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Neural Tube Defect Genes and Maternal Diabetes during Pregnancy

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Abstract

BACKGROUND—Maternal diabetes during pregnancy is a well-known teratogen that increases the risk for birth defects, such as neural tube defects (NTDs). We have previously shown that maternal diabetes profoundly affects gene expression in the developing embryo, in particular a suite of known NTD genes. In rodent experimental systems, NTDs present as phenotypes of incomplete penetrance in diabetic pregnancies. This property is difficult to reconcile with observations of consistently altered gene expression in exposed embryos. We here show that maternal diabetes increases the overall variability of gene expression levels in embryos.

RESULTS—Altered gene expression and increased variability of gene expression together may constitute the molecular correlates for incomplete phenotype penetrance.

DISCUSSION—Based on this model, we suggest that maternal diabetes reduces the precision of gene regulation in exposed individuals. Loss of precision in embryonic gene regulation may include changes to the epigenome via deregulated expression of chromatin-modifying factors. Unraveling the mechanisms underlying such epigenetic modifications in diabetic pregnancies will help to understand how teratogenic insults compromise embryonic development and possibly provide avenues for therapeutic intervention.

Keywords

gene expression; variability; incomplete penetrance; signaling pathways; epigenetic modifications

INTRODUCTION

The prevalence of diabetes is increasing in the United States (National Diabetes Data Group, 1995) and worldwide. In addition to severely compromising the health of the afflicted individual, maternal diabetes during pregnancy also impacts embryonic development with well-documented teratogenic effects (Greene, 1999). Given the increasing incidence of diabetes in younger women (Dunne, 2005), prenatal exposure of the next generation becomes a major health concern. Exposure in utero to maternal diabetes constitutes a risk for diabetic embryopathy (Mills, 1982; Goto and Goldman, 1994; Casson et al., 1997; Aberg et al., 2001), a spectrum of severe birth defects including cardiovascular malformations and neural tube defects (NTDs) (Kucera, 1971; Martinez-Frias, 1994; Martinez-Frias et al.,

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1998). The underlying mechanisms are not well understood, but are thought to involve various responses of the embryonic genome to the adverse intrauterine environment (Greene, 2001; Loeken, 2008).

To explore how conditions of maternal diabetes affect gene expression in the embryo, we recently conducted expression profiling experiments on embryos from diabetic dams compared to embryos from normal dams (Pavlinkova et al., 2009). We were able to demonstrate that maternal diabetes has a profound impact on gene expression levels. It is reasonable to assume that these deregulated genes are the most likely candidates to contribute to the molecular etiology for maternal diabetes–induced birth defects. Among these affected genes (Pavlinkova et al., 2009) are several that are known NTD genes (Harris and Juriloff, 2007). Because NTDs are one of the cardinal birth defects in diabetic embryopathy (Mills, 1982; Goto and Goldman, 1994; Casson et al., 1997; Aberg et al., 2001), we therefore focused our attention on known NTD genes and their interaction with maternal diabetes.

NTDs in diabetic pregnancies typically occur in only a fraction of exposed embryos (Otani et al., 1991). In the widely used model of chemical induction of diabetes by streptozotocin, the frequency of NTDs is typically in the range of 10–15% in mice (Phelan et al., 1997; Pavlinkova et al., 2008). This is evident even in inbred strains, where the genetic background is thought to be constant. Furthermore, NTDs elicited by maternal diabetes display a phenotypic diversity that may range from a wavy neural tube to spina bifida in various locations to exencephaly and craniorachischisis. Together, these observations characterize NTDs in diabetic pregnancies as a phenotype of incomplete penetrance and variable expressivity (Grüneberg, 1952b). However, gene expression profiling experiments are typically performed with the goal to discover genes with highly consistent change in expression when exposed groups are compared to controls (Kappen et al., 2010). This poses an apparent conundrum: How can genes that are consistently changed in exposed embryos generate a phenotype that appears only in a small percentage of such cases? In this article, we investigate this question by reanalyzing existing gene profiling data, by determining which known NTD genes are altered in their embryonic expression by maternal diabetes, and by considering how maternal diabetes, in addition to changing specific gene expression levels, may exert genome-wide effects on the embryo. We propose that variation in gene expression could be a relevant parameter underlying penetrance and discuss the implications for the etiology of NTD phenotypes in diabetic pregnancies.

METHODS

We used the Mouse Genome Informatics database MGI (Jackson Laboratory, http://www.informatics.jax.org) as the base for annotations. As of August 2009, a search of the Mammalian Phenotype Ontology database at MGI with the term “abnormal neural tube closure” yielded 373 genotypes (432 annotations), including the terms “delayed neural tube closure” (32 genotypes, 32 annotations), “incomplete cephalic closure” (50 genotypes, 50 annotations), and “open neural tube” (281 genotypes, 295 annotations). We further included the terms “craniorachischisis” (25 genotypes, 25 annotations), “kinked neural tube” (61 genotypes, 61 annotations), “wavy neural tube” (31 genotypes, 31 annotations), “spina bifida” (95 genotypes, 98 annotations), “spina bifida occulta” (12 genotypes, 12 annotations), “exencephaly” (315 genotypes, 315 annotations), “holoprosencephaly” (43 genotypes, 44 annotations), as well as “anencephaly” (22 genotypes, 22 annotations). Consolidating the output from these searches yielded a total of 383 unique gene names, comprising 347 genes that were associated with genome coordinates. The 36 loci where genome coordinates were not available were excluded from further consideration.

Gene names were compared against the Affymetrix microarray platform database using the NetAffx Query tools (https://www.affymetrix.com/analysis/netaffx) to retrieve probe identification numbers. Gene pathway analyses were conducted using the DAVID tools (http://david.abcc.ncifcrf.gov). Transcription factor binding site presence was determined using the Whole Genome rVISTA (http://genome.lbl.gov/vista/index.shtml) program based upon the comparison of mouse (February 2006, mm8) and human (March 2006, hg18) genome assemblies. Statistical analyses were performed using Microsoft Excel, GraphPad Prism (Wilcoxson signed-rank test), and online resources for a Kolmogorov-Smirnov comparison (http://www.physics.csbsju.edu/stats/KS-test.html).

RESULTS

Database and Literature Studies

To relate results from our transcriptome studies to NTDs, we first generated a compilation of known NTD genes as well as genes associated with NTD. Queries of the MGI database yielded 347 genes; we refer to this group as NTD-associated genes, as this group encompasses two types of genes: (1) genes in which a targeted (or other type of) mutation results in a NTD phenotype and (2) genes that can act as modifiers of NTD phenotypes caused by other loci, or genes that give rise only to NTD phenotypes in combination with other mutations. The standard reference paper with respect to NTD genes (Harris and Juriloff, 2007) yielded a total of 197 gene symbols; of those, 179 genes were associated with current genome coordinates. For comparison to our expression data, we focused on genes with known genomic locations. Of the 179 genes, 154 were represented within the list of 347 NTD-associated genes obtained from the MGI database. Combining results from the MGI database and the paper by Harris and Juriloff resulted in a total of 372 gene symbols that we treated as NTD-associated genes for our further analyses. These genes are listed in Table 1.

