Molecular Patterns of Neurodevelopmental Preconditioning: A Study of the Effects of Antenatal Steroid Therapy in a Protein-Restriction Mouse Model

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Introduction. Prenatal programming secondary to maternal protein restriction renders an inherent susceptibility to neural compromise in neonates and any addition of glucocorticosteroids results in further damage. This is an investigation of consequent global gene activity due to effects of antenatal steroid therapy on a protein restriction mouse model. Methods. C57BL/6N pregnant mice were administered control or protein restricted diets and subjected to either 100 μg/Kg of dexamethasone sodium phosphate with normosaline or normosaline alone during late gestation (E10–E17). Nontreatment groups were also included. Brain samples were collected on embryonic day 17 and analyzed by mRNA microarray analysis. Results. Microarray analyses presented 332 significantly regulated genes. Overall, neurodevelopmental genes were overrepresented and a subset of 8 genes allowed treatment segregation through the hierarchical clustering method. The addition of stressor steroids greatly affected gene regulation through glucocorticoid receptor and stress signaling pathways. Furthermore, differences between dexamethasone-administered treatments implied a harmful effect during conditions of high stress. Microarray analysis was validated using qPCR. Conclusion. The effects of antenatal steroid therapy vary in fetuses according to maternal-fetal factors and environmental stimuli. Defining the key regulatory networks that signal either beneficial or damaging corticosteroid action would result in valuable adjustments to current treatment protocols.

1. Introduction

The concept of the fetal genome is no longer that of a static framework inherited from paternal and maternal sources but a malleable scaffold constantly adapting to stimuli. This is most evident in studies involving fetal programming due to the effects of nutritional variation and glucocorticoid exposure [1]. Here, we examined the resulting fetal molecular preconditioning due to antenatal steroid therapy using a protein-restriction mouse model. This model was first designed in a previous study [2] as a novel approach to evaluate postnatal adaptive responses due to varied prenatal nutritional conditions and the addition of stress or steroids. Molecular evidence revealed that prenatal programming secondary to maternal protein restriction rendered an inherent susceptibility to neural compromise in neonates and any further addition of antenatal steroids may be detrimental to these already injury-prone offspring. Thus, an examination of underlying molecular mechanisms in the fetus was warranted to elucidate the effects seen postnatally.

Understanding any subtle changes in the fetus induced by these factors and their correlation with phenotypic outcomes in the adult would facilitate early detection of either wellbeing or disease. Current biomolecular techniques such as microarray analysis have allowed the investigation of global gene expression and subsequently, the parallel data mining
of gene transcripts of interest as well as the discovery of new gene involvement. Moreover, gene expression profiles through clustering of significant genes have shown promising potential as diagnostic panels. All these have led to the rapid identification of biomarkers for disease conditions and their associated regulatory pathways [3]. Using these advancements, a panoramic view of genetic movement in utero is presented.

2. Materials and Methods

2.1. Experimental Animals. Female C57BL/6N mice about 6 weeks old provided by the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine, were maintained under controlled lighting (12-hour light cycles) and temperature (24°C). These were allowed for free access to food (AIN-93G: Oriental Yeast Co., Ltd., Tokyo, Japan) and water during a 2-week acclimatization period after which each female was time mated with a male.

2.2. Treatment Groups. Pregnant females (n = 36) were then housed singly and administered either control (C) or protein restricted (PR) diets ad libitum all throughout pregnancy (Embryonic stage, E0 to E17). These were then further subdivided into 6 groups and subjected to either plain normosaline solution (C-S, PR-S) or 100 μg/Kg dexamethasone sodium phosphate (Decadron, MSD K.K., Tokyo, Japan) in normosaline solution (C-D/S, PR-D/S) by subcutaneous injection daily during late gestation (E10 to E17). Nontreatment groups were also included (C, PR). All injections were performed between 12 nn and 2 pm. Maternal weights on days E0, E10, and E17 were recorded, as well as fetal weights on E17. On embryonic day 17, whole brain samples collected from 2 male and 2 female fetuses from each litter were supercooled in liquid nitrogen and stored at –80°C.

2.3. DNA Chip Analysis. A total of 6 Toray 3D-Gene Mouse Oligo chip 24 K (Toray Industries, Inc., Tokyo, Japan) microarrays were analyzed per treatment. Each chip utilized a 0.5μg portion of combined total RNA from a matched pair of male and female samples. RNA was amplified and labeled using an Amino Allyl MessageAmp II aRNA Amplification kit (Life Technologies Japan Ltd.) according to the manufacturer's instructions. Each sample of aRNA was labeled with fluorescence Cy3 or Cy5 and cohybridized at 37°C for 16 hours. These were subsequently washed and dried. Hybridization signals were scanned using Scan Array Express (Perkin Elmer, MA, USA) and global background analysis was performed using GenePix Pro (MDS Analytical Technologies, CA, USA). All 36 arrays were then normalized together as one experiment to reduce nonbiological variability.

2.4. Quantitative PCR (qPCR). To validate microarray results, qPCR was performed on 2 selected genes, microtubule-associated protein 1b (Map1b) and 3-hydroxy-3-methylglutaryl-CoA synthase 1 (Hmgcs1), using the C and C-D/S treatments. Total RNA was extracted from whole fetal brains (n = 6 per treatment) using QIAzol Lysis Reagent (QIAGEN, Hilden, Germany) and cleaned with an AllPrep DNA/RNA Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Complimentary DNA was synthesized using the Superscript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) and quantitative PCR was conducted with EXPRESS SYBR GreenER Supermix with Premixed ROX (Invitrogen, Carlsbad, CA) on an Eppendorf Realplex Mastercycler (Eppendorf, Hamburg, Germany). Amplified transcripts were quantified and normalized against hypoxanthine phosphoribosyltransferase 1 (Hprt1). Primer sequences of the selected genes and housekeeping gene are provided.

2.5. Statistical Analysis. Microarray data were subjected to t-test analyses with standard Bonferroni correction for multiple comparisons. The P value was set at 0.05 and a threshold of 1.5-fold was applied to determine significantly regulated genes. These were subjected to an ontological review and a subset of neurodevelopmental genes for genetic profiling was determined by hierarchical clustering. Targeted gene transcripts of interest on the microarrays were treated to one-way ANOVA with post-hoc analysis (Bonferroni post-test). Confirmation of selected genes by qPCR was validated through Fold Change Analysis (threshold of 1.5-fold). Data management, statistical analysis, and gene ontology were performed using geWorkbench software (https://gforge.nci.nih.gov/frs/?group_id=78) and MGI Gene Ontology Tool (http://www.informatics.jax.org/gotoools/).

3. Results

3.1. Mouse Model. Mean maternal weight gain patterns, between groups, were similar before (E0 to E10) and during treatment (E10 to E17). Mean fetal brain to body weight indices on sampling day E17 were not significantly different (Figure 1).

3.2. Global Gene Changes. Microarray analyses of 23,522 probe transcripts presented 10,946 genes without absent calls or unreadable hybridization signals. There were more upregulated genes as compared to downregulated genes across all treatment groups (Figure 2, Table 1). The combined number of significant genes regulated from all treatment groups versus the control was 332 (Figure 3). Subsequent gene ontology analysis revealed that ongoing cell organization and biogenesis, developmental processes, and transport were most rampant in the global expression survey. The discovery of genes uniquely activated per treatment and sharing similar ontologies facilitated individual treatment characterization (Table 2(a)). Associated genes were found for protein restriction, cell adhesion genes in both PR-D/S only (Col1a1, Atp1b2, Ctnnd1, Rpsa, and Fat4), and PR-S only (Cdh2, Edil3, and Astn1); for dexamethasone treatment, stress response genes in both C-D/S only (Brskl, Rarres2), and PR-D/S only (Klk8, Myo6, Ndufa6, Col1a1, Mapk8, and Phlda3); and for both protein restriction, dexamethasone treatment, and DNA metabolism genes for PR-D/S only (Tcf3, Mapk8).
Overall, neurodevelopmental genes were overrepresented among those significantly regulated (Table 2(b)) and were associated with nervous system development (Table 3). Two-way ANOVA indicates a significant treatment effect ($P < 0.0001$) and time effect ($P < 0.0001$). Bonferroni posttest indicates that all treatments were similar to the C group. (b) Mean fetal brain to body weight indices across treatments $\pm$ SEM were not significantly different by one-way ANOVA ($n = 96$).

### 3.3. Gene Expression Profiling
A subset of 8 genes out of 332 was filtered through the hierarchical clustering method allowing segregation of treatments (Figure 4). An assessment of individual biological themes within the subset revealed neurodevelopmental roles and distinct causal relationships with glucocorticoid treatment and protein restriction (Table 3).

### 3.4. Targeted Genes of Interest
The addition of stress or steroids (-S and -D/S groups) greatly affected gene regulation leading to further investigation of genes related to glucocorticoid and stress signaling pathways. Mapk8, Fkbp5, Mkp-1, Pp2A, Akt, and Gsk3 exhibited expression patterns across treatment groups that corresponded to an overall reduction in glucocorticoid receptor (GR) activity in the -D/S and -S groups. Most significant of which were the marked differences between C-D/S and PR-D/S.

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**Table 1:** Summary of significantly regulated genes. Determination of upregulated and downregulated significant genes based on paired $t$-tests with standard Bonferroni correction for multiple comparisons ($P < 0.05$; thresholds of $>1.5$ for upregulated genes and $< -1.5$ for downregulated genes).

| $t$-test* | Regulated genes | Fold change ($\uparrow>1.5$) | Fold change ($\downarrow<1.5$) |
|-----------|-----------------|-----------------------------|-----------------------------|
| C versus PR | 4 | 0 | 0 |
| C versus C-D/S | 751 | 91 | 24 |
| C versus PR-D/S | 974 | 158 | 44 |
| C versus C-S | 896 | 141 | 25 |
| C versus PR-S | 1125 | 184 | 40 |

* $P < 0.05$.
Table 2: Ontological review of biologic processes.

(a) Comparison of gene ontology slim terms of biological processes involved for the total number of genes on the microarray as compared to the combined significantly regulated genes and the genes uniquely regulated per treatment

