |                               | Bcl11b, Dyrk2, Rfx7        |      |       |         |                        |
|          |                               | B3gmt7, Zfp422, Elf2       |      |       |         |                        |
|          |                               | Suv420h1, Cdk2, Phf3       |      |       |         |                        |
|          |                               | Hdac4, Impact, Foxj2       |      |       |         |                        |
|          |                               | Rabggta, Rasd1             |      |       |         |                        |
| GO: 0009058 | Biosynthetic process      |                               |      |       |         |                        |
|          |                               | Sqstm1, Xtp3tpa,           |      |       |         |                        |
|          |                               | Vamp2, Cox18, Tinf2        |      |       |         |                        |
|          |                               | RGD1562997, Eif3s10        |      |       |         |                        |
|          |                               | Srpk2, Mll1, Enth, Pfn2    | 12   | 883   | 1.36%   | 0.0299                  |
| GO: 0044085 | Cellular component biogenesis | Sqstm1, Xtp3tpa,           |      |       |         |                        |
|          |                               | Vamp2, Cox18, Tinf2        |      |       |         |                        |
|          |                               | RGD1562997, Eif3s10        |      |       |         |                        |
|          |                               | Srpk2, Mll1, Enth, Pfn2    | 12   | 883   | 1.36%   | 0.0299                  |
| GO: 0014874 | Response to stimulus involved in regulation of muscle adaptation | Hdac4 | 1    | 4     | 25.00%  | 0.0359                  |
| GO: 0043233 | Organelle lumen            | Sqstm1, Mutyh, Itp2r1      |      |       |         |                        |
|          |                               | Tinf2, RGD1562997          |      |       |         |                        |
|          |                               | Srpk2, Tspyl2, Zrsr2       | 16   | 1360  | 1.18%   | 0.0416                  |
|          |                               | Nrip1, Kif20b, Mll1        |      |       |         |                        |
|          |                               | Zfp422, Setx, Cdk2        |      |       |         |                        |
|          |                               | Hdac4, Resp18              |      |       |         |                        |
| GO: 0051716 | Cellular response to stimulus | Ube2n, Mutyh, Dyrk2       | 8    | 528   | 1.52%   | 0.0422                  |
|          |                               | Mll1, Setx, Cdk2          |      |       |         |                        |
Table 4: Continued.

| GO Id   | Name                                | Symbol                  | Hits | Total | Percent | Enrichment test P value |
|---------|-------------------------------------|-------------------------|------|-------|---------|-------------------------|
|         | Pdcd6ip, Rnf168                      |                         |      |       |         |                         |
| GO:0016740 Transferase activity | Crcp, Mat2a, Pfkb2        |                         |      |       |         |                         |
|         | Gart, Mgst2, Srpk2                   |                         |      |       |         |                         |
|         | RGD1304822, Dyrk2                    |                         |      |       |         |                         |
|         | Fastkd1, MIL1, B3gnt7                |                         |      |       |         |                         |
|         | Suv420h1, Cdk2, Fgfr11, RGD1560523   |                         |      |       |         |                         |
|         | Rabggta, Csnk1g1                     |                         |      |       |         |                         |
|         | **GO:0031974 Membrane enclosed lumen** | Sqstm1, Mutyh, Itpr1    |       |       |         |                         |
|         | Tinf2, RGD1562997                    |                         |      |       |         |                         |
|         | Srpk2, Tpsy2, Zsr2                   |                         |      |       |         |                         |
|         | Nrip1, Kif20b, MIL1                  |                         | 16   | 1392  | 1.15%   | 0.0495                 |
|         | Zfp422, Setx, Cdk2,                  |                         |      |       |         |                         |
|         | Hdac4, Resp18                         |                         |      |       |         |                         |
| GO:0031077 Postembryonic camera-type eye development | Bcl11b               | 1    | 6     | 16.67%  | 0.0499                |

KEGG annotation. As showed in Figure 6(a), Irf7, Ninj2, and Isca1 were significantly hypoexpressed in ESG (FC < 0.5); however, the gene expression of Plxn1 did not match the RT-PCR validation; in Figure 6(b), the four genes were not significantly hypoexpressed in HG versus CG (0.5 < FC < 2), and the RT-PCR validation showed an obviously reduced ΔΔCt values compared with those in Figure 6(a). The gene expression profile chip outcomes showed a favorable match with the RT-PCR result.

4. Discussion

Substantial evidence from preclinical laboratory studies indicates that PS affects the hormonal and behavioral development of offspring. PS has been found to alter baseline and stress-induced responsivity of the HPA axis and levels and distribution of regulatory neurotransmitters, such as norepinephrine, dopamine, serotonin, and acetylcholine and to modify key limbic structures and to retard intrauterine growth [15]. In this study, ESG demonstrated differences from CG on body weight, hormone levels, and gene expressions, and HG differed from the ESG group on body weight, hormone levels, and gene expressions. From the perspective of Chinese medicine, once parental kidney is injured from PS, manifestations are handed down to offspring, showing development retardation and OFT performance reduction. JKSQW is a typical herbal formula for kidney qi supplementing, which recovers the physiological functions of kidney. In this study, the body weight and OFT performance were improved by JKSQW, supporting the effectiveness of Chinese herb remedy in rodents in lab [13].

Experimentally, PS in animal models mal-programs offspring physiology, resulting in increasing the likelihood of disorders of HPA axis activity and anxiety-related behaviors in adulthood [16]. PS increases plasma levels of corticosterone and corticotrophin releasing hormone in the mother and fetus, which may contribute to insulin resistance and behavior disorders in their offspring that include attention and learning deficits, generalized anxiety and depression [17]. We demonstrated that the serum corticosterone of ESG were significantly higher than CG and slightly higher than HG, which was in accordance with previous reports [18–20]. Animal studies indicate that PS can affect the activity of the placental barrier enzyme 11-βHSD2 (11β-hydroxysteroid dehydrogenase type 2), which metabolizes corticosterone [2, 17]. 5-HT level of ESG was significantly higher than CG and HG. Alterations in activity of the central 5-HT system play an essential role in many of these behavioral aberrations due to PS [21, 22]. During pregnancy, the 5-HT system has a fundamental role in the fetus’ development of the central nervous system, and 5-HT neurotransmission is involved in the activation and feedback of HPA axis throughout life [23]. Huang et al. [14] reported that levels of 5-HT were higher in rat hippocampus and hypothalamus of fetuses in the CUS group, that is, chronic unpredictable stress maternally performed than in the controls. Increased 5-HT signaling increases the expression of key transcription factors, notably
Table 5: KEGG Pathway annotation of the 120 differentially expressed genes ($P < 0.05$, $q < 0.05$) (↓ refers downregulation, ↑ refers upregulation).