Microarray Gene Expression Analyses

To survey gene expression changes in the developing embryo that were brought about by the adverse uterine environment of maternal diabetes, we had performed transcriptome profiling by microarray (Pavlinkova et al., 2009; Kappen et al., 2010). These experiments were performed using the streptozotocin (STZ) model of diabetes induction in dams of the FVB strain of mice. Embryos were collected at gestation day 10.5, and three sample pools (each of three embryos, each embryo from a different pregnancy) were used to compare expression profiles between embryos from normal and diabetic pregnancies. Although our initial interpretation was aimed at detecting overall gene expression changes, the detailed analysis of the array data in this report is focused on the effects of maternal diabetes on NTD-associated genes. This was done to evaluate the hypothesis that NTDs in diabetic pregnancies may have their etiology in changes of expression of already known NTD genes. We therefore queried the 372 NTD-associated genes against the Affymetrix database of microarray probes on the Mouse Genome 430 2.0 array. We obtained a total of 870 Affymetrix Probe Set IDs representing 366 of the 372 NTD-associated genes; six genes of this group (*Cyp26c1, Gdf7, Pax2, Rpgrip1l, Tctn1, Terc*) were not represented on the array. From the 870 probes, 726 probes showed at least a single “present” call among the six arrays; these 726 probes represented 343 genes. With respect to statistically significant changes ($p < 0.05$, total of 379 probes, 245 genes) between diabetes-exposed and control embryos, we detected 161 probes representing 128 genes that showed a greater than twofold change in expression; all exhibited decreased expression levels under conditions of a diabetic pregnancy. Change of expression between 1.5- and twofold was found for 103 probes, representing 64 genes. The tendency toward decreased gene expression in diabetic pregnancy conditions is also evident for this group: 81 probes show reduced expression,

whereas only 22 probes display an increase in gene expression levels. The changes in gene expression levels of NTD-associated genes (with a cutoff at 2.0) as well as the associated *p* value obtained in the statistical evaluation of the microarray data are listed in Table 2. These results indicate that only a subset of all currently known NTD-associated genes are deregulated, and that maternal diabetes elicits a specific genomic response in the embryo.

NTD-Associated Genes and Cellular Pathways

To determine how maternal diabetes–induced expression changes of NTD-associated genes may affect specific cellular processes of the embryo, we conducted pathway analyses using the DAVID platform (Huang et al., 2009). For this approach, all NTD-associated genes were grouped together that showed at least 1.5-fold change of expression on the microarray (deregulated by maternal diabetes, 192 genes) and were compared to genes we deemed unaffected in their expression by maternal diabetes (change of expression <1.5-fold, or no significant change; 180 genes). The results of these studies are summarized in Table 3. These analyses revealed that genes associated with Wnt signaling (19 genes), Hedgehog signaling (11 genes), Notch signaling (10 genes), MAPK signaling (20 genes), and focal adhesion (16 genes) pathways were deregulated by maternal diabetes, whereas many genes associated with the TGFβ signaling pathway (15 genes) were not affected by maternal diabetes. However, it is notable that *Tgfβ1, Tgfβ2*, and *Tgfβr1* are deregulated, suggesting that a specific aspect of TGFβ signaling may be affected by maternal diabetes. In fact, assignment of the TGFβ signaling pathway to the unaffected group of genes is largely due to genes encoding ligands of the TGFβ family. Together, these results suggest that the Wnt, Hedgehog, Notch, and MAPK pathways and the focal adhesion mechanism may preferentially contribute to the etiology of NTDs in diabetic pregnancies.

Transcription Control and NTD-Associated Genes

Although the pathway analyses highlight potential mechanisms in phenotype etiology in diabetic pregnancies, they do not address the question how maternal diabetes perturbs gene expression in the developing embryo. We have shown previously (Pavlinkova et al., 2009) that transcription factors and DNA- or chromatin-interacting factors are enriched among genes with altered expression in embryos from diabetic pregnancies. Table 4 summarizes the genes encoding chromatin-modifying factors that are affected by maternal diabetes. However, exactly how transcription factor genes respond to maternal diabetes, how their target genes respond, and whether known NTD-associated genes are direct or indirect targets, is largely unknown. Furthermore, the regulatory elements that drive correct developmental expression for each gene, in most cases, are also unknown.

In the absence of gene-specific regulatory information, we therefore evaluated whether any of the recently described p300-associated enhancers, which have been shown to be associated with transcriptional activity in the embryonic forebrain or midbrain (Visel et al., 2009), were located near any of the NTD-associated genes. For this purpose, we analyzed an envelope (i.e., from 50 kb upstream of the annotated transcription start to 50 kb downstream of the transcription stop) around each NTD-associated gene for the presence of an annotated p300-associated enhancer. The results are summarized in Table 5. Although more p300-associated forebrain enhancers are present in the group of NTD-associated genes that are deregulated by maternal diabetes, this association does not reach statistical significance ($p = 0.076$). In fact, the fraction of genes that have such an enhancer in the 100 kb envelope is nearly identical between the two groups (19.7 vs. 17.2%). Similarly, the distribution of enhancer sequences with midbrain activity is not different between the two groups of NTD-associated genes. In this context, however, it was interesting to note that p300 is the product of the *Ep300* gene, which is a NTD gene itself. In our array experiment, we classified *Ep300* as unaffected by maternal diabetes, with a statistically significant ($p = 0.006$) yet slight

(−1.38 fold) decrease of expression in embryos from diabetic dams. Whether this rather moderate decrease of *Ep300* expression could have consequences on enhancer activities is unknown, but it stands to reason that it would affect all p300-associated enhancers equally and may therefore not account for the diabetes-induced differences in gene expression that we observed. Nevertheless, these enhancer sequences could serve as experimental inroads toward understanding in vivo regulatory events at NTD-associated genes on a molecular level.

We further performed analyses of potential transcription factor binding sites for a 5 kb region upstream of the transcription start of each gene. We used WholeGenome rVista software (Loots and Ovcharenko, 2004), which identifies transcription factor binding sites that (1) are conserved between mouse and human and (2) are significantly overrepresented in a given group of genes when compared to the entire genome (p value below 0.005). The analyses were performed separately for the group of NTD-associated genes that were affected by maternal diabetes (192 genes) and for the group of unaffected genes (180 genes). We found 142 transcription factors to be associated with the affected genes; of these 142 factors, 114 were also found to be associated with the group of unaffected genes. Twenty-eight transcription factors were found to be only overrepresented in the group of affected genes, suggesting that these transcription factors may contribute to the differential response to maternal diabetes between affected and nonaffected genes. However, detailed inspection showed that only small subsets of genes within the entire group of 192 deregulated NTD-associated genes actually carry the respective transcription factor binding sites, and not a single transcription factor binding site could be regarded as diagnostic for the whole group. The broadest coverage is attributable to the two forkhead-family transcription factors Foxj1 and Foxq1, binding site motifs for which are overrepresented in the group of affected genes ($p = 6.4\times10^{-14}$ for Foxj1 and $p = 1.99\times10^{-7}$ for Foxq1), but not in the group of unaffected genes. Yet, as with other transcription factor motifs, binding sites for Foxj1 and Foxq1 appear only in 69 and 62 of the 192 affected genes, respectively; 41 of these genes carry sites for both forkhead factors. Therefore, although Foxj1 and Foxq1 may represent good candidates to mediate part of the transcriptional response elicited by maternal diabetes, our results do not provide evidence for a unified transcriptional mechanism on the basis of shared transcription factor binding sites in a many NTD-associated genes.

Effects of Maternal Diabetes on Variability of Gene Expression

The discovery that known NTD genes are consistently decreased in their expression by maternal diabetes lends support to the idea that insufficient expression of these specific genes may represent the cause of NTDs in diabetic pregnancy (Chappell et al., 2009; Pavlinkova et al., 2009). However, from a mechanistic perspective, such consistent changes of gene expression are difficult to reconcile with a phenotype of incomplete penetrance. If genes with consistent change of expression were the cause for the phenotype, we would expect that the phenotype should occur in all individuals with the same extent of exposure. Yet this is not the case in any of the maternal diabetes models (Otani et al., 1991; Phelan et al., 1997; Machado et al., 2001; Pavlinkova et al., 2008, 2009). The low phenotype incidence therefore implies either that (1) specific changes in gene expression occur only in the limited number of embryos that are eventually afflicted with the birth defect, or that (2) in addition to changes in gene expression in all embryos, further factors contribute to diabetes-induced NTDs, and that these factors are variable between exposed embryos. Having observed no evidence to support the first possibility from microarray results for individual embryos with or without NTDs (Pavlinkova et al., 2009), we therefore reanalyzed our microarray data with particular focus on the variability of gene expression.

When we compared the variability of gene expression levels in embryos from diabetic dams to the variability in embryos from normal dams, we noticed that, in general, the diabetic

samples display a higher degree of variability (Fig. 1). This was detected regardless of absolute expression level, and irrespective of up- or down-regulation for any particular gene. The increase in variability was visualized by plotting the standard deviations associated with each gene probe (Fig. 1A), as well as by plotting the coefficient of variation (Fig. 1B). Statistical analyses of the distributions for the ln-transformed coefficient of variation demonstrate that the two data distributions are statistically significantly different from each other (Kolmogorv-Smirnov test, $p = 0.007$). In all our analyses, the diabetic dams had glucose levels exceeding 250 mg/dl at the start of pregnancy, and often exceeding 400 mg/dl by the time of sacrifice at gestational day 10.5 (Kappen et al., submitted). We found a comparable outcome in a separate analysis (Fig. 1C) of microarray data from an independent experiment (Pavlinkova et al., 2009; Kappen et al., 2010) where gene expression profiling was conducted on individual embryos from the same normal pregnancy and compared to individual embryos that were raised in the same diabetic pregnancy (Pavlinkova et al., 2009). In this dataset, gene expression in the diabetic embryos was also characterized by a higher degree of variability (data not shown). Taken together, these findings suggest that maternal diabetes not only alters absolute gene expression levels in the embryo, but also has the additional effect of increasing the variability in gene expression levels. Such an increase in variability would be consistent with the idea that the incomplete penetrance of NTDs in diabetic pregnancies could be due to increased variability of gene expression. This could explain why some embryos, while exposed to the same adverse uterine environment, exhibit defects, whereas others develop normally.

To determine whether the diabetes-elicited increase in the variability of gene expression was limited to these two gene expression-profiling experiments, or whether it was specifically associated with embryonic gene expression, we evaluated independent datasets from other diabetes-related gene expression profiling experiments. We first applied the same analytical approach to microarray data obtained at E10.5 from placentas of diabetic and normal pregnancies (Salbaum et al., in preparation). These placenta samples did not correspond to the embryonic samples used for the embryo gene expression profiling experiments, but were prepared from independent pregnancies. Comparison of the coefficient of variation for gene expression in diabetic and normal placentas revealed that the distribution of data from diabetic samples was shifted toward higher variability (Fig. 2A). We also analyzed a diabetes paradigm unrelated to embryonic development: Gene expression profiling data were obtained from a published experiment on human diabetic nephropathy (Baelde et al., 2004), where gene expression in normal human kidney glomeruli was compared to expression in glomeruli from human diabetic kidney. Expression data were analyzed for variability as described above. Similar to the experiments with embryos and placentas, the data distribution obtained from diabetic samples was shifted toward increased variability compared to normal samples (Fig. 2B). On the other hand, there was no evidence for increased variability in gene expression when we compared results from profiling of normal placentas at different gestational time points (Knox and Baker, 2008), nor from profiling of gene expression in normal and *Hox*-gene transgenic mouse cartilage from late-term mouse embryos (Kruger and Kappen, 2010). Taken together, these results indicate that increased variability of gene expression in diabetes-exposed samples is detectable in three separate tissues and is not limited to NTD-associated genes or to embryonic development. The fact that the same trend toward higher variability was found in experiments with diabetes-exposed embryos and placentas (our own work) and in results from human adult diabetic tissues, but not in other paradigms, suggests that increased variability of gene expression may be a general feature associated with the diabetic condition.

DISCUSSION

Maternal diabetes exerts a significant effect on embryonic gene expression. Among the deregulated genes is a large group of genes that were previously recognized to play a role for NTDs in genetic paradigms such as targeted mutations. We find that a distinct subgroup of known NTD-associated genes, with causal roles for NTDs, or genes with modifying functions, responded in their embryonic expression to maternal diabetes. This suggests that maternal diabetes does in fact elicit specific responses at the level of embryonic gene expression, but does not misregulate all NTD-associated genes. It is notable that all those NTD-associated genes affected by maternal diabetes showed a significant decrease in expression: a change in the same direction as the corresponding loss-of-function mutants. This suggests that thresholds (Grüneberg, 1952a; Fraser, 1980) of gene expression exist that are crucial for correct closure of the neural tube. Experimental control of gene expression levels, for example, with hypomorphic or conditional alleles, will be required to test this hypothesis.

Findings that maternal diabetes alters embryonic gene expression in different experimental paradigms have been reported by several laboratories (Phelan et al., 1997; Chang and Loeken, 1999; Chan et al., 2002; Reece et al., 2006; Kumar et al., 2007; Jiang et al., 2008; Pavlinkova et al., 2009) and for various stages of development under diabetic conditions. How these changes come about is not well understood, although specific transcription factors are thought to contribute. A parsimonious assumption is that transcriptional control mechanisms are affected, leading to the assumption that coordinated changes in expression across multiple genes could be attributable to shared transcriptional control mechanisms in such a group of genes (Pavlinkova et al., 2009; Kappen et al., 2010). Therefore, we would expect to find transcription factor binding sites that are common among the suite of coordinately deregulated genes; indeed, we detected, in a subset of genes, enrichment of motifs for transcription factors that regulate response to oxidative stress or hypoxia, including transcription factors of the forkhead family (Pavlinkova et al., 2009). For the suite of NTD-associated genes that respond to maternal diabetes and are the subject of this article, we also implicate fork-head transcription factors as mediators. Nevertheless, the presence of those potential transcription factor binding sites in only a fraction of the deregulated genes indicates that the overall transcriptional response is not based on readily identifiable or simple mechanisms. It therefore remains unclear whether preventive interventions could be devised, short of prevention of maternal diabetes itself, that act “upstream” of the gene regulation in the embryo to bring gene expression profiles back in line with normal development.

The alternative to “upstream” interventions would be to target interventions “downstream” of altered gene expression, with particular focus on the NTD-associated genes that are decreased in their expression. The identification of affected pathways (Pavlinkova et al., 2008) will prove useful toward such a strategy, as it substantially reduces the number of potential targets: from many deregulated genes to a small number of affected signaling pathways. It will now be important to identify the nodes of convergence for these pathways in diabetes-exposed embryos. Genes acting at sites of input into, and in integration of, the signaling pathways may represent gating points for the flow of signaling information. Therefore, any intervention focused at these particular genes would conceptually benefit from the reduced complexity compared to “upstream” interventions and may be able to buffer the detrimental effects of several dampened cellular signaling pathways simultaneously. Targeting of gating and convergence points and their downstream effectors is a strategy that can be tested experimentally.

In the effort to understand cause and etiology of maternal diabetes–induced birth defects, two cardinal problems remain: One is the question how a systemic condition, such as diabetes and the associated hyperglycemia, can cause morphogenetic defects in the embryo that are restricted to particular regions or tissues. This is difficult to reconcile with a systemic insult such as hyperglycemia, hypoxia, or oxidative stress, which, in theory, should exert its effects everywhere. The second question is how these phenotypes arise with incomplete penetrance despite exposure of all embryos in a litter. Particularly perplexing is that incomplete penetrance is found even among genetically identical individuals, such as in inbred mouse strains. We posit that a model to explain the etiology of maternal diabetes-induced birth defects on the basis of gene expression would have at least two components: (1) deregulation of gene expression and (2) increased variability of gene expression. The first component, deregulation of gene expression, leads to the consistent decease in expression of known NTD genes in all exposed embryos. Although this constitutes an increased susceptibility to NTDs, and this increased susceptibility is conferred to all exposed embryos, it is not sufficient to cause NTDs by itself. The second component, increased variability of gene expression, produces discrete differences between exposed embryos and interacts with the first component in that it represents the trigger for pathogenic events to happen. There are two possibilities how this interaction might occur: First, variability affects specific NTD genes directly by lowering their expression below a critical (but as of yet undefined) threshold in some embryos but not others. Consequently, only these embryos would suffer a NTD, resulting in incomplete penetrance. Second, variability affects expression of other genes that are not NTD genes themselves, but can interact with NTD genes and their pathways. This would lead to lower signaling output in some embryos but not others, again triggering pathogenesis with incomplete penetrance. Overall, the endogenous region-specific expression patterns of the affected genes represent the spatial confines where the interaction of the first two components of the model can lead to a pathogenic outcome. In this way, increased variability can explain the incomplete penetrance of specific NTDs in embryos exposed to the adverse conditions of a diabetic pregnancy.

Our analysis of various expression profiling data sets suggests that, in the respective paradigms (mouse embryo, mouse placenta, and human kidney), diabetes leads to an increase in the variability of gene expression, possibly by affecting the precision of gene regulation in general. Although this would be consistent with our model for maternal diabetes-elicited NTD etiology, it is important to note that the currently available gene-profiling surveys were never designed to capture variability of gene expression as an explicit experimental parameter. In fact, microarray experiments are typically structured to eliminate variability as a confounding element as much as possible, such as through the use of pooled samples. To directly measure the extent of variability of gene expression brought about by maternal diabetes, it would be necessary to conduct expression-profiling experiments with individual embryo samples, and with a higher number of samples for each side of the experimental paradigm. In this way, it would be possible to not only classify genes according to their change in expression, but also according to their change in variability of gene expression. Such experiments would define which genes exhibit increased variability in expression levels. According to our model, these would be candidate genes to trigger birth defect pathogenesis. Functional assays will then be required to test which genes of this “highly variable” group are able to interact with the “susceptibility” component-NTD genes with consistent change of expression in all exposed individuals.

How maternal diabetes would affect the regulatory precision is unclear as of yet. Control of transcription not only is exerted through the action of transcription factors at their target binding sites, but also involves chromatin structure and histone modification. In fact, several prominent histone modification factors are among the group of genes that are deregulated by

maternal diabetes (see Table 4). Therefore, we submit that the loss of regulatory precision may be mediated through alterations in the embryonic epigenome. *Ehmt2* (Tachibana et al., 2002; Wagschal et al., 2008), encoding a histone methyltransferase responsible for histone H3K9 methylation that serves as guidance for DNA methylation during development, and *Kat2a* (*Gcn5*), encoding a histone acetyltransferase (Yamauchi et al., 2000; Lin et al., 2008), are two NTD genes that show decreased expression (Table 1). Other such deregulated genes encode histone deacetylases, histone methyltransferases, and histone demethylases, consistent with the idea that the epigenome may be altered in the exposed embryos. Transcripts for all of these chromatin-modifying factors exhibit decreased expression in the embryo under conditions of maternal diabetes. Interestingly, these results encompass seemingly opposing chromatin-based transcriptional control mechanisms: Genes for enzymes regulating epigenetic modifications associated with activation of transcription (e.g., *Mll1, Setd1a*, or *Kdm1*, which affect histone H3 lysine 4 trimethylation) show a reduced expression, but at the same time, genes encoding proteins that regulate epigenetic modifications correlated with repression of transcription (e.g., *Ehmt2* and *Kdm4b*, which affect histone H3 lysine 9 trimethylation) are also decreased. There are two plausible scenarios: (1) A shift in the balance between activating and repressing mechanisms toward repression would explain why the majority of genes that are deregulated under conditions of maternal diabetes show decreased expression. (2) Generally decreased expression of chromatin-modifying factors could result in a net reduction of *both* activating and repressing epigenetic marks. This would cause loss of precision in the regulation of gene expression in the exposed embryo and could account for the increased variability in gene expression levels. Thus, the basis of pathogenic processes that ultimately lead to NTDs in diabetic pregnancy could be altered epigenetic mechanisms. With the recent finding that epigenetic modifications may be subject to dietary modulation (Waterland and Jirtle, 2003; Davison et al., 2009; Wellen et al., 2009), this opens new avenues for strategies that are aimed at preventing NTDs by targeting epigenetic mechanisms.

