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Imprinted gene expression in fetal growth and development

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ABSTRACT

Experimental studies showed that genomic imprinting is fundamental in fetoplacental development by timely regulating the expression of the imprinted genes to overlook a set of events determining placenta implantation, growth and embryogenesis.

We examined the expression profile of 22 imprinted genes which have been linked to pregnancy abnormalities that may ultimately influence childhood development. The study was conducted in a subset of 106 placenta samples, overrepresented with small and large for gestational age cases, from the Rhode Island Child Health Study.

We investigated associations between imprinted gene expression and three fetal development parameters: newborn head circumference, birth weight, and size for gestational age. Results from our investigation show that the maternally imprinted/paternally expressed gene *ZNF331* inversely associates with each parameter to drive smaller fetal size, while paternally imprinted/maternally expressed gene *SLC22A18* directly associates with the newborn head circumference promoting growth. Multidimensional Scaling analysis revealed two clusters within the 22 imprinted genes which are independently associated with fetoplacental development. Our data suggest that cluster 1 genes work by assuring cell growth and tissue development, while cluster 2 genes act by coordinating these processes. Results from this epidemiologic study offer solid support for the key role of imprinting in fetoplacental development.

1. Introduction

The role of imprinted genes in fetoplacental development has been widely reported in the literature [1,2]. Genomic imprinting is predicted to involve only about 1% (~200 genes) of the expressed genome with only about 90 imprinted genes that are well characterized at present [3–5]. This gene set shares the unique characteristic of expressing from only one of two parental alleles in a parental specific fashion. Silencing of the inactive allele is thought be achieved by epigenetic mechanisms including DNA methylation, histone modification and long non-protein-coding RNAs (lncRNAs) that act upon specific imprinting control regions (ICRs) or ICR-like elements within promoters and enhancers [6,7]. Imprinted genes often aggregate in clusters under the control of specific ICRs; single imprinted genes regulated by dedicated ICR-like elements are however not infrequent [6]. Interestingly imprinted genes are functionally haploid in those tissues/organs that perpetuate the imprinting epigenetic signal while they otherwise behave as normal diploid genes.

Imprinted genes, as indicated by the Ingenuity Pathway Analysis (IPA) (Ingenuity® Systems, www.ingenuity.com), belong to gene networks critical for the proper cellular and organ development; perturbations of these networks have been associated with developmental, neurological, endocrine and muscular disorders as well as cancer (IPA) (Table 1). Consistently, by using animal models, imprinted gene have been shown to: 1) regulate the exchange of resources between mother and fetus; 2)

Abbreviations: AGA, Appropriate for Gestational Age; ICR, Imprinting Control Region; IPA, Ingenuity Pathway Analysis; IUGR, Intrauterine Growth Restriction; LGA, Large for Gestational Age; MDS, Multidimensional Scaling; RCN, RNA Copy Number; RICHS, Rhode Island Child Health Study; SGA, Small for Gestational Age.

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Table 1
List of 22 candidate imprinted genes and their putative functions by network analysis.[a]

Gene Network[b]	Networks	
	Functional areas	Associated disease
1. *DLK1*, *H19*, *HOXD10*, *IGF2*, *NNAT*, *TP73*	Cellular growth and proliferation	Developmental disorders Genetic disorders
2. *HOXA11*, *PEG3*, *SNRPN*	Nervous system development and function	Cancer Skeletal and muscular disorders Reproductive system diseases
3. *CD44*, *EPS15*, *ILK*	Cellular development Cell cycle	Cardiovascular diseases
4. *CCDC86*, *CDKAL1*	Small molecule biochemistry	Gastrointestinal diseases Hepatic system diseases
5. *MEST*, *PLAGL1*	Gene expression	Cancer Connective tissue disorders
6. *DHCR24*, *PEG10*, *ZNF331*	Cell cycle Cellular development	Cancer
7. *PHLDA2*	Organ development Respiratory system development and function Cellular assembly and organization	–
8. *MEG3*	Connective tissue development and function	Behavioral syndromes
9. *SLC22A18*	–	Cancer, developmental disorders

[a] Elaborated by using the Ingenuity Pathway Analysis informatics tool.
[b] Networks includes several other not imprinted genes which are not reported here for ease of reading.

program the metabolism in the early postnatal period to determine growth and metabolic phenotype; and 3) participate in the development of metabolically important organs such as the pituitary, pancreas, liver, fat, the hypothalamus and the placenta [8,9].

Additionally, imprinted gene expression demonstrated low transcriptional noise, as shown in placenta [10]. This finding is in agreement with the imprinted genes functional importance that, as shown for other such genes [11,12], once altered, can lead to prominent phenotypic changes [13,14] such as lethality [15,16] that is further enhanced by their constitutional haploinsufficiency [17].

In this framework the placenta is considered as the main determinant of the fetal phenotype and therefore represents the appropriate tissue for analyzing the imprinted gene expression profile in relation to growth and developmental outcomes. The placenta indeed: 1) supplies the much needed fetomaternal interface, and functions as immune and endocrine organ [18,19]; 2) overlooks and coordinates the embryonic growth [20]; 3) synchronises with the brain development by the coordinated expression of many imprinted genes [21,22]; and 4) supplies serotonin to the developing brain to support neuronal differentiation [23,24]. The placenta is also of predominantly fetal origin as it originates from the outer layer of the blastocyst [25] and therefore provides a unique snapshot of the fetal epi/genetic status.

Expression profiling of the placenta has already provided hints about the activity of imprinted genes in correlation with pregnancy outcomes such as intrauterine growth restriction (IUGR) [10,26] and preeclampsia (PE) [27]. These disorders of pregnancy have been independently linked to chronic and developmental abnormalities in children as mostly neurodevelopmental, consistent with the Barker hypothesis [28–30]. These findings are supportive of the theory that links perturbations of imprinting regulation in the placenta to chronic and developmental disorders through an altered fetal phenotype. Nevertheless the correlation between imprinted gene expression and fetal growth has been previously tested only in a small study by our group with limited information on the newborns [10]. In the current study, we analyzed the expression of a panel of imprinted genes (Table 1) in 106 human term placenta samples from a birth cohort of infants, the Rhode Island Child Health Study (RICHS) and examined the correlation between imprinted gene expression and fetal development.

2. Material and methods

2.1. Study population

Placenta samples were collected in the framework of the RICHS, which enrolls mother–infant pairs at Women and Infants Hospital of Rhode Island. Every term small for gestational age (SGA) (<10th percentile) and large for gestational age (LGA) (>90th percentile) infant enrolled, as calculated from the Fenton growth chart based on birth weight and gestational age [31], was matched for gender, gestational age (±3 days), and maternal age (±2 years) with one appropriate for gestational age (AGA) newborn.

Exclusion criteria adopted were: multiple pregnancies, maternal age <18 years, life-threatening medical complications of the mother, and congenital or chromosomal abnormalities of the infant. Data on maternal ethnicity, age and insurance were obtained through both a structured chart review and an interviewer-administered questionnaire. Data on gestational week, delivery method, infant gender, head circumference, birth weight and size for gestational age were abstracted from charts. For this study, the first 106 subjects enrolled between September 2009 and May 2010, were selected. All subjects were consented accordingly to the specific protocol approved by the Institutional Review Boards for Women and Infants' Hospital and Brown University.

2.2. Placental tissue collection and RNA isolation

Placental tissue was biopsied from each of the 4 placenta quadrants midway from the cord insertion and the placental rim, within 2 h from the delivery. The maternal decidua was then excised and the biopsies placed in RNAlater (Qiagen – Valencia, CA, USA) for 72 h at 4 °C. Tissue was then blotted dry, snap-frozen in liquid nitrogen, homogenized with mortar and pestle and stored in ultrafreezer at −80 °C. RNA was later extracted by using the RNeasy kit (Qiagen – Valencia, CA, USA) supplemented by double DNase I (Qiagen – Valencia, CA, USA) on column digestion in order to clear any DNA contamination. Extracted RNA was finally quantified with Nanodrop spectrophotometer and stored at −80 °C.

2.3. Gene expression analysis

The list of imprinted genes was populated by consolidating data of 3 previous experiments: 1) the analysis of the expression of 52 imprinted genes expressed in placenta from pregnancies diagnosed with severe intrauterine growth restriction (IUGR) which returned 9 dysregulated genes [10]; 2) the investigation of the loss of genomic imprinting (LOI) profile at the RNA level in 22 severe IUGR placentas by using a quantitative assay that we developed [10,32]; 3) a test we ran on the expression of 2 homeobox genes in a subset of 60 samples from the RICHS cohort (unpublished data) (see Table 1).

Gene expression was measured by the Mount Sinai School of Medicine Real-Time PCR facility using quantitative real-time PCR (qRT-PCR) with a robotized