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The role of imprinted genes in fetal growth abnormalities

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Abstract

Epigenetics, and in particular imprinted genes, play a critical role in the development and function of the placenta, which in turn, plays a central role in the regulation of fetal growth and development. A unique characteristic of imprinted genes is their expression from only one allele, maternal or paternal, dependent on parent-of-origin. This unique expression pattern may have arisen as a mechanism to control the flow of nutrients from the mother to the fetus/offspring, with maternally expressed imprinted genes reducing the flow of resources and paternally expressed genes increasing resources to the fetus. As a result, any epigenetic deregulation affecting this balance can result in fetal growth abnormalities. In humans, imprinting-associated disorders such as Beckwith-Wiedemann and Angelman syndrome, support the role of imprinted genes in fetal growth. Similarly, assisted reproductive technologies in animals have been shown to affect the epigenome of the early embryo as well as expression of imprinted genes. Their role in disorders such as intrauterine growth restriction (IUGR) appears to be more complex in that imprinted gene expression can be seen as both causative and protective of fetal growth restriction. This protective or compensatory effect needs to be more fully explored.

Epigenetics

Epigenetics refers to heritable, but potentially reversible, modifications to the genome, representing the most fundamental aspect of gene regulation aside from the DNA sequence. Epigenetic modifications imparted onto DNA and chromatin can be classified into two categories: those that create an active chromatin state, positively regulating gene expression; and those that create a repressive chromatin state, negatively regulating gene expression. Methylation of a cytosine nucleotide positioned 5' to a guanine nucleotide (CpG methylation) and, in some instances adenine methylation (CpA methylation), represents the only epigenetic modifications to DNA. CpG methylation of promoters and regulatory elements negatively regulates gene expression through the recruitment of repressive protein complexes and specific patterns of histone modifications (Goll and Bestor, 2005). Covalent modifications to histones are a more complex form of epigenetics, as they are diverse in their type (e.g., methylation, phosphorylation, acetylation, and ubiquitination), position (e.g., lysine 4, 9, 27, and 36), and the numbers of modification per amino acid residue (e.g., mono-, di-, or trimethylation). The "histone code", which represent the collective array of histone modifications, including the type, position, and number, results in either an active, repressive, or bivalent chromatin state by altering the net charge of the DNA backbone or through the recruitment of various active or repressive protein complexes (Berger, 2007; Kouzarides, 2007; Wang et al., 2008). Epigenetics plays a pivotal role establishing genomic imprinting, as it is the different patterns of epigenetic modifications between the maternal and paternal chromosomes that allows expression of one allele and repression of the other in

a parent of origin dependent manner. Thus genomic imprinting is established by differential epigenetic modifications imparted onto the maternal and paternal chromosomes. These modifications, and their location in the genome, are dictated by unique genetic elements that establish germline-specific “imprints”, which are stably maintained throughout development. Most imprinted genes are found in clusters and in most cases contain a CpG rich imprinting control region (ICR) that establishes genomic imprinting throughout the region. ICRs are essential regulatory elements that direct differential allelic expression patterns of multiple imprinted genes (Li et al., 1993). In addition, imprinted genes can contain secondary differentially methylated regions (DMRs) that control genomic imprinting of single genes. ICRs and DMRs can be identified by overlapping patterns of trimethylated histone H3 lysine 4 (H3K4me3 [active]) and histone H3 lysine 9 (H3K9me3 [repressive]) modifications and differential DNA methylation between gametes and somatic tissue (Dindot et al., 2009). Thus DMRs and ICRs establish active and repressive chromatin domains on their respective homologous chromosomes and modulate the allele specific patterns of expression of imprinted genes.

Genomic imprinting

The discovery of genomic imprinting came from studies aimed at understanding why some species could develop through parthenogenesis while others could not. Through a series of pronuclear transfer experiments in mice, it was observed that zygotes generated from either two sets of maternally-derived chromosomes (gynogenotes or parthenotes) or two sets of paternally derived chromosomes (androgenotes) could not survive to term (Solter et al., 1985). Gynogenetic embryos yielded intrauterine growth-restricