Associations between imprinted gene expression in the placenta, human fetal growth, and preeclampsia

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Abstract

Genomic imprinting is essential for normal placental and fetal growth. One theory to explain the evolution of imprinting is the kinship theory (KT), which predicts that genes that are paternally expressed will promote fetal growth, whereas maternally expressed genes will suppress growth. We investigated the expression of imprinted genes using microarray measurements of expression in term placentae. Correlations between birthweight and the expression levels of imprinted genes were more significant than for non-imprinted genes, but did not tend to be positive for paternally expressed genes and negative for maternally expressed genes. Imprinted genes were more dysregulated in preeclampsia (a disorder associated with placental insufficiency) than randomly-selected genes, and we observed an excess of patterns of dysregulation in preeclampsia that would be expected to reduce nutrient allocation to the fetus, given the predictions of the KT. However, we found no evidence of coordinated regulation among these imprinted genes. A few imprinted genes have previously been shown to be associated with fetal growth and preeclampsia, and our results indicate that this is true for a broader set of imprinted genes.

Keywords: placenta, imprinting, preeclampsia, intrauterine growth restriction, kinship theory

Introduction

Genomic imprinting is a phenomenon where the expression of an allele depends on whether it was inherited from the mother or the father. Many known imprinted genes in humans are expressed in the placenta and are essential for its normal function [1,2]. One theory to explain imprinting is the parental conflict theory, or kinship theory (KT), which proposes that there is a potential genetic conflict of interest between maternally and paternally inherited alleles [3,4]. Because of the possibility of multiple paternity, maternally inherited alleles are more likely to be related to alleles found in siblings who share a mother than are paternally inherited alleles. Therefore, paternally expressed genes (PEGs) are expected to promote the growth of the fetus, whereas maternally expressed genes (MEGs) are expected to constrain fetal growth to conserve resources so that they can be allocated more equally among a fetus and its siblings [1,5].

The expression levels of some imprinted genes have been correlated with fetal growth [6] and pregnancy complications associated with placental insufficiency such as preeclampsia (PE) and intrauterine growth restriction (IUGR) [5,7,8]. However, no study has systematically compared the expression of imprinted and non-imprinted genes in terms of associations with birthweight or pregnancy complications. Since the KT posits that imprinted genes are involved in nutrient allocation, this theory predicts that the placental expression of imprinted genes will be more strongly correlated with birthweight than non-imprinted genes. Imprinted genes are also expected to be dysregulated more than non-imprinted genes in PE and IUGR placentae, where nutrient delivery to the fetus is thought to be impaired. Furthermore, the KT predicts that increased expression of MEGs and decreased expression of PEGs will limit maternal investment in the fetus, whereas the opposite patterns will promote investment. The expression of imprinted genes might drive fetal growth and/or contribute to placental pathology, but imprinted genes

might also take part in compensation for poor fetal growth and/or placental pathology, in which case the opposite expression patterns would be observed (Fig. 1). Furthermore, the expression of PEGs might counterbalance the expression of MEGs and vice versa, in which case no correlation between gene expression and birthweight or pathology would be observed (Fig. 1).

We used microarray measurements of gene expression in term placentae to test multiple predictions that follow from the KT. We tested (1) whether imprinted genes are more strongly correlated with birthweight than non-imprinted genes, (2) whether imprinted genes are more dysregulated in PE and IUGR than non-imprinted genes, and (3) whether there is an excess of patterns of expression expected to drive fetal growth/ contribute to PE, or compensate for poor placental growth/placental insufficiency in PE (Fig. 1). We also hypothesized that genes showing the same patterns (i.e., expected to limit investment according to the KT or expected to promote investment) would be regulated by the same transcription factors. Therefore, we also tested (4) whether there is evidence of coordinated regulation among genes showing limiting patterns according to the KT, and among genes showing promoting patterns, but not between these two sets, e.g., we would not expect a transcription factor in a pathway promoting maternal investment to downregulate some maternally expressed genes and upregulate others.

Material and methods

A list of 120 unique genes imprinted in humans was compiled [9–12] (Supplementary Tables 1 and 2). We obtained expression data for 19882 genes in 157 placental samples [13], all of which were used for analyses of birthweight. However, since PE and IUGR have diverse pathophysiology, we reduced heterogeneity by restricting our analyses to PE and IUGR samples from a single cluster (cluster 2) defined by clustering of gene expression; this cluster exhibits

histopathological features of insufficient placental development [13]. Hence, the sample size was 117 for analyses of PE and IUGR (Table 1). We removed genes annotated as “not detected” in the placenta by Protein Atlas [14], leaving 14986 genes, including 28 MEGs and 33 PEGs (Supplementary Table 3). Clinical data included gestational age at delivery, occurrence of PE, occurrence of IUGR, birthweight (correcting for gestational age and sex) and offspring sex [13].

For each gene, we performed a general linear model with gene expression as the dependent variable and terms for birthweight (or occurrence of IUGR), PE, fetal sex, and gestational age. This yielded, for each gene, F-statistics for the effects of birthweight (or IUGR) and preeclampsia on gene expression (Supplementary Table 3). As measures of effect size, we obtained the slope of the regression of gene expression on birthweight or the fold change in gene expression between IUGR or PE and controls.

We tested whether the significance and effect size of imprinted genes were greater than would be expected for random genes. We selected genes at random from the complete set, such that the number of genes in the random sample was the same as the number of imprinted genes. We repeated this sampling 10000 times to calculate null distributions for the F-statistic and effect size, to which we compared the values for imprinted genes.

To identify sets of imprinted genes showing an investment-limiting or -promoting pattern for PE or IUGR, we selected imprinted genes with significant effects (alpha = 0.05). PEGs with increased expression and MEGs with decreased expression in PE or IUGR were categorized as promoting investment whereas those showing the opposite pattern were categorized as limiting. To test for coordinated regulation among genes showing the same pattern, we calculated the proportion of variation explained by the first principal component within sets of genes and assessed whether this was greater within sets than between sets. To achieve this, we combined

the limiting and promoting genes, divided them into two sets at random, and calculated the proportion of variation explained by the first principal component within each. The actual proportion of variation explained in each of the sets was compared with the null distribution from 10000 randomizations. All analyses were performed in SAS, Version 9.4.

Results

The correlation between birthweight and the expression levels of imprinted genes was greater than that for randomly-selected genes (P = 0.011; Supplementary Figure 1). However, the directions of the correlations between imprinted genes and birthweight did not deviate from random, i.e., there was not an excess of the expected pattern (positive correlations between PEGs and birthweight, and negative correlations between MEGs and birthweight) or the reverse pattern (P = 0.21). If the expression of PEGs were counterbalancing the expression of MEGs or vice versa, we would expect an excess of positive correlations among PEGs and MEGs, but this was not observed (Supplementary Table 4).

Imprinted genes were more dysregulated in PE than randomly-selected genes (P = 0.032), but were not more dysregulated in IUGR than expected due to chance (P = 0.13; Supplementary Figure 1). The direction of effects was consistent with imprinted genes limiting investment in pregnancies complicated by PE (i.e., PEGs downregulated and MEGs upregulated in PE; P = 0.021; Table 2), with a similar but marginally non-significant pattern for IUGR (P = 0.082; Table 2).

We tested for coordinated regulation among sets of genes showing limiting or promoting patterns, as predicted by the KT (Supplementary Table 3). For PE, the correlation in expression levels was not greater within promoting genes (P = 0.28) or within limiting genes (P = 0.50) than

between these two sets of genes. Similarly, there was no evidence of increased co-regulation in promoting or limiting genes (P = 0.84 and P = 0.22, respectively) categorized by dysregulation in IUGR.

Discussion

In general, imprinted genes had stronger correlations with birthweight and were more differentially expressed in PE than randomly-selected genes. However, the directions of the correlations between birthweight and gene expression did not show an excess of patterns predicted to drive fetal growth according to the KT, and the pattern of up- and downregulation in IUGR was not significantly different than that of random genes.