| Name                                           | Symbol       | Total | Percent | Enrichment test P value | q value |
|------------------------------------------------|--------------|-------|---------|-------------------------|---------|
| Oocyte meiosis                                 | Itpr2↓ Ppp2r5e↓ Cdk2↑ | 116   | 0.0345  | 0.0008                  | 0.0048  |
| Vascular smooth muscle contraction             | Ppp1r12a↑ Itpr1↑ Ppp1r12b↑ | 128   | 0.0313  | 0.0011                  | 0.0048  |
| RIG-I-like receptor signaling pathway          | Irf7↓ Dhx58↓ Mavs↓ | 64    | 0.0469  | 0.0016                  | 0.0048  |
| Long-term potentiation                         | Ppp1r12a↑ Itpr1↑ Itpr2↑ | 72    | 0.0417  | 0.0022                  | 0.0049  |
| Ubiquitin mediated proteolysis                 | Ube2n↓ Herc1↑ Cul5↑ | 132   | 0.0227  | 0.0111                  | 0.0176  |
| Cytosolic DNA-sensing pathway                  | Irf7↓ Mavs↓ | 49    | 0.0408  | 0.0131                  | 0.0176  |
| Biotin metabolism                              | Hlcs↓       | 3     | 0.3333  | 0.0135                  | 0.0176  |
| RNA degradation                                | Cnot6↓ Dcp1a↓ | 61    | 0.0328  | 0.0196                  | 0.0223  |
| Long-term depression                           | Itpr1↑ Itpr2↑ | 69    | 0.029   | 0.0245                  | 0.0245  |
| Ubiquinone and other terpenoid-quinone biosynthesis | Coq6↑   | 7     | 0.1429  | 0.0269                  | 0.0245  |
| Phosphatidylinositol signaling system          | Itpr2↑ Itpr1↑ | 77    | 0.026   | 0.0299                  | 0.0247  |
| Gap junction                                   | Itpr2↑ Itpr1↑ | 87    | 0.023   | 0.0371                  | 0.0281  |
| GnRH signaling pathway                         | Itpr1↑ Itpr2↑ | 99    | 0.0202  | 0.0467                  | 0.0326  |

Nerve growth factor induced protein A, which binds to and regulates activation of the GR promoter [24]. No difference of the dopamine level between ESG and CG were obtained, indicating earthquake may not impact on the growth hormone of offspring. Interestingly, however, JKSQW in HG significantly elevated the dopamine level of ESG, which might be explained by the function of kidney that governs development. Shen and Cai [26] reported that growth hormone genes were downregulated in a kidney-qi deficiency rat model and Chinese formula supplementing kidney qi could correct the downregulation. Mak et al. [27] found that chronic kidney disease in children was
Table 6: Differentially expressed genes in ESG versus HG, among which 60 genes were upregulated and 28 genes downregulated.

| Gene ID   | P values | Fold change | Symbol | Remark       |
|-----------|----------|-------------|--------|--------------|
| 287881    | 0.006042 | 0.220799    | Dyfip1 | Downregulated|
| 25405     | 0.004824 | 0.344631    | Ccn1   | Downregulated|
| 24237     | 0.003207 | 0.40894     | C6     | Downregulated|
| 313219    | 0.003811 | 0.410283    | Zfp189 | Downregulated|
| 287343    | 0.008194 | 0.499299    | Olr1454| Downregulated|
| 293156    | 0.008272 | 0.508908    | Lrtomt | Downregulated|
| 405143    | 0.009972 | 0.5345      | Olr803 | Downregulated|
| 116724    | 0.000512 | 0.546672    | Epb4.1L3| Downregulated|
| 313917    | 0.00383  | 0.578297    | Abhd1  | Downregulated|
| 83681     | 0.004251 | 0.581219    | Cish   | Downregulated|
| 301346    | 0.007628 | 0.609505    | Sema4c | Downregulated|
| 315346    | 0.003519 | 0.619843    | Ilga5  | Downregulated|
| 56825     | 0.009009 | 0.625224    | Cym    | Downregulated|
| 690810    | 0.007066 | 0.637375    | Adat1  | Downregulated|
| 313982    | 0.009162 | 0.653927    | RGD1561890 | Downregulated|
| 363285    | 0.004745 | 0.660307    | Scly   | Downregulated|
| 316090    | 0.003533 | 0.683347    | Fam198a| Downregulated|
| 24513     | 0.003494 | 0.687818    | Ivd    | Downregulated|
| 303384    | 0.007792 | 0.703077    | Mmp28  | Downregulated|
| 246074    | 0.009445 | 0.718762    | Scd1   | Downregulated|
| 500011    | 0.008188 | 0.726294    | RGD1563091 | Downregulated|
| 362943    | 0.004839 | 0.735253    | Adck5  | Downregulated|
| 500420    | 0.008119 | 0.744282    | LOC500420 | Downregulated|
| 399489    | 0.006413 | 0.763541    | E2f1   | Downregulated|
| 311716    | 0.004912 | 0.77549     | Col20a1| Downregulated|
| 113894    | 0.007846 | 0.78406     | Sqtstm1| Downregulated|
| 266609    | 0.005228 | 0.798742    | Bles03 | Downregulated|
| 246766    | 0.00514  | 0.821038    | Gta1   | Downregulated|
| 288518    | 0.008613 | 1.136098    | RGD1311660 | Upregulated|
| 499430    | 0.008063 | 1.148146    | Lrrc20 | Upregulated  |
| 317399    | 0.000156 | 1.156541    | Ddx21  | Upregulated  |
| 306182    | 0.00808  | 1.160148    | Lpo5   | Upregulated  |
| 301038    | 0.00729  | 1.178184    | Ubp1   | Upregulated  |
| 310806    | 0.006399 | 1.178549    | Cdc14a | Upregulated  |
| 287954    | 0.003091 | 1.181263    | Dgcr8  | Upregulated  |
| 260321    | 0.008611 | 1.181875    | Fkbp4  | Upregulated  |
| 305828    | 0.006609 | 1.182203    | Socs4  | Upregulated  |
| 64161    | 0.005932 | 1.183779    | Pia4k  | Upregulated  |
| 290679    | 0.009165 | 1.186593    | Ints10 | Upregulated  |
| 298429    | 0.006198 | 1.188777    | Rad54l | Upregulated  |
| 474154    | 0.005077 | 1.190852    | Rbm4b  | Upregulated  |
| 288717    | 0.006268 | 1.196619    | Srrd   | Upregulated  |
| 296312    | 0.004568 | 1.197256    | RGD1311066 | Upregulated|
| 312640    | 0.005739 | 1.198178    | Tmeme111 | Upregulated|
| 83624     | 0.009311 | 1.200882    | Ppip   | Upregulated  |
| 288778    | 0.001749 | 1.22319     | Pa2g4  | Upregulated  |
| 362851    | 0.004166 | 1.224723    | Cds20  | Upregulated  |
| 308404    | 0.006579 | 1.227818    | Irf2bp1| Upregulated  |
| 363760    | 0.005704 | 1.237527    | Arl6   | Upregulated  |
| 296076    | 0.007529 | 1.238081    | Srp14  | Upregulated  |
| 291787    | 6.57E-05 | 1.242186    | Rbbp8  | Upregulated  |
| Gene ID | P values | Fold change | Symbol | Remark      |
|---------|----------|-------------|--------|-------------|
| 500727  | 0.00344  | 1.246021    | Cda4   | Upregulated |
| 306587  | 0.008906 | 1.255527    | Tcta   | Upregulated |
| 29541   | 0.000917 | 1.259108    | Nh1l   | Upregulated |
| 360855  | 0.004605 | 1.26267     | Smg7   | Upregulated |
| 362317  | 0.008649 | 1.284527    | Kr1t   | Upregulated |
| 313757  | 0.004801 | 1.294664    | RGD1565591 | Upregulated |
| 499370  | 0.009663 | 1.326682    | Itpr1  | Upregulated |
| 288259  | 0.009472 | 1.335197    | Gart   | Upregulated |
| 29704   | 0.002213 | 1.349013    | Pacsin1| Upregulated |
| 84472   | 0.006393 | 1.366251    | Iff3   | Upregulated |
| 363210  | 0.006023 | 1.388566    | Phf3   | Upregulated |
| 680451  | 0.005563 | 1.419061    | Nrbp2  | Upregulated |
| 311112  | 0.001699 | 1.426768    | Fastkd1| Upregulated |
| 54323   | 0.001608 | 1.4509      | Arc    | Upregulated |
| 309136  | 0.006405 | 1.452428    | Oraov1 | Upregulated |
| 363169  | 0.005748 | 1.472567    | Toag1  | Upregulated |
| 29642   | 0.004937 | 1.475875    | Slc3a2 | Upregulated |
| 305461  | 0.004104 | 1.475879    | Fam53a | Upregulated |
| 304813  | 0.00934  | 1.481691    | Ppp1r12b | Upregulated |
| 680006  | 0.007932 | 1.484512    | Mad1I1 | Upregulated |
| 304474  | 0.001635 | 1.497221    | Pitpm2 | Upregulated |
| 115768  | 0.009088 | 1.509009    | Zfp37  | Upregulated |
| 301513  | 0.001268 | 1.512431    | Rqc1d  | Upregulated |
| 363273  | 0.009331 | 1.521116    | Cops7b | Upregulated |
| 293511  | 0.008749 | 1.533752    | Znf688 | Upregulated |
| 245966  | 0.004372 | 1.544613    | Tmem150a | Upregulated |
| 291409  | 0.003844 | 1.552189    | Zfp236 | Upregulated |
| 84607   | 0.007931 | 1.552588    | Soc52  | Upregulated |
| 306344  | 0.007778 | 1.569477    | Arrdc2 | Upregulated |
| 309828  | 0.006302 | 1.584851    | Tspyl4 | Upregulated |
| 501095  | 0.009284 | 1.589281    | Rftn1  | Upregulated |
| 81531   | 0.008017 | 1.606129    | Pfn2   | Upregulated |
| 293152  | 0.007896 | 1.613085    | Art2b  | Upregulated |
| 497040  | 0.006162 | 1.71037     | Prss36 | Upregulated |
| 171454  | 0.009816 | 1.850404    | Nacc1  | Upregulated |
| 363827  | 0.00216  | 1.948295    | LOC363827 | Upregulated |
| 364361  | 0.001905 | 4.479744    | RGD1563700 | Upregulated |

Table 7: Significant GO Annotation of the 5 differentially expressed genes and the genes included (P < 0.05).

| GO ID   | Name                        | Symbol       | Hits | Total | Percent | Enrichment test P value |
|---------|-----------------------------|--------------|------|-------|---------|-------------------------|
| GO: 0031503 | Protein complex localization | Fkbp4        | 1    | 5     | 20.00%  | 0.0309                  |
| GO: 0022607 | Cellular component assembly | Sqstm1, Nacc1, Ivd, Fkbp4, Tspyl4, Itga5, Pfn2 | 8    | 786   | 1.02%   | 0.0548                  |
| GO: 0044085 | Cellular component biogenesis | Sqstm1, Nacc1, Ivd, Fkbp4, Tspyl4, Itga5, Pfn2 | 8    | 883   | 0.91%   | 0.0926                  |
| GO: 0010926 | Anatomical structure formation | Sqstm1, Nacc1, Ivd, Fkbp4, Ubp1, Tspyl4, Itga5, Pfn2 | 9    | 1049  | 0.86%   | 0.0993                  |
| GO: 0043233 | Organelle lumen               | Pa2g4, Ints10, Nh1l, Ddx21, E2f1, Rhm4b, Ppig | 11   | 1360  | 0.81%   | 0.0994                  |
Table 8: KEGG Pathway annotation of the 120 differentially expressed genes ($P < 0.05$, $q < 0.05$) (↓ refers downregulation, ↑ refers upregulation).

| Name                                | Symbol | Total | Percent | Enrichment test P value | q value |
|-------------------------------------|--------|-------|---------|-------------------------|---------|
| Cell cycle                          |        |       |         |                         |         |
|                                     | Cdc14a↑| 132   | 0.0227  | 0.0044                  | 0.0067  |
|                                     | E2f1↓  |       |         |                         |         |
|                                     | Mad1↓  |       |         |                         |         |
| Jak-STAT signaling pathway          |        |       |         |                         |         |
|                                     | Socs4↑ | 149   | 0.0201  | 0.0062                  | 0.0067  |
|                                     | Cish↓  |       |         |                         |         |
|                                     | Socs2↓ |       |         |                         |         |
| Type II diabetes mellitus           |        |       |         |                         |         |
|                                     | Socs4↑ | 53    | 0.0377  | 0.008                   | 0.0067  |
|                                     | Socs2↑ |       |         |                         |         |
| One carbon pool by folate           |        |       |         |                         |         |
|                                     | Gart↑  | 17    | 0.0588  | 0.0429                  | 0.0158  |
| Insulin signaling pathway           |        |       |         |                         |         |
|                                     | Socs4↑ | 140   | 0.0143  | 0.0471                  | 0.0158  |
|                                     | Socs2↑ |       |         |                         |         |

Figure 1: Mean plot of body weight. According to the ANOVA for Repeated Measurement, the body weight of ESG offspring were statistically all inferior to the CG offspring despite in Day 10 ($P < 0.05$). The body weight HG offspring were statistically superior to the ESG offspring despite in Day 5 ($P < 0.05$); The body weight HG in Day 15, Day 20 and Day 25 were statistically superior to the CG ($P < 0.05$).

Figure 2: Box plot of OFT in the comparison between CG, ESG, and HG. ESG showed less scores than CG ($P < 0.05$) and HG ($P < 0.05$).

Mechanisms underlying the interaction between PS and adult mental disorders suggest the involvement of multiple neurotransmitter systems [29, 30]. Findings of the hormones alterations suggest manual earthquake is a liable model modulating the fear from natural earthquake involving development retardation and neurotransmitter systems disorder. Meanwhile, from the perspective of Chinese medicine, kidney function is disturbed by the earthquake and recovered by JKSQW.

We found 81 genes upregulated and 39 genes downregulated in ESG versus CG, from which 14 significant GO and 12 KEGG pathways were annotated, indicating diversified and complicated physiological and psychological impacts on offspring left by the prenatal earthquake as a prenatal stress, for example, long-term depression and long-term potentiation. Mychasiuk et al. [31] reported that significant gene expression level changes in 558 different
| Gene ID | P   | Fold change | Symbol | Description                                                                                           |
|--------|-----|-------------|--------|-------------------------------------------------------------------------------------------------------|
| 287443 | 0.0414 | 2.0120     | Acap1  | ArfGAP with coiled-coil, ankyrin repeat, and PH domains 1                                              |
| 316628 | 0.0044 | 0.2748     | Asb1   | Ankyrin repeat and SOCS box-containing 1 (Asb1), mRNA                                                  |
| 307970 | 0.0397 | 0.3289     | Atxn1l | PREDICTED: similar to Ataxin-1 (Spinocerebellar ataxia type 1 protein homolog)                        |
| 304127 | 0.0266 | 0.4310     | Bach1  | BTR and CNC homology 1, basic leucine zipper transcription factor 1                                    |
| 94342  | 0.0368 | 0.4621     | Bat3   | HLA-B-associated transcript 3, transcript variant 2,                                                  |
| 308588 | 0.0241 | 0.4679     | Car11  | Carbonic anhydrase-related XI protein                                                                |
| 81780  | 0.0349 | 2.6298     | Ccl5   | Chemokine (C-C motif) ligand 5                                                                        |
| 25405  | 0.0303 | 0.3845     | Car11  | Carboxytetramerisation domain containing 12                                                           |
| 362217 | 0.0393 | 0.4273     | Cenpb  | PREDICTED: centromere protein B                                                                        |
| 314004 | 0.0237 | 0.3330     | Cmpk2  | Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial, nuclear gene encoding mitochondrial protein    |
| 24273  | 0.0401 | 0.4750     | Cryaa  | Crystallin, alpha A                                                                                    |
| 361729 | 0.0183 | 0.4488     | Cybasc3| Cytochrome b, ascorbate dependent 3                                                                   |
| 308942 | 0.0369 | 0.3530     | Dennd5a| DENN/MADD domain containing 5A                                                                       |
| 360583 | 0.0296 | 0.4192     | Dhrs11 | Dehydrogenase/reductase (SDR family) member 11                                                         |
| 362293 | 0.0203 | 0.4955     | Dnajb6 | DnaJ (Hsp40) homolog, subfamily B, member 6                                                            |
| 81655  | 0.0336 | 0.4654     | Dyncl1i| Dynein, cytoplasmic 1 light intermediate chain 2                                                        |
| 59117  | 0.0343 | 0.3116     | Eif2c2 | Eukaryotic translation initiation factor 2C, 2                                                         |
| 497983 | 0.0476 | 0.4848     | Fam117a| Family with sequence similarity 117, member A                                                           |
| 365083 | 0.0074 | 2.5213     | Fbxl22 | F-box and leucine-rich repeat protein 22                                                                |
| 29292  | 0.0293 | 0.4455     | Ftl    | Ferritin, light polypeptide                                                                           |
| 54281  | 0.0281 | 0.3897     | Furin  | Furin (paired basic amino acid cleaving enzyme)                                                        |
| 25172  | 0.0185 | 0.3991     | Gata1  | GATA binding protein 1                                                                                 |
| 293267 | 0.0274 | 0.3516     | Hbc1   | Hemoglobin, epsilon 1                                                                                 |
| 94164  | 0.0175 | 0.4161     | Hbg1   | Hemoglobin, gamma A                                                                                  |
| 498008 | 0.0335 | 2.2484     | Hexim1 | Hexamethylene bis-acetamide inducible 1                                                                |
| 365895 | 0.0417 | 0.3894     | Hipk1  | Homeodomain interacting protein kinase 1                                                               |
| 288240 | 0.0022 | 0.3449     | Hlcs   | PREDICTED: holocarboxylase synthetase (biotin-([proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase) |
| 293624 | 0.0080 | 0.3642     | Irf7   | Interferon regulatory factor 7                                                                        |
| 290985 | 0.0079 | 0.4919     | Isca1  | Iron-sulfur cluster assembly 1 homolog (S. cerevisiae)                                                |
| 298693 | 0.0462 | 0.3402     | Isg15  | ISG15 ubiquitin-like modifier                                                                         |
| 25118  | 0.0351 | 2.9262     | Ilga1  | Integrin, alpha 1                                                                                    |
| 300317 | 0.0493 | 0.4873     | Kctd17 | Potassium channel tetramerisation domain containing 17                                                |
| 25110  | 0.0410 | 2.6060     | Klrd1  | Killer cell lectin-like receptor, subfamily D, member 1                                               |
| Gene ID     | P      | Fold change | Symbol | Description                                                                 |
|------------|--------|-------------|--------|-----------------------------------------------------------------------------|
| 245955     | 0.0120 | 0.4700      | Lgals3bp | Lectin, galactoside-binding, soluble, 3 binding protein                     |
| 25476      | 0.0214 | 0.4406      | Lgals9  | Lectin, galactoside-binding, soluble, 9                                     |
| 100365370  | 0.0172 | 0.4588      | LOC100365370 | PREDICTED: nuclear LIM interactor-interacting factor 2-like                  |
| 498145     | 0.0213 | 0.3006      | LOC498145 | Similar to RIKEN cDNA 2810453106                                            |
| 679596     | 0.0155 | 0.4814      | LOC679596 | PREDICTED: similar to GABA(A) receptor-associated protein like 2             |
| 684112     | 0.0121 | 0.4067      | LOC684112 | PREDICTED: similar to KIAA0999 protein                                       |
| 293156     | 0.0090 | 0.4140      | Lrptomt | Leucine rich transmembrane and 0-methyltransferase domain containing        |
| 294241     | 0.0443 | 0.2072      | Ly6g6c  | Lymphocyte antigen 6 complex, locus G6C                                      |
| 117558     | 0.0498 | 0.3267      | Mylk2   | Myosin light chain kinase 2                                                 |
| 85482      | 0.0360 | 0.4205      | Nbn     | Nibrin                                                                       |
| 366998     | 0.0309 | 0.4486      | Nfe2    | Nuclear factor, erythroid derived 2                                          |
| 59115      | 0.0355 | 0.3302      | Ninj2   | Ninjurin 2                                                                   |
| 245980     | 0.0238 | 0.4878      | Nr2f6   | Nuclear receptor subfamily 2, group F, member 6                               |
| 287328     | 0.0292 | 0.4931      | Olr1439 | Olfactory receptor 1439                                                      |
| 287520     | 0.0498 | 0.4482      | Olr1516 | Olfactory receptor 1516                                                      |
| 366104     | 0.0175 | 0.4251      | Olr541  | Olfactory receptor 541                                                       |
| 246294     | 0.0120 | 0.3491      | Optn    | Optineur                                                                     |
| 362973     | 0.0467 | 0.4896      | Parvb   | Parvin, beta                                                                |
| 24649      | 0.0147 | 0.3899      | Pim1    | Pim-1 oncogene                                                               |
| 64534      | 0.0423 | 2.1733      | Pim3    | Pim-3 oncogene                                                               |
| 301173     | 0.0478 | 0.3759      | Plc1    | Phospholipase C-like 2                                                       |
| 310674     | 0.0473 | 0.4134      | Plekho1 | Pleckstrin homology domain containing, family O member 1                     |
| 362873     | 0.0062 | 0.4404      | Plxncl  | Plexin C1                                                                    |
| 362248     | 0.0215 | 0.4759      | Procr   | Protein C receptor, endothelial                                             |
| 309381     | 0.0286 | 2.2397      | Pyroxd2 | Pyridine nucleotide-disulphide oxidoreductase domain 2                       |
| 171452     | 0.0460 | 0.3652      | Rab3il1 | RAB3A interacting protein                                                    |
| 56820      | 0.0334 | 0.1273      | Ramp3   | Receptor (G protein-coupled) activity modifying protein 3                   |
| 498659     | 0.0473 | 7.0377      | RatNP-3b| Defensin RatNP-3 precursor                                                  |
| 296408     | 0.0259 | 0.4348      | RGD1311378 | Similar to RIKEN cDNA 201001120                                            |
| 501644     | 0.0175 | 0.4259      | RGD1561055 | PREDICTED: similar to Ferritin light chain 2 (Ferritin L subunit 2) (Ferritin subunit LG) |
| 65190      | 0.0454 | 0.3257      | Rsad2   | Radical S-adenosyl methionine domain containing 2                           |
| 24974      | 0.0165 | 0.4619      | RT1-A2  | RT1 class Ia, locus A2 (RT1-A2)                                             |
Table 9: Continued.