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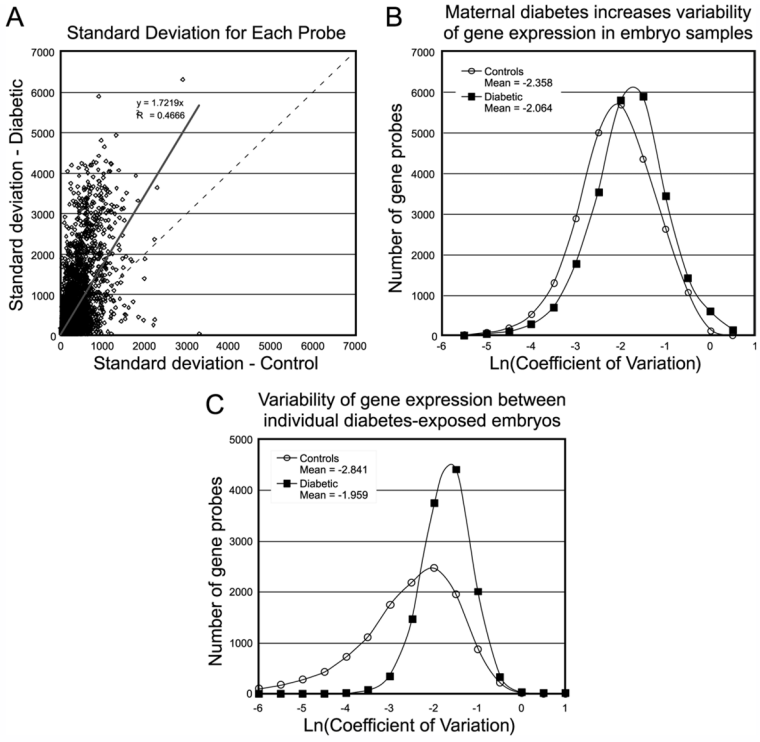

Figure 1.

Maternal diabetes increases the variability of gene expression levels in the developing embryo. (**A**) Standard deviations from the mean expression level were calculated for each gene probe on the microarray that showed a “Present” signal in all samples. Standard deviations were then plotted for control as well as diabetic samples; each data point represents a single gene probe and its standard deviations. The cloud of data points deviates from the diagonal (dotted black line) that depicts the hypothetical regression line for equal variability under both metabolic conditions. The blue solid line for the experimental results displays a shift toward the y-axis (diabetic condition), indicating that standard deviations are generally higher (on average by 1.72-fold) in embryo samples from diabetic dams. (**B**) Although the SD plot shows absolute values regardless of expression level of each gene, the coefficient of variation shows variability of gene expression normalized for the respective expression levels. After logarithmic transformation of the coefficient of variation for each gene probe, a histogram was obtained, which shows that the curve representing the results for embryos from diabetic dams is shifted to the right toward higher values. Again, this visualization indicates that the variability of gene expression levels is higher in embryos obtained from diabetic dams compared to embryos from control dams. These results were obtained from microarrays in which each sample consisted of pools of three embryos that each came from an independent diabetic or control pregnancy, respectively. (**C**) Variability of gene expression levels between individual embryos from the same control or the same diabetic pregnancy, respectively, following the same analysis and transformations as for panel B.

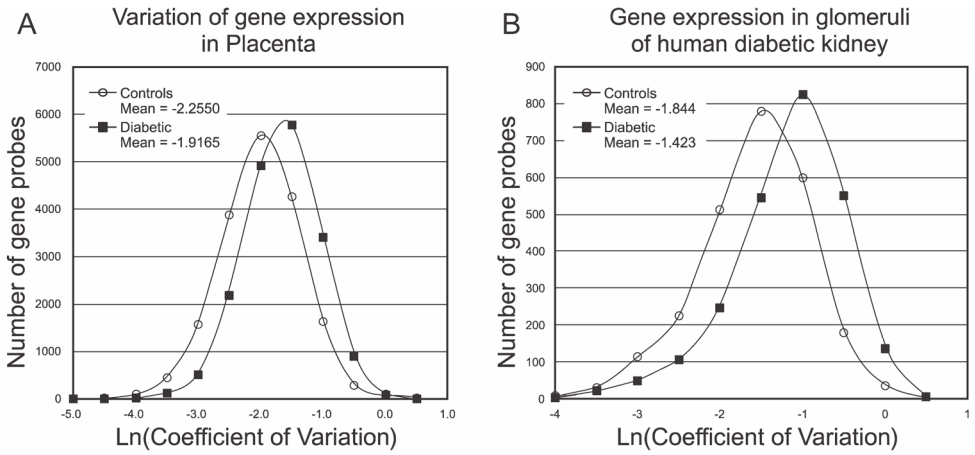

Figure 2.

Diabetes increases the variability of gene expression levels in other experimental paradigms. (**A**) Microarray data from gene expression profiling in placentas from normal compared to diabetic pregnancies (Salbaum and Kappen, unpublished data) were processed as shown in Figure 1B: the coefficient of variation was determined for each gene probe, and a histogram was obtained after logarithmic transformation. The curve representing the diabetic placenta samples was shifted to higher values, similar to the results obtained in embryos from diabetic pregnancies. (**B**) Publicly available microarray data from diabetic versus normal human kidney (GEO record GSE1009) were treated in the same fashion as described for embryonic or placental gene expression data. Similar to our own datasets, the curve representing the coefficients of variation for the diabetic samples is shifted toward higher values, again implying that the variability of gene expression levels is higher in diabetic samples compared to control samples.

Table 1

Neural Tube Defect-Associated Genes

Abi1[HJ]
Abl1[1,4,HJ]
Abl2[1,4,HJ]
Acvr1b[6]
Adnp[1,9]
Adsl[HJ]
Aldh1a2[1,9,HJ]
Alx1[1,2,5,9,HJ]
Alx4[5]
Ambra1[1,5,9,10]
Apaf1[1,4,5,9,10,11,HJ]
Aph1a[8]
Apob[5,HJ]
Apoh[HJ]
Arid3b[12]
Arl13b[HJ]
Arnt[HJ]
Atp2c1[1,5,9]
Axin1[1,7,8,HJ]
Bard1[1,9]
Bat3[5]
Bbs4[HJ]
Bcl10[1,5,9,HJ]
Bcl2l1[5]
Bmp2[1,5,7,9]
Bmp4[6]
Bmp5[HJ]
Bmp7[5,HJ]
Bmper[5]
Brca1[1,5,9,10,HJ]
Brca2[1,5,9]
Brd2[1]
C2cd3[5]
Calr[1,5,9,HJ]

Card10[2]

Casp3[5,HJ]

Casp7[5]

Casp8[8,12]

Casp9[1,5,HJ]

Ccnf[1,8]

Cdc42[6]

Cdh2[12]

Cdon[6]

Cecr2[5,HJ]

Celsr1[1,3,7,HJ]

Cfl1[1,9,HJ]

Chrd[6]

Chuk[1,9,HJ]

Cited2[1,5,9,HJ]

Cobl[5,HJ]

Coq7[1,9]

Crebbp[1,5,9,HJ]

Csda[5]

Csk[1,7,9,12,HJ]

Csnk2a1[1]

Ctbp1[1]

Ctbp2[1]

Cthrc1[1]

Ctnnbip1[5,HJ]

Cycs[5,HJ]

Cyp26a1[1,5,9,10,HJ]

Cyp26c1[1,9]