| Biological process                          | Total number of genes | Significant genes | C-D/S only (frequency) | PR-D/S only (frequency) | C-S only (frequency) | PR-S only (frequency) |
|---------------------------------------------|-----------------------|-------------------|------------------------|-------------------------|----------------------|-----------------------|
| Cell adhesion                               | 460 (0.04202)         | 21 (0.06325301)   | 0 (0.00000)            | 5 (0.08621)             | 0 (0.00000)          | 3 (0.06818)           |
| Cell-cell signaling                         | 403 (0.03682)         | 22 (0.06626506)   | 2 (0.01765)            | 5 (0.08621)             | 2 (0.015385)         | 0 (0.00000)           |
| Cell cycle and proliferation                | 1329 (0.12141)        | 35 (0.10542619)   | 0 (0.00000)            | 6 (0.10345)             | 2 (0.015385)         | 0 (0.00000)           |
| Death                                       | 946 (0.08642)         | 29 (0.08734940)   | 2 (0.01765)            | 5 (0.08621)             | 0 (0.00000)          | 2 (0.04545)           |
| Cell organization and biogenesis            | 2327 (0.21259)        | 96 (0.28915663)   | 6 (0.24138)            | 14 (0.24138)            | 4 (0.30769)          | 11 (0.25000)          |
| Protein metabolism                          | 2199 (0.20090)        | 61 (0.18373494)   | 3 (0.17746)            | 13 (0.22414)            | 2 (0.15385)          | 7 (0.15909)           |
| DNA metabolism                              | 438 (0.04001)         | 20 (0.06024096)   | 0 (0.00000)            | 2 (0.03448)             | 0 (0.00000)          | 0 (0.00000)           |
| RNA metabolism                              | 2020 (0.18454)        | 65 (0.19578313)   | 7 (0.41176)            | 14 (0.24138)            | 3 (0.23077)          | 7 (0.15909)           |
| Other metabolic processes                   | 1657 (0.15138)        | 57 (0.17168675)   | 4 (0.23529)            | 10 (0.17241)            | 2 (0.15385)          | 9 (0.20455)           |
| Stress response                             | 1156 (0.10561)        | 29 (0.08734940)   | 2 (0.11765)            | 6 (0.10345)             | 0 (0.00000)          | 0 (0.00000)           |
| Developmental processes                     | 2000 (0.18272)        | 83 (0.25000000)   | 5 (0.29412)            | 13 (0.22414)            | 5 (0.38462)          | 10 (0.22727)          |
| DNA metabolism                              | 1929 (0.17623)        | 72 (0.21686747)   | 4 (0.23529)            | 13 (0.22414)            | 4 (0.30769)          | 5 (0.1164)            |
| Unknown biological processes                | 0 (0.00000)           | 0 (0.00000000)    | 0 (0.00000)            | 0 (0.00000)             | 0 (0.00000)          | 0 (0.00000)           |
| All biological processes                    | 10946 (1.00000)       | 332 (1.0000000)   | 17 (1.00000)           | 58 (1.00000)            | 13 (1.00000)         | 44 (1.00000)          |

† Only genes without any absent calls were included in the analysis from 23,522 transcripts on the microarray.
‡ Genes determined by up or down regulation within the 1.5 fold change threshold.
§ Cell adhesion genes: PR-D/S only (Col1a1, Atp1b2, Ctnnd1, Rpsa, and Fat4); PR-S only (Cdh2, Edil3, and Astn1).
¶ DNA metabolism: PR-D/S only (Tcf3, Mapk8).
ß Stress response genes: C-D/S only (Brsk1, Rarres2); PR-D/S only (Klk8, Mys6, Ndufa6, Col1a1, Mapk8, and Phlda3).

(b) Top 25 biological processes among the significantly regulated genes based on P values