| Gene ID   | P       | Fold change | Symbol | Description                                      |
|-----------|---------|-------------|--------|-------------------------------------------------|
| 414779    | 0.0105  | 0.4766      | RT1-CE2| RT1 class I, locus CE2 (RT1-CE2)                 |
| 266758    | 0.0163  | 2.6183      | Sec11c | SEC11 homolog C (S. cerevisiae)                  |
| 313057    | 0.0446  | 0.4886      | Serinc2 | Serine incorporator 2                           |
| 498546    | 0.0120  | 0.1863      | Serp2  | Stress-associated endoplasmic reticulum protein family member 2 |
| 360636    | 0.0484  | 0.4722      | Slc25a39| Solute carrier family 25, member 39 (Slc25a39)   |
| 192208    | 0.0472  | 2.6183      | Slc38a5| Solute carrier family 38, member 5 (Slc38a5)     |
| 300191    | 0.0457  | 0.4485      | Slc48a1| Solute carrier family 48 (heme transporter), member 1 |
| 64630     | 0.0330  | 0.4620      | Snap23 | Synaptosomal-associated protein 23               |
| 314251    | 0.0353  | 0.4407      | Sptb   | Spectrin, beta, erythrocytic                     |
| 113894    | 0.0230  | 0.4367      | Sqtstn1| STE20-related kinase adaptor beta                |
| 501146    | 0.0449  | 0.3749      | Stradb | Tropomyosin 1, alpha                            |
| 24851     | 0.0449  | 0.3944      | Tpm1   | Predicted: tripartite motif-containing 58        |
| 303167    | 0.0390  | 0.3720      | Trim58  | UBA domain containing 1                         |
| 362089    | 0.0450  | 0.3958      | Ubac1  | Ubiquitin-conjugating enzyme E2L 6              |
| 295704    | 0.0234  | 0.3510      | Ube2l6 | Ubiquitin 4                                     |
| 310633    | 0.0316  | 0.3751      | Ubqln4 | Von Hippel-Lindau tumor suppressor               |
| 289229    | 0.0240  | 0.3468      | Vangl2 | Vang-like 2                                    |
| 24874     | 0.0262  | 2.6865      | Vhl    | Zinc finger protein 36, C3H type-like 2         |
| 298765    | 0.0209  | 2.4995      | Zfp36l2| Zinc finger protein 36, C3H type-like 2          |

genes, associated with overrepresentation of 36 biological processes and 34 canonical pathways indicating prenatal stress did not have to be experienced by the mother herself to influence offspring brain development. Among the GO annotations Itpr1 and Itpr2 appeared in almost all the affected pathways. In nonexcitable cells, the inositol 1,4,5-trisphosphate receptor (IP3R) is an intracellular Ca\(^{2+}\) channel, which plays a major role in Ca\(^{2+}\) signalling. Three isoforms of IP3R have been identified (IP3R-1, IP3R-2, and IP3R-3) and most cell types express different proportions of each isoform [31]. IP3Rs play major roles in agonist-induced intracellular Ca\(^{2+}\) release and also in store operated Ca\(^{2+}\) entry, a process whereby the depletion of intracellular Ca\(^{2+}\) stores causes the opening of intracellular Ca\(^{2+}\) channels in the plasma membrane [32]. The intracellular Ca\(^{2+}\) elevations induced by BDNF required a signaling pathway consistent with the activation of the Trk-IP3R cascade, which was also necessary for the activation of the membrane conductance IBDNF [33, 34]. Amaral and Pozzo-Miller [35] reported that Trk receptors, IP3Rs, full intracellular Ca\(^{2+}\) stores and Ca\(^{2+}\) influx are all required for BDNF-induced Ca\(^{2+}\) elevations and membrane currents. Opposing influences of mBDNF and proBDNF on long-term potentiation and long-term depression might contribute to the dichotomy of BDNF actions on behaviors mediated by the brain stress and reward systems [36, 37]. Twelve KEGG pathways were annotated, including oocyte meiosis, vascular smooth muscle contraction, RIG-I-like receptor signaling pathway, long-term potentiation, ubiquitin mediated proteolysis, and long-term depression, Titterness and Christie [38] prenatal ethanol and prenatal stress produce sex-specific alterations in synaptic plasticity in the adolescent hippocampus. Calpains, which belong to a family of at least 14 members of calcium-dependent cysteine proteases and are involved in apoptosis are implicated in a wide range of physiological functions including cell motility, differentiation, signal transduction, including cell survival pathways, cell cycle progression, regulation of gene expression, and long-term potentiation [39, 40]. Yang et al. [41] reported that prenatal stress (10 unpredictable, 1 s, 0.8 mA foot shocks per day during gestational days 13–19) impaired long-term potentiation (LTP) but facilitated long-term depression (LTD) in hippocampal CA1 region in slices of the prenatal stressed offspring (5 weeks old). Proteolysis by the ubiquitin-proteasome pathway has attained prominence as a new molecular mechanism which regulates varied important functions of the nervous system, including development of synaptic connections and synaptic plasticity.
Figure 3: ELISA outcomes of corticosterone, dopamine, 5-HT, and growth hormone. (a) ANOVA test for the corticosterone showed $P = 0.027$ in CG versus ESG, $P = 0.491$ in CG versus HG, and $P = 0.111$ in ESG versus HG. (b) ANOVA test for the dopamine showed $P = 0.065$ in CG versus ESG, $P = 0.805$ in CG versus HG, and $P = 0.039$ in ESG versus HG. (c) ANOVA test for 5-HT showed $P = 0.000$ in CG versus ESG, $P = 0.004$ in CG versus HG, and $P = 0.013$ in ESG versus HG. (d) ANOVA test for the growth hormone showed $P = 0.135$ in CG versus ESG, $P = 0.034$ in CG versus HG, and $P = 0.001$ in ESG versus HG.