Deaf1[1,5,9,HJ]

Dlc1[1,9,HJ]

Dll3[8]

Dlx5[1,2,5,9,HJ]

Dlx6[1,2,5,9]

Tgif1[5,6]

Dnmt1[1,9]

Dnmt3b[1,9,HJ]

Dnmt3l[1,5,9,HJ]

Dvl1[1,3,5,9,HJ]

Dvl2[1,3,5,9,HJ]

Dvl3[1,3,5]

Dync2h1[5,HJ]

Dync2li1[1,9,HJ]

E2f1[5]

Efna5[1,2,7,HJ]

Ehmt1[1,9]

Ehmt2[1,9]

Eif2c2[1,9,12,HJ]

Enah[1,2,5,7,HJ]

Enpp2[1,8,9,12,HJ]

Ep300[1,5,8,9,HJ]

Epha7[2,HJ]

Epor[HJ]

Erf[1,9]

Evl[5]

Fam48a[1,5,9,10,HJ]

Fat1[6]

Fbxw7[1,9]

Fdft1[1,HJ]

Fen1[1,9]

Fgfr1[1,3,8,9,10,HJ]

Fign[1,9]

Fkbp1a[1,5,9,HJ]

Fkbp8[1,5,9,10,HJ]

Flrt3[1]

Fn1[8]

Folr1[1,9,HJ]

Foxa2[8]

Foxb1[1,9,HJ]

Foxc1[1,9,HJ]

Foxc2[1,8,9,10,11,HJ]

Foxg1[6]

Foxh1[6]

Frem1[HJ]

Frem2[1,9,HJ]

Furin[5,12]

Fzd3[1,7,9,HJ]

Fzd6[1,9,HJ]

Gadd45a[5,HJ]

Gas1[6]

Gatad2a[1,7]

Gbx2[5]

Gdf1[6]

Gdf7[1,9]

Gja1[HJ]

Gja5[HJ]

Gli2[1,5]

Gli3[1,4,5,7,12,HJ]

Glra1[HJ]

Gpr161[1,9,10,HJ]

Grhl3[1,5,9,10,HJ]

Grip1[1,9,HJ]

Grlf1[1,5,HJ]

Gtf2i[HJ]

Gtf2ird1[HJ]

Hdac4[5,HJ]

Hdac8[5]

Hectd1[5,HJ]

Hes1[1,2,5,7,8,HJ]

Hes3[5]

Hgs[1,9,12]

Hhat[6]

Ttc21b[HJ]

Hhip[1,5,7,9,10]

Hic1[5,6,HJ]

Hif1a[1,9,HJ]

Hipk1[5,HJ]

Hipk2[5,HJ]

Hira[1,8,9]

Hmx1[5]

Hoxa1[1,4]

Hsd17b2[5]

Hspg2[5,HJ]

Htt[5]

Hus1[1,7,8]

Ift172[1,5,6,9,HJ]

Ift57[1,5,7,9]

Ift88[1,5,8,9,12]

Ikbkap[1,7]

Ikbkb[1,9,HJ]

Inpp5e[2,5]

Ipmk[1,8,9]

Itga3[1,HJ]

Itga5[8]

Itga6[1,HJ]

Itgb1[1,5,8,9,10,12,HJ]

Itpk1[1,5,9,10]

Jag1[8]

Jarid2[1,4,7,HJ]

Jmjd6[5,HJ]

Kat2a[1,5,7,9]

Kif3a[1,9,10,11,12]

Kif3b[1,5,9,HJ]

Kif7[5]

Kitl[10]

Klkb1[HJ]

Lama5[1,4,5,HJ]

Lamc1[1,5]

Lats2[12]

Lbx1[1,9]

Lbx2[1,5,9]

Ldb1[8]

Lmo4[1,2,5,9,10,HJ]

Lmx1a[1,9,10]

Lpar1[5,HJ]

Lrp2[1,4,5,6]

Lrp6[1,5,9,10,HJ]

Luzp1[1,5,9]

Map3k4[1,3,5,9,10,HJ]

Map3k7[1,9,12,HJ]

Mapk8[1,5,7,HJ]

Mapk9[1,5,7,HJ]

Marcks[1,5,9,HJ]

Marcksl1[1,5,9,10,HJ]

Mdfi[1,4,9,10,12]

Mdm2[5]

Mdm4[1,5,HJ]

Med24[1,9]

Men1[1,5,HJ]

Mesp1[1,4]

Mesp2[1,9,10]

Mib1[8]

Mib2[5]

Mkks[5]

Mll2[1,8,9]

Msgn1[1,8,HJ]

Msx1[1,5,9,10,HJ]

Msx2[1,5,9,10,HJ]

Mttp[5,HJ]

Myc[1,9]

Vangl1[3]

Nap1l2[HJ]

Nat2[1]

Nck1[1,7,12]

Nck2[1,7,12]

Nckap1[1,9]

Ncstn[8]

Ndst1[6]

Ndst3[6]

Neurog2[HJ]

Nf1[5,HJ]

Nodal[6]

Nog[1,5,6,7,8,9,10,11,12,HJ]

Notch1[1,8,9]

Notch4[1,9]

Nphp3[5]

Nr6a1[1]

Nuak2[5]

Numb[1,9,HJ]

Nup133[5,8]

Nup50[5,8,HJ]

Odz4[1,8,9]

Otx2[1,2,5,6,9]

Ovol2[1,9,HJ]

Palld[1,5,9,HJ]

Pax1[1,9,10,11]

Pax2[1,4,5,7,HJ]

Pax3[1,5,7,9,10,HJ]

Pax6[5]

Pdgfa[1,9,10,12]

Pdgfc[1,9,10,11,12,HJ]

Pdgfra[1,7,9,10,12,HJ]

Pdgfrb[1,9]

Pfn1[1,2,5,7,HJ]

Phactr4[1,5,9,12]

Phgdh[5]

Piga[5,HJ]

Pip5k1c[1,5,7]

Pitx2[5,HJ]

Pkd1[1,9,10,11,HJ]

Plxnb2[1,5,7,9,10]

Pnpla6[1]

Pofut1[8]

Por[1,5,7,HJ]

Ppap2b[1,8,9]

Prkaca[1,5,9,10,HJ]

Prkacb[1,5,9,10,HJ]

Prrx1[1,9,10,HJ]

Prrx2[1,9,10]

Psen1[1,4,8]

Psen2[1,4,8]

Ptch1[1,5,6,7,9,10,HJ]

Pten[1,8,9,12]

Ptk7[1,3,9,10,HJ]

Ptpn11[1,8,9,HJ]

Ptpn12[8]

Ptpn9[5,HJ]

Ptprf[5]

Ptprs[5]

Pygo1[5]

Pygo2[5]

Qk[1,9,12]

Rab23[1,5,9,10,11,HJ]

Rapgef2[1,2,9,10,12]

Rara[HJ]

Rarg[HJ]

Rax[1,4]

Rbpj[HJ]

Xbp1[12]

Rere[1,9,HJ]

Rgma[1,5,7,HJ]

Rpgrip1l[5]