| GO ID      | GO term                                      | Frequency | Genome frequency | P value     | Corrected P value |
|------------|----------------------------------------------|-----------|-----------------|-------------|------------------|
| GO:0009987 | Cellular process                             | 0.68072   | 0.39346         | 2.0856E-26  | 2.6196E-23       |
| GO:0071841 | Cellular component organization or biogenesis at cellular level | 0.27108 | 0.08456 | 8.0847E-24 | 1.0154E-20 |
| GO:0071842 | Cellular component organization at cellular level | 0.25904 | 0.08100 | 1.2822E-22 | 1.6105E-19 |
| GO:0071840 | Cellular component organization or biogenesis | 0.30120 | 0.10864 | 6.9965E-22 | 7.6573E-19 |
| GO:0016943 | Cellular component organization               | 0.28916  | 0.10476         | 7.5917E-21  | 9.3531E-18       |
| GO:0008152 | Metabolic process                             | 0.50301   | 0.26565         | 1.9862E-20  | 2.4947E-17       |
| GO:0044237 | Cellular metabolic process                    | 0.43818   | 0.22384         | 2.2263E-20  | 2.7962E-17       |
| GO:0044238 | Primary metabolic process                     | 0.43976   | 0.22525         | 2.6340E-18  | 3.3082E-15       |
| GO:0007399 | Nervous system development                    | 0.15060   | 0.04413         | 2.9821E-15  | 3.7455E-12       |
| GO:0043170 | Macromolecule metabolic process               | 0.37048   | 0.18900         | 5.7101E-15  | 7.1719E-12       |
| GO:0051179 | Localization                                 | 0.26807   | 0.11543         | 1.3853E-14  | 1.7399E-11       |
| GO:0048699 | Generation of neurons                         | 0.11747   | 0.02708         | 1.8462E-14  | 2.3188E-11       |
| GO ID       | GO term                                      | Frequency | Genome frequency | $P$ value | Corrected $P$ value |
|------------|----------------------------------------------|-----------|------------------|-----------|--------------------|
| GO:0030182 | Neuron differentiation                        | 0.1145    | 0.02443          | 1.9738E−14 | 2.4790E−11        |
| GO:0044260 | Cellular macromolecule metabolic process      | 0.33434   | 0.16512          | 2.9036E−14 | 3.6469E−11        |
| GO:0022008 | Neurogenesis                                  | 0.12048   | 0.02896          | 3.2387E−14 | 4.0678E−11        |
| GO:0007275 | Multicellular organismal development          | 0.25000   | 0.10479          | 3.3420E−14 | 4.1975E−11        |
| GO:0006996 | Organelle organization                        | 0.16867   | 0.05448          | 4.7022E−14 | 5.9060E−11        |
| GO:0048666 | Neuron development                            | 0.09639   | 0.01882          | 5.6471E−14 | 7.0927E−11        |
| GO:0065007 | Biological regulation                         | 0.45783   | 0.27182          | 2.5651E−13 | 3.2218E−10        |
| GO:0034641 | Cellular nitrogen compound metabolic process  | 0.28614   | 0.13631          | 5.9844E−13 | 7.5165E−10        |
| GO:0009058 | Biosynthetic process                          | 0.27108   | 0.12543          | 6.0854E−13 | 7.6433E−10        |
| GO:0032502 | Developmental process                         | 0.25904   | 0.11705          | 6.6540E−13 | 8.3574E−10        |
| GO:0006807 | Nitrogen compound metabolic process           | 0.28986   | 0.13916          | 7.8812E−13 | 9.8988E−10        |
| GO:0006139 | Nucleobase, nucleoside, nucleotide, and nucleic acid metabolic process | 0.27108 | 0.12602 | 7.9334E−13 | 9.9643E−10 |
| GO:0031175 | Neuron projection development                 | 0.08434   | 0.01588          | 9.5266E−13 | 1.1965E−09        |
| Gene symbol | Functional summary                                           | Reference number | MGI ID     | Fold change versus C |
|-------------|-------------------------------------------------------------|------------------|------------|----------------------|
| Sox11       | Modulator of neocortical development                        | [4]              | 98359      | ↑3.84                |
| Tcf3        | Neural differentiation                                       | [5]              | 1202876    | ↓1.62                |
| Mapk8       | Neuron projection development                                | [6]              | 1346861    | ↑1.50                |
| Btd6        | Neuronal differentiation                                    | [7]              | 3026623    | ↓1.96                |
| Mtap1b      | Axonal guidance and neuronal migration                       | [8]              | 1306778    | ↑3.83                |
| Eif2b5      | Myelination and glial development                            | [9]              | 2446176    | ↓1.65                |
| Hmgcs1      | Brain cholesterol pathways in myelination                    | [10]             | 107592     | ↑2.03                |
| Camk2n1     | Neuronal development                                         | [11]             | 1913509    | ↓1.81                |
(Mapk8, Gsk3, and Mtap1b) emphasizing the disparate effect on varying nutritional conditions due to dexamethasone administration (Figures 5(a) and 5(b)).

3.5. Quantitative PCR Validation. qPCR results demonstrated a good agreement with the microarray data for Mtap1b and Hmgcs1 (Figure 6).