through control of axonal growth, axonal and dendritic pruning, and regulation of synaptic size and number [42].

We found 60 genes upregulated and 28 genes downregulated in HG versus ESG, from which five significant GO and five KEGG pathways were annotated, indicating diversified cellular biological process and signaling pathways. Interestingly, Socs 2 and Socs 4 of Socs (suppressors of cytokine signaling) family appeared in three of the KEGG pathways. SOCS family consists of eight structurally similar proteins (SOCS-1 to SOCS-7 and CIS), which have been implicated as potential inhibitors of tissue growth during both prenatal and postnatal life [43] and their actions clearly now extend to other intracellular pathways, they remain key negative regulators of cytokine and growth factor signaling [44]. Cytokine-mediated JAK/STAT signaling, that is, Janus kinase/signal transducers and activators of transcription, controls a number of vital biologic responses, including immune function, cellular growth, differentiation, and hematopoiesis [45]. The SOCS Family—The SOCS proteins were identified as STAT target genes that directly antagonize STAT activation, resulting in a classic “feedback loop” [46]. PS in rats induced lifespan reduction of neurogenesis in the dentate gyrus and produced impairment in hippocampal-related spatial tasks through blocking the increase of learning-induced neurogenesis [47]. Previous research reported that male rats exposed to stress in utero are characterized by a decrease in hippocampal cell proliferation, and consequently neurogenesis, from adolescence to senescence [48]. PS has been reported to alter cytokine levels. Coussons-Read et al. [49] reported that stress-related neural immune
interactions may contribute to pregnancy complications and poor outcome. Collier et al. [50] found that PS changed typical proinflammatory cytokines including tumor necrosis factor (TNF)-α, and interleukin (IL)-6. As mentioned above, JKSQW recovered the dysfunction of kidney due to fear from earthquake, which could be supported by gene profile experiment outcome. In other words, cytokine conduction pathways, for example, JAK/STAT are involved in the prenatal kidney deficiency, and key molecules like Socs-2 and Socs-4 are the regulating targets of Chinese medicine treatment. The underlying mechanism that JKSQW improves development and behavior might attribute to the upregulation of Socs-2 and Socs-4 which suppress the pathway of JAK/STAT, resulting in reduction certain cytokines’ secretion. diabetes is considered as Xiao-ke in Chinese medicine, whose major pattern is kidney deficiency. JKSQW plays an important role in the composition of prescriptions treating Diabetes in Chinese medicine [51]. Promisingly, our findings revealed insulin related pathways were involved in the outcome of herbal intervention in HG, supporting the hypnosis that JKSQW recovery the dysfunction of kidney.

Four genes (Irf7, Ninj2, Plxnc1, and Isca1) were validated with RT-PCR, showing a favorable match (75%) between the gene expression profile chip and RT-PCR result. It is reported that all elements of IFN responses, whether the systemic production of IFN in innate immunity or the local action of IFN from plasmacytoid dendritic cells in adaptive immunity, are under the control of Irf7 [52]. Hannah et al. [53] reported that induction of pattern recognition receptors (PRRs; Tlr7 and Rig-1), expression of antiviral genes (Myd88, Visa, Jun, Irf7, Ifnbeta, Ifnar1, Jak2, Stat3, and Mx2), and production of Mx protein was elevated in the lungs of intact females compared with intact males. Ninjurin2 (Ninj2) is a transmembrane protein that mediates cell-to-cell and cell-to-extracellular matrix interactions during development, differentiation, and regeneration of the nervous system [54]. Recently, Ninj2 was reported to be a vascular susceptibility gene and associated with Alzheimer’s disease risk [55].

In conclusion, together with our own recent data, the findings of this body of work demonstrate the earthquake as a prenatal stressor during the pregnancy could negatively retard the body and nervous system development, and Chinese herbal remedy could correct the retardation, which could attribute to neurohormones alteration and altered gene expression profile. The gene pathways involved have been tied to signaling pathway, long-term potentiation, ubiquitin mediated proteolysis, and long-term depression relating to disruptions from prenatal stress; Jak-STAT signaling pathway could play a key role in improving the function of JKSQW. This study demonstrates that negatively prenatal experiences have the ability to significantly retard
**Figure 5:** Heat map of the differently expressed genes. R2.1, NS, R2.2, NS, and R2.3, NS refer to ESG and R3.1, NS, R3.2, NS, R3.3, NS to HG.

**Figure 6:** RT-PCR validation of the selected four genes from gene expression profile chips, that is, Irf7, Ninj2, and Plxnc1, and Isca1. \( \Delta \Delta Ct < 0 \) indicates the target genes were hyperexpressed in ESG/HG comparing with CG while \( \Delta \Delta Ct > 0 \) indicates the target genes were hypoexpressed in ESG/HG comparing with CG. FC > 2 indicates the target genes were hyperexpressed in ESG/HG comparing with CG while FC < 0.5 indicates the target genes were hypoexpressed in ESG/HG comparing with CG.
offspring developmental and immunity trajectories, which can be corrected by Chinese herbal remedy.

**Conflict of Interests**

The authors declare no conflict of interests.

**Acknowledgments**

This study was under the support of National Science Funds of China with the Grant no. 81072719. The authors thank Sheri L. Johnson, Ph.D. and Zeyiad Elias, Ph.D. USA for English editing.

**References**

[1] J. Simpson and J. P. Kelly, “The impact of environmental enrichment in laboratory rats—Behavioural and neurochemical aspects,” *Behavioural Brain Research*, vol. 222, no. 1, pp. 246–264, 2011.