Rpl24[5,HJ]

Rybp[1,4,5,HJ]

Sall1[1,5,9,HJ]

Sall2[1,5,9]

Sall4[1,5,9,HJ]

Scrib[1,3,9,10,HJ]

Scube1[1,5]

Serpinh1[1,4]

Sfrp1[1,3,9,10]

Sfrp2[1,3,9,10]

Sfrp5[1,3,9,10]

Shb[1,9]

Shh[1,5,6,9,10]

Shroom3[1,5,9,12,HJ]

Sirt1[5,HJ]

Ski[1,5,9,HJ]

Slc25a19[1,5,9,12]

Slc31a1[1,4]

Slc39a4[5]

Slc40a1[1]

Smad1[1,7]

Smad2[5,6]

Smad3[6]

Smad5[1,4,5,7,9,HJ]

Smarca4[HJ]

Smarcc1[5,HJ]

Smo[1,5]

Smurf1[1,5,9,10]

Smurf2[1,5,9,10]

Snai1[1,9]

Snai2[1,9]

Snx1[1,5,HJ]

Snx13[1,9]

Snx2[1,5,HJ]

Sobp[HJ]

Sox10[1,9]

Sp8[1,5,9,10,HJ]

Sphk1[1,5,HJ]

Sphk2[1,5,HJ]

Spry2[6]

Spry4[6]

Ss18[1,8,9]

Stil[1,4,6]

Stk11[1]

Strap[1]

Sufu[1,9,HJ]

Suz12[1,9,10]

T[8,HJ]

Tbx6[8]

Tcf12[5]

Tcf3[1,9,10]

Tcfap2a[1,2,5,7,9,HJ]

Tcof1[1,4,5,9,HJ]

Tctn1[6]

Tdgf1[6]

Tead1[1,5,9]

Tead2[1,5,9]

Terc[1,9,HJ]

Tfb1m[1,9]

Tfdp1[5]

Tfrc[8]

Tgfb1[1,9]

Tgfb2[1,9,10,11,HJ]

Tgfbr1[8]

Zfp568[1,7,9]

Tnfrsf1a[1,9]

Traf4[1,9,10,11,HJ]

Traf6[HJ]

Trp53[1,5,9,HJ]

Trpm6[1,5,9,10,11,HJ]

Tsc1[1,5,9,HJ]

Tsc2[1,9,HJ]

Tulp3[1,5,7,9,10,11,HJ]

Twist1[1,5,9,HJ]

Twsg1[6]

Txn2[1,5,7,HJ]

Ubr1[8]

Ubr2[8]

Unc5b[1,7]

Vangl2[1,3,4,5,9,10,HJ]

Vasp[5,HJ]

Vax1[6]

Vcl[1,7,12,HJ]

Vps26a[5]

Wasl[12]

Wnt3a[1,8,9,10,HJ]

Xrcc2[1,5,HJ]

Ybx1[1,5,7,HJ]

Yy1[5]

Zeb1[1,5,9,HJ]

Zeb2[1,5,9]

Zfp148[1,9]

Zfp36l1[1,9,HJ]

Zic1[HJ]

Zic2[1,2,5,6,9,10,HJ]

Zic3[1,2,4,5,9,HJ]

Zic5[1,5,9,HJ]

NTD-associated genes were compiled from the MGI database and from the literature. Underlined gene names are modifiers of NTD phenotypes, but do not cause NTDs directly.

Database search terms are represented as follows:

[1] abnormal neural tube closure;

[2] anencephaly;

[3] craniorachischisis;

[4] delayed neural tube closure;

[5] exencephaly;

[6] holoprosencephaly;

[7] incomplete cephalic closure;

[8] kinked neural tube;

[9] open neural tube;

[10] spina bifida;

[11] spina bifida occulta;

[12] wavy neural tube;

[HJ] annotated in Harris and Juriloff (2007).

Table 2

NTD-Associated Genes with More than Twofold Change in Expression Between Diabetes-Exposed and Control Embryos

Gene	*p* value	Fold
Apaf1	0.001769	−5.76
Axin1	0.000067	−2.37
Bat3	0.000717	−4.05
Bbs4	0.004033	−3.67
Bcl2l1	0.033851	−8.09
Brca1	0.002077	−6.28
Brd2	0.001314	−2.44
C2cd3	0.006726	−2.08
Casp3	0.016579	−10.99
Casp7	0.022795	−2.22
Cdon	0.013209	−6.20
Celsr1	0.000041	−3.61
Cited2	0.012445	−2.31
Csk	0.003739	−2.59
Ctbp2	0.001518	−2.14
Dlc1	0.016911	−4.75
Dll3	0.002935	−2.26
Dnmt3b	0.002354	−2.55
Dvl1	0.000090	−2.24
Dvl2	0.000352	−3.04
Efna5	0.000862	−2.04
Ehmt2	0.000635	−4.30
Enah	0.014821	−2.40
Epha7	0.004288	−4.11
Evl	0.000723	−2.23
Fam48a	0.010687	−3.29
Fbxw7	0.031085	−2.02
Fgfr1	0.000050	−2.28
Fign	0.009486	−3.41
Foxb1	0.010951	−2.06
Foxc1	0.000008	−2.95
Foxg1	0.000055	−2.91
Gas1	0.000107	−2.21
Gatad2a	0.002907	−5.35
Gja1	0.031859	−8.25
Gli2	0.000084	−2.19
Gli3	0.026003	−7.70
Glra1	0.021907	−2.30

Gene	*p* value	Fold
Grip1	0.021470	−2.95
Gtf2i	0.005501	−12.12
Gtf2ird1	0.001476	−2.26
Hic1	0.023267	−3.02
Hif1α	0.018226	−22.58
Hipk2	0.022998	−2.70
Hira	0.000969	−3.85
Hmx1	0.008225	−2.07
Hspg2	0.000459	−2.30
Ikbkb	0.002197	−13.23
Itga3	0.002004	−14.42
Itga6	0.010260	−4.62
Itgb1	0.030799	−2.39
Jarid2	0.003987	−2.39
Kat2a	0.003468	−3.70
Kif3a	0.014521	−2.49
Kif3b	0.004527	−4.39
Kitl	0.008918	−2.30
Lama5	0.000742	−3.60
Lats2	0.008473	−2.26
Lbx1	0.002760	−2.96
Ldb1	0.004923	−4.22
Lpar1	0.046567	−3.35
Map3k4	0.019136	−5.00
Map3k7	0.001850	−2.53
Mapk8	0.011017	−3.85
Mapk9	0.010986	−2.34
Mdm2	0.000886	−5.40
Men1	0.000063	−2.37
Msx1	0.000197	−2.34
Nck2	0.008556	−3.81
Ndst1	0.000360	−2.07
Neurog2	0.000747	−2.05
Nf1	0.008377	−3.43
Notch1	0.000646	−3.64
Nr6a1	0.025669	−2.75
Numb	0.002260	−6.62
Nup133	0.004533	−3.38
Nup50	0.011223	−4.85
Odz4	0.000050	−2.32
Pax6	0.003037	−8.54
Pdgfra	0.024074	−3.46