4. Discussion

The maternal-fetal compartment serves to cushion the fetus from environmental stimuli, but beyond normal circumstances, prenatal conditioning invariably occurs. Microarray analysis allowed for a panoramic view of gene activity along two levels: through the global expression of genes and through individual treatment groups and their association with one another. In general, ongoing cell organization and biogenesis, developmental processes, and transport were most rampant in the global expression survey (Table 2(a)). The dominance of these particular gene groups is expected in the developing fetus just as genes for growth and maturation are more likely activated in neonates. Regardless, these increased gene frequencies most likely demonstrate consequent fetal reactions to acquired insults from protein restriction and glucocorticoid exposure either as compensatory regulation or protective feedback. They signaled changes
multipotent progenitor differentiation and neuronal migration. Their increased expression over other gene systems emphasized the significance of fetal neuroplasticity even to the detriment of visceral organ growth similar to physiologic brain sparing. This also underlined the early dependence on brain-controlled pathways that trigger bodily functions during stages when less developed organs have not yet attained full functional independence.

Our mouse model simulated conditions of multifactorial environmental impact. The isolation of distinct genes associated with individual factors of protein restriction, dexamethasone, and stress were complimentary to known gene networks.

4.1. Protein Restriction Associated with Cell Adhesion Genes. Maternal nutrition plays a critical role in fetal growth and development. Studies of these genes uniquely regulated in both PR-D/S (Col1a1, Atp1b2, Ctnnd1, Rpsa, and Fat4) and PR-S only (Cdh2, Edil3, and Astn1) emphasized the crucial role of nutritional factors in maintaining the integrity of cell interaction. Col1a1, a known marker of fibrosis and aging, has been linked to alterations in oxidative and antioxidant defense capacity in cells due to poor maternal nutrition [13]. On the other hand, modifications in Atp1b2, Edil3, and Astn1 during development lead to glial dysfunction [14–16]. Moreover, various studies on nutritional factor effects and progenitor cell differentiation included Ctnnd1, Fat4, and Cdh2 [17–19].

4.2. Dexamethasone Associated with Stress Response Genes. Fetal dexamethasone exposure impairs development in various cell types eliciting a dose-dependent stress response [20]. In the brain, the pituitary is the site of action of administered dexamethasone in the blockade of stress induced hypothalamic-pituitary axis (HPA) activation. The latter involves the stimulation of brain receptors, primarily, those of glucocorticoid receptors (GR) by both exogenous and endogenous corticosterone. During conditions of stress, the HPA axis releases reactive feedback which suppresses increased excitability allowing recovery from stress induced activation and facilitation of memory storage. The addition of dexamethasone can partially deplete the brain of corticosterone and in turn suppress the fetal HPA axis. Studies that included the genes uniquely regulated in C-D/S (Brsk1, Rarres2) and PR-D/S (Klk8, Myo6, Ndufa6, Collal, Mapk8, and Phlda3) report their important roles in neuroregulation and adaptation to stress responses during brain development [21–25]. Furthermore, expression patterns of targeted genes related to stress signaling pathways revealed decreased GR activity: Mapk8 (mitogen activated protein kinase 8) and Fkbp5 (FK506 binding protein 5), both GR inhibitors [26, 27], were increased in the -D/S and -S groups; Mkp1 (mitogen activated protein kinase phosphatase 1) and Pp2a (protein phosphatase 2), both Map kinase inhibitors [28, 29], were decreased in the -D/S and -S groups (Figure 5(a)).

4.3. Convergent Effects of Maternal Nutrition and Dexamethasone Associated with DNA Metabolism. Genes associated with PR-D/S only (Tcf3 and Mapk8) on microarray analysis, within a fetus long previously believed to be immune prior to the conception of the Barker theory [12].

Interestingly, neurodevelopmental genes were overrepresented among the significantly regulated genes. This was exemplified by recurring themes in biological processes related to ongoing brain development during fetal stages:
Figure 5: Targeted genes of interest related to stress signaling pathways. (a) One-way ANOVA of gene expression patterns revealing an overall reduction of glucocorticoid receptor (GR) activity included *Mapk8*, *Fkbp5*, *Mkp1*, and *Pp2a*. (b) One-way ANOVA of gene expression patterns for *Akt* and *Gsk3* and *Mtap1b* revealing ongoing neurodysgenesis (n = 6 per treatment; mean ± SEM; one-way ANOVA P < 0.0001; Bonferroni *P < 0.05 and **P < 0.1).
as well as two targeted genes (Akt and Gsk3) all function in metabolic gene networks, especially for glucose metabolism in DNA synthesis. Here, their specific expression patterns between -D/S groups underscored the most significant observation in this study, which was the apparent harmful effect of dexamethasone to fetuses in highly stressed conditions (PR-D/S). A diagram interrelating the targeted genes of interest with regard to GR activity is shown in Figures 7(a) and 7(b). Previous reports have stated that a loss of GR activity reduces dexamethasone inhibition of Akt (thymoma viral protooncogene 1), which in turn decreases Akt inhibition of Gsk3 (glycogen synthase kinase 3), a proapoptotic gene [30–32]. This beneficial effect was seen in the C-D/S group. But in the PR-D/S group, a highly stressed condition, dexamethasone was evidently harmful. This disparate pattern between C-D/S and PR-D/S, significant in Mapk8, Gsk3, and Mtap1b (P < 0.0001), denotes the possibility of ongoing altered neurodevelopment or even neurodysgenesis (Figure 5(b)).

The process of data mining revealed the association between regulated genes unique to individual treatment groups and certain biologic processes. Their correlation provided a better understanding of underlying pathophysiology and a glimpse of key pathways for future focused studies. One possible application is the development of gene panels for genetic expression profiling as diagnostic tools. Hierarchical clustering programs currently allow the generation of gene maps to be capable of distinguishing between phenotypes. In our simulation, highly regulated neurodevelopmental genes were used and these successfully segregated treatments between microarrays (Figure 4).

Our findings strengthen our previous study’s assertion that fetal programming secondary to maternal protein restriction renders an inherent susceptibility to neural compromise in offspring and that the addition of dexamethasone to this vulnerable group results in further injury. In future studies, the investigation of both sex-specific and transgenerational effects is necessary as glucocorticoids influence endocrinological pathways differently in males and females. Also, timing of exposure to glucocorticoids as well as dosage studies is no less relevant especially in the light of reported

![Microarray and qPCR](image-url)

**Figure 6:** qPCR validation of microarray analysis. Two neurodevelopmental genes, Mtap1b and Hmgcs1, that were significantly changed between C and C-D/S groups in the microarray analysis (represented as mean hybridization signals + SEM) were compared with qPCR (mean ± SEM transcript signals normalized against Hprt1). When comparing non-logged fold changes by the ratio method, there was an overall good agreement between qPCR and microarray analysis with a fold change of at least 1.5∗ observed. (n = 6 per treatment).
Dexamethasone is an agonist for the glucocorticoid receptor (GR).

- Dexamethasone inhibits Akt.
- Akt inhibits proapoptotic gene Gsk3.

Extremely stressful conditions, such as maternal protein restriction, highly activate MAP kinase signal pathways.

- Mkp1 and Pp2a are reduced and fail to inhibit Mapk8.
- Mapk8 and Fkbp5 reduce GR activity.
- Reduced GR activity inhibits dexamethasone.
- Akt is inhibited by dexamethasone.
- Gsk3 activity is unchecked by Akt leading to neurodysgenesis, an example of which is the upregulation of Mtap1b.

In C-D/S, there is less reduction of GR activity, less dexamethasone inhibition of Akt, and less neurodysgenesis.

**Conflict of Interests**

The authors hereby disclose that there was no financial or personal conflict of interests involved.

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