In PE, the pattern of up- and downregulation was consistent with imprinted genes limiting maternal investment in complicated pregnancies (i.e., PEGs downregulated and MEGs upregulated in PE). A potential explanation for this result is that imprinted genes are contributing to placental pathology. Imprinted genes are often coordinately regulated [15], and if some imprinted genes were contributing to placental pathology, it would not be expected that each gene would be independently regulated; some genes would be regulated by the same transcription factors. However, we found no evidence of coordinated regulation among genes predicted to limit maternal investment based on the KT. This suggests that imprinted genes predicted by the KT to have similar effects in pathological pregnancies are not more likely to be part of a common pathway. Alternatively, some of these genes may not play roles in limiting or promoting maternal investment as predicted by the KT, potentially because the KT does not explain the imprinting of these genes.

Our study examined gene expression at the end of pregnancy, whereas nutrient allocation is determined much earlier. However, this limitation does not explain why imprinted genes

showed stronger correlations with birthweight and greater dysregulation in PE than expected due to chance. While some imprinted genes have previously been shown to play important roles in placental development and function [16–18], our results suggest that this is true for some other, less-studied imprinted genes. Further investigation into the expression patterns of imprinted genes will improve our understanding of the mechanisms underlying poor fetal growth and severe pregnancy complications.

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Author contributions

JKC conceived of the study, carried out statistical analyses, and drafted the manuscript. KL and BJC contributed to analyses and interpretations, and helped draft the manuscript. All authors approve and are accountable for the final version.

Data accessibility

Data are available from the GEO database (accession number GSE75010).

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Competing interests

We have no competing interests.

Ethical statement

This study required no ethical permit; all data were previously published.

Table 1. Gestational age at delivery (weeks).

	PE+IUGR	PE only	IUGR only	Control
N	19	31	4	63
Mean (SD)	30.8 (2.0)	31.9 (3.5)	32.6 (3.2)	34.9 (4.9)
Range	27.0 – 34.1	26.9 – 39.3	29.4 – 37.1	25.9 – 41.1

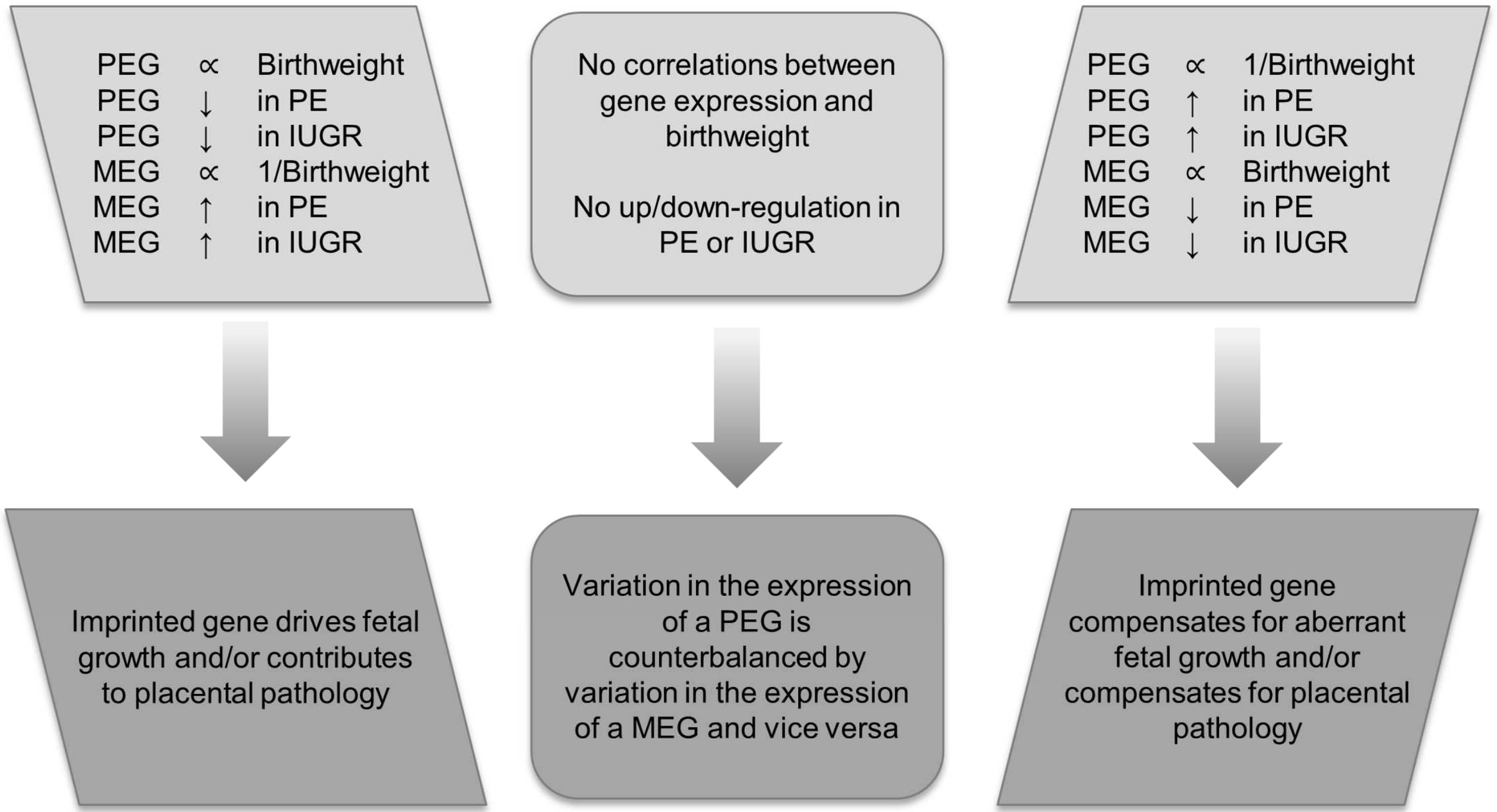
Table 2. Statistical significance and effects of birthweight, preeclampsia, and intrauterine growth restriction (means ± SE; * = imprinted genes significantly different from all genes at alpha = 0.05).

	Parameter	All genes (N = 14986)	Imprinted genes (N = 61)
Birthweight (continuous)	F-statistic	3.88 ± 0.04	5.61 ± 0.88*
	Effect size[1]	1.01 ± 0.00	1.00 ± 0.00
Preeclampsia	F-statistic	7.36 ± 0.10	10.56 ± 1.78*
	Effect size[1]	1.00 ± 0.00	1.03 ± 0.02*
Intrauterine growth restriction	F-statistic	1.86 ± 0.02	2.24 ± 0.35
	Effect size[1]	0.99 ± 0.00	1.01 ± 0.01

[1]For all genes, a value less than 1 indicates a negative correlation between gene expression and birthweight, reduced expression in PE compared with controls, or reduced expression in IUGR compared with controls, whereas values greater than 1 indicate the opposite patterns. For

229 imprinted genes, values less than 1 indicate patterns expected to promote investment in response
230 to low birthweight/pathology, while values greater than 1 indicate patterns expected to drive fetal
231 growth or contribute to PE/IUGR. More detailed description is provided in Supplementary Table
232 3.

Figure 1. Patterns of gene expression for individual imprinted genes, and interpretations under the KT.


PEG ∝ Birthweight
PEG ↓ in PE
PEG ↓ in IUGR
MEG ∝ 1/Birthweight
MEG ↑ in PE
MEG ↑ in IUGR
No correlations between gene expression and birthweight
No up/down-regulation in PE or IUGR
PEG ∝ 1/Birthweight
PEG ↑ in PE
PEG ↑ in IUGR
MEG ∝ Birthweight
MEG ↓ in PE
MEG ↓ in IUGR
Imprinted gene drives fetal growth and/or contributes to placental pathology
Variation in the expression of a PEG is counterbalanced by variation in the expression of a MEG and vice versa
Imprinted gene compensates for aberrant fetal growth and/or compensates for placental pathology