Gene	*p* value	Fold
Pdgfrb	0.001263	−2.34
Piga	0.008180	−5.32
Pkd1	0.001758	−2.06
Plxnb2	0.009202	−2.33
Prrx1	0.004261	−4.74
Psen1	0.009795	−2.56
Pten	0.005512	−2.17
Ptpn12	0.002044	−3.86
Ptprf	0.000039	−2.76
Ptprs	0.000557	−2.33
Rab23	0.024663	−7.78
Rara	0.029550	−3.73
Rbpj	0.005694	−2.65
Rgma	0.004018	−3.38
Sall1	0.005773	−6.30
Scrib	0.000070	−3.22
Sfrp1	0.002989	−2.41
Shb	0.000265	−3.26
Shh	0.003495	−14.22
Shroom3	0.000153	−2.17
Ski	0.000002	−2.49
Smad1	0.034504	−2.92
Smarcc1	0.028614	−13.37
Smo	0.000290	−4.22
Smurf2	0.003683	−4.01
Sox10	0.000842	−2.09
Sp8	0.002138	−2.17
Ss18	0.003118	−3.46
Stk11	0.001391	−2.29
Suz12	0.001322	−3.93
Tcf12	0.002147	−3.80
Tcof1	0.009709	−7.50
Tfrc	0.037479	−3.82
Tgfb1	0.005918	−5.19
Tgfb2	0.022009	−2.28
Tgfbr1	0.037189	−79.00
Traf6	0.012046	−2.20
Trp53	0.044230	−2.98
Tsc2	0.002076	−4.52
Twsg1	0.008026	−10.81
Ubr1	0.001154	−6.38
Vangl2	0.025849	−4.37

Gene	*p* value	Fold
Vcl	0.024067	−22.66
Ybx1	0.005300	−15.80
Yy1	0.006151	−2.28
Zeb2	0.001213	−2.41
Zfp36l1	0.003150	−4.27
Zic5	0.006330	−7.02

NTD-associated genes with expression changes in excess of twofold as response to maternal diabetes. *P* values were obtained from the statistical evaluation of the microarrays (Pavlinkova et al., 2009), and fold change was computed on the basis of array data.

Table 3

Pathways of NTD-Associated Genes Deregulated in the Embryo by Maternal Diabetes

Pathway	*p* value	Enrichment	Genes
Wnt signaling	1.0×10^{-8}	5.2	*Axin1, Crebbp, Csnk2a1, Ctbp2, Ctnnbip1, Dvl1, Dvl2, Lrp6, Map3k7, Mapk8, Mapk9, Prkaca, Prkacb, Psen1, Sfrp1, Tcf3, Trp53, Vangl1, Vangl2*
Hedgehog signaling	4.9×10^{-7}	8.2	*Gas1, Gli2, Gli3, Hhip, Lrp2, Prkaca, Prkacb, Rab23, Shh, Smo, Sufu*
Notch signaling	1.4×10^{-6}	8.6	*Crebbp, Ctbp2, Dll3, Dvl1, Dvl2, Notch1, Notch4, Numb, Psen1, Rbpj*
MAPK signaling	1.0×10^{-5}	3.1	*Casp3, Cdc42, Chuk, Fgfr1, Ikbkb, Map3k4, Map3k7, Mapk8, Mapk9, Nf1, Pdgfra, Pdgfrb, Prkaca, Prkacb, Rapgef2, Tgfb1, Tgfb2, Tgfbr1, Traf6, Trp53*
Focal adhesion	6.7×10^{-5}	3.3	*Cdc42, Itga3, Itga5, Itga6, Itgb1, Lama5, Lamc1, Mapk8, Mapk9, Pdgfc, Pdgfra, Pdgfrb, Pip5k1c, Pten, Vasp, Vcl*
Unaffected in the embryo by maternal diabetes			
TGFβ signaling	1.3×10^{-10}	9.7	*Acvr1b, Bmp2, Bmp4, Bmp5, Bmp7, Chrd, Gdf7, Myc, Nodal, Nog, Pitx2, Smad2, Smad3, Smad5, Tfdp1*

Results from DAVID pathway analyses on NTD-associated genes. *P* values indicating statistical significance and enrichment factors were obtained from DAVID software.

Table 4

Embryonic Genes for Chromatin-Modifying Factors Affected by Maternal Diabetes

Gene	Gene title/function	*p* value	Fold change
Chd3	Chromodomain helicase DNA binding protein 3	0.00062	−3.41
Dot1l	DOT1-like, histone H3 methyltransferase, *H3K79*	0.022	−2.74
Ehmt2	Euchromatic histone-lysine N-methyltransferase 2, G9a, *H3K9*	0.00064	−4.30
Hdac5	Histone deacetylase 5	0.0041	−2.48
Hdac6	Histone deacetylase 6	0.0017	−1.88
Hdac7	Histone deacetylase 7	0.012	−2.97
Kat2a	K(lysine) acetyltransferase 2A	0.0035	−3.70
Kdm1	Lysine (K)-specific demethylase 1, *H3K4*	0.0022	−2.14
Kdm2a	Lysine (K)-specific demethylase 2A	0.0014	−2.14
Kdm4b	Lysine (K)-specific demethylase 4B, *H3K9*	0.0009	−2.55
Kdm6a	lysine (K)-specific demethylase 6A	0.019	−2.05
Kdm6b	Lysine (K)-specific demethylase 6B, *H3K27*	0.0088	−7.64
Mll1	Trithorax; histone-lysine N-methyltransferase, *H3K4*	0.0029	−2.46
Ring1	Component of polycomb repressive complex 1	0.010	−2.04
Setd1a	Histone-lysine N-methyltransferase, *H3K4*	0.00003	−3.60
Suv420h1	Histone-lysine N-methyltransferase, *H4K20*	0.005	−4.18
Suz12	Component of polycomb repressive complex 2	0.0013	−3.93

Genes for chromatin-modifying factors that are changed in their embryonic expression by maternal diabetes *P* value and fold change refer to the results obtained in microarray gene expression profiling.

G9a = traditional name for *Ehmt2; H3K4* = histone H3 lysine 4; *H3K9* = histone H3 lysine 9; *H3K27* = histone H3 lysine 27; *H3K79* = histone H3 lysine 79; *H4K20* = histone H4 lysine 20.

Table 5

p300 Enhancers within a 100 kb Envelope around a Gene

	p300 enhancer-forebrain	Genes with forebrain enhancer	p300 enhancer-midbrain	Genes with midbrain enhancer
Affected genes	58	38	18	15
Unaffected genes	39	31	11	11
Total	97	69	29	26

Presence of enhancer sequences characterized by binding of the p300 cofactor within a 50 kb envelope around NTD-associated genes. The table lists for affected NTD-associated genes, unaffected NTD-associated genes, and all NTD-associated genes (1) the total number of forebrain enhancers found, (2) the number of genes where those forebrain enhancers were detected, (3) the total number of midbrain enhancers found, and (4) the number of genes where those enhancers were detected.