[2] K. O’Donnell, T. G. O’Connor, and V. Glover, “Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta,” *Developmental Neuroscience*, vol. 31, no. 4, pp. 285–292, 2009.

[3] S. Morley-Fletcher, M. Rea, S. Maccari, and G. Laviola, “Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats,” *European Journal of Neuroscience*, vol. 18, no. 12, pp. 3367–3374, 2003.

[4] E. J. H. Mulder, P. G. Robles De Medina, A. C. Huizink, B. R. H. Van Den Bergh, J. K. Buitelaar, and G. H. A. Visser, “Prenatal maternal stress: effects on pregnancy and the (unborn) child,” *Early Human Development*, vol. 70, no. 1-2, pp. 3–14, 2002.

[5] S. Maccari, M. Darnaudery, S. Morley-Fletcher, A. R. Zuena, C. Cinque, and O. Van Reeth, “Prenatal stress and long-term consequences: implications of glucocorticoid hormones,” *Neuroscience and Biobehavioral Reviews*, vol. 27, no. 1-2, pp. 119–127, 2003.

[6] S. Maccari and S. Morley-Fletcher, “Effects of prenatal restraint stress on the hypothalamic-pituitary-adrenal axis and related behavioural and neurobiological alterations,” *Psychoneuroendocrinology*, vol. 32, pp. S10–S15, 2007.

[7] J. Lesage, F. Del-Faverio, M. Leonhardt et al., “Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat,” *Journal of Endocrinology*, vol. 181, no. 2, pp. 291–296, 2004.

[8] S. King, R. G. Barr, A. Brunet, J. F. Saucier, M. Meaney et al., “The ice storm: an opportunity to study the effects of prenatal stress on the baby and the mother,” *Santé Mentale au Québec*, vol. 25, no. 1, pp. 163–185, 2000.

[9] C. E. Tan, H. J. Li, X. G. Zhang et al., “The impact of the Wenchuan earthquake on birth outcomes,” *PLoS ONE*, vol. 4, no. 12, Article ID e8200, 2009.

[10] C. Oyarzo, P. Bertoglia, R. Avendaño et al., “Adverse perinatal outcomes after the February 27th 2010 Chilean earthquake,” *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 25, no. 10, pp. 1868–1873, 2012.

[11] R. Yaka, S. Salomon, H. Matzner, and M. Weinstock, “Effect of varied gestational stress on acquisition of spatial memory, hippocampal LTP and synaptic proteins in juvenile male rats,” *Behavioural Brain Research*, vol. 179, no. 1, pp. 126–132, 2007.

[12] P. Leung, M. Cheung, and V. Tsui, “Help-seeking behaviors among Chinese Americans with depressive symptoms,” *Social Work*, vol. 57, no. 1, pp. 61–71, 2012.

[13] A. Kolasaki, H. Xu, and M. Millikan, “Determination and comparison of mineral elements in traditional Chinese herbal formulae at different decoction times used to improve kidney function—chemometric approach,” *The African Journal of Traditional, Complementary and Alternative Medicines*, vol. 8, supplement 5, pp. 191–197, 2011.

[14] X. G. Zhang, Y. Q. Yang, S. T. Li et al., “Further study on the thought of terror impairing kidney by simulating the earthquake experiment,” *J Sichuan Tradit Chin Med*, vol. 26, no. 12, pp. 27–28, 2008.

[15] O. Kofman, “The role of prenatal stress in the etiology of developmental behavioural disorders,” *Neuroscience and Biobehavioral Reviews*, vol. 26, no. 4, pp. 457–470, 2002.

[16] E. C. Cottrell and J. R. Seckl, “Prenatal stress, glucocorticoids and the programming of adult disease,” *Frontiers in Behavioral Neuroscience*, vol. 3, article 19, 2009.

[17] M. Weinstock, “The long-term behavioural consequences of prenatal stress,” *Neuroscience and Biobehavioral Reviews*, vol. 32, no. 6, pp. 1073–1086, 2008.

[18] Y. Kotozaki and R. Kawashima, “Effects of the Higashi-Nihon earthquake: posttraumatic stress, psychological changes, and corticosterone levels of survivors,” *PLoS ONE*, vol. 7, no. 4, Article ID e34612, 2012.

[19] R. S. Goland, S. Jozak, W. B. Warren, I. M. Conwell, R. I. Stark, and P. J. Tropper, “Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses,” *Journal of Clinical Endocrinology and Metabolism*, vol. 77, no. 5, pp. 1174–1179, 1993.

[20] Y. Huang, H. Xu, H. Li, H. Yang, Y. Chen, and X. Shi, “Pre-gestational stress reduces the ratio of 5-HIAA to 5-HT and the expression of 5-HT1A receptor and serotonin transporter in the brain of foetal rat,” *BMC Neuroscience*, vol. 13, article 22, 2012.

[21] S. Spinelli, S. Chefer, R. E. Carson et al., “Effects of early-life stress on serotonin1a receptors in juvenile rhesus monkeys measured by positron emission tomography,” *Biological Psychiatry*, vol. 67, no. 12, pp. 1146–1153, 2010.

[22] M. K. Brown and Y. Luo, “Bilobalide modulates serotonin-controlled behaviors in the nematode Caenorhabditis elegans,” *BMC Neuroscience*, vol. 10, article 62, 2009.

[23] S. Davidson, D. Prokonov, M. Taler et al., “Effect of exposure to selective serotonin reuptake inhibitors. In Utero on fetal growth: potential role for the IFG-I and HPA axes,” *Pediatric Research*, vol. 65, no. 2, pp. 236–241, 2009.

[24] I. C. G. Weaver, A. C. D’Alessio, S. E. Brown et al., “The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming; altering epigenetic marks by immediate-early genes,” *Journal of Neuroscience*, vol. 27, no. 7, pp. 1756–1768, 2007.

[25] E. Carboni, V. G. Barros, M. Ibba, A. Silvagni, C. Mura, and M. C. Antonelli, “Prenatal restraint stress: an in vivo microdialysis study on catecholamine release in the rat prefrontal cortex,” *Neuroscience*, vol. 168, no. 1, pp. 156–166, 2010.

[26] Z. Y. Shen and D. P. Cai, “Study on the regulative rule of reinforcing shen principle on sexual precocity and senescence of developmental behavioural disorders,” *Neuroscience and Biobehavioral Reviews*, vol. 31, no. 3, pp. S10–S15, 2007.

[27] R. H. Mak, W. W. Cheung, and C. T. Roberts Jr., “The growth hormone-insulin-like growth factor-I axis in chronic kidney disease,” *Growth Hormone and IGF Research*, vol. 18, no. 1, pp. 17–25, 2008.
[28] Y. H. Yang and Z. Li, “Gene chip study of cerebral genome of effect of jinkui shenqi pill in mice model with kidney-yang asthma induced by excessive physical and sexual activity,” *Liaoning Journal of Traditional Chinese Medicine*, vol. 35, no. 5, pp. 733–739, 2008.

[29] W. Gao, J. Paterson, M. Abbott, S. Carter, and L. Iusitini, “Maternal mental health and child behaviour problems at 2 years: findings from the Pacific Islands Families Study,” *Australian and New Zealand Journal of Psychiatry*, vol. 41, no. 11, pp. 885–895, 2007.

[30] C. J. Ewell Foster, J. Garber, and J. A. Durlak, “Current and past maternal depression, maternal interaction behaviors, and children’s externalizing and internalizing symptoms,” *Journal of Abnormal Child Psychology*, vol. 36, no. 4, pp. 527–537, 2008.

[31] R. Mychasiuk, N. Schmol, S. Ilnytskyj, O. Kovachuk, B. Kolb, and R. Gibb, “Prenatal bystander stress alters brain behavior, and the epigenome of developing rat offspring,” *Developmental Neuroscience*, vol. 33, no. 2, pp. 159–169, 2011.

[32] G. Arguin, Y. Regimbald-Dumas, M. O. Fregeau, A. Z. Caron, and G. Guilleulmet, “Protein kinase C phosphorylates the inositol 1,4,5-trisphosphate receptor type 2 and decreases the mobilization of Ca2+ in pancreatoma AR4-2J cells,” *Journal of Endocrinology*, vol. 192, no. 3, pp. 659–668, 2007.

[33] J. T. Smyth, W. I. DeHaven, B. F. Jones et al., “Emerging perspectives in store-operated Ca2+ entry: roles of Orai, Stim and TRP,” *Biochimica et Biophysica Acta*, vol. 1763, no. 11, pp. 1147–1160, 2006.

[34] H. Nakata and S. Nakamura, “Brain-derived neurotrophic factor regulates AMPA receptor trafficking to post-synaptic densities via IP3R and TRPC calcium signaling,” *FEBS Letters*, vol. 581, no. 10, pp. 2047–2054, 2007.

[35] M. D. Amaral and L. Pozzo-Miller, “TRPC3 channels are necessary for brain-derived neurotrophic factor to activate a nonselective cationic current and to induce dendritic spine formation,” *Journal of Neuroscience*, vol. 27, no. 19, pp. 5179–5189, 2007.

[36] M. D. Amaral and L. Pozzo-Miller, “BDNF induces calcium elevations associated with IBDNF, a nonselective cationic current mediated by TRPC channels,” *Journal of Neurophysiology*, vol. 98, no. 4, pp. 2476–2482, 2007.

[37] K. Martinowich, H. Manji, and B. Lu, “New insights into BDNF function in depression and anxiety,” *Nature Neuroscience*, vol. 10, no. 9, pp. 1089–1093, 2007.

[38] A. K. Titterness and B. R. Christie, “Prenatal ethanol exposure enhances NMDAR-dependent long-term potentiation in the adolescent female dentate gyrus,” *Hippocampus*, vol. 22, no. 1, pp. 69–81, 2012.

[39] S. J. Franco and A. Huttenlocher, “Regulating cell migration: calpains make the cut,” *Journal of Cell Science*, vol. 118, no. 17, pp. 3829–3838, 2005.

[40] D. E. Goll, V. F. Thompson, H. Li, W. Wei, and J. Cong, “The calpain system,” *Physiological Reviews*, vol. 83, no. 3, pp. 731–801, 2003.

[41] J. Yang, H. Han, J. Cao, L. Li, and L. Xu, “Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring,” *Hippocampus*, vol. 16, no. 5, pp. 431–436, 2006.

[42] A. N. Hegde and S. C. Upadhya, “The ubiquitin-proteasome pathway in health and disease of the nervous system,” *Trends in Neurosciences*, vol. 30, no. 11, pp. 587–595, 2007.

[43] S. Gentili, J. S. Schwartz, M. J. Waters, and I. C. McMillen, “Prolactin and the expression of suppressor of cytokine signaling-3 in the sheep adrenal gland before birth,” *American Journal of Physiology*, vol. 291, no. 5, pp. R1399–R1405, 2006.

[44] B. A. Croker, H. Kiu, and S. E. Nicholson, “SOCS regulation of the JAK/STAT signalling pathway,” *Seminars in Cell and Developmental Biology*, vol. 19, no. 4, pp. 414–422, 2008.

[45] R. N. Cooney, “Suppressors of cytokine signaling (SOCS): inhibitors of the JAK/STAT pathway,” *Shock*, vol. 17, no. 2, pp. 83–90, 2002.

[46] W. S. Alexander and D. J. Hilton, “The role of Suppressors of Cytokine Signaling (SOCS) proteins in regulation of the immune response,” *Annual Review of Immunology*, vol. 22, pp. 503–529, 2004.

[47] V. Lemaire, M. Koehl, M. Le Moal, and D. N. Abrous, “Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 11032–11037, 2000.

[48] M. Koehl, V. Lemaire, M. Le Moal, and D. N. Abrous, “Age-dependent effect of prenatal stress on hippocampal cell proliferation in female rats,” *European Journal of Neuroscience*, vol. 29, no. 3, pp. 635–640, 2009.

[49] M. E. Coussons-Read, M. L. Okun, M. P. Schmitt, and S. Giese, “Prenatal stress alters cytokine levels in a manner that may endanger human pregnancy,” *Psychosomatic Medicine*, vol. 67, no. 4, pp. 625–631, 2005.

[50] C. T. Collier, P. N. Williams, J. A. Carroll, T. H. Welsh, and J. C. Laurezn, “Effect of maternal restraint stress during gestation on temporal lipopolysaccharide-induced neuroendocrine and immune responses of progeny,” *Domestic Animal Endocrinology*, vol. 40, no. 1, pp. 40–50, 2011.

[51] H. Zhang, C. E. Tan, H. Z. Wang, S. B. Xue, and M. Q. Wang, “Study on the history of traditional Chinese medicine to treat diabetes,” *European Journal of Integrative Medicine*, vol. 2, no. 1, pp. 41–46, 2010.

[52] K. Honda, H. Yanai, H. Negishi et al., “IRF-7 is the master regulator of type-I interferon-dependent immune responses,” *Nature*, vol. 434, no. 7034, pp. 772–777, 2005.

[53] M. F. Hannah, V. B. Bajic, and S. L. Klein, “Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats,” *Brain, Behavior, and Immunity*, vol. 22, no. 4, pp. 503–516, 2008.

[54] T. Araki and J. Milbrandt, “Ninjurin2, a novel homophilic adhesion molecule, is expressed in mature sensory and enteric neurons and promotes neurite outgrowth,” *European Journal of Neuroscience*, vol. 17, no. 2, pp. 83–90, 2002.

[55] K. P. Lin, S. Y. Chen, L. C. Lai et al., “Genetic polymorphisms of eNOS and calpain system,” *Frontiers in Cell and Developmental Biology*, vol. 30, no. 11, pp. 587–595, 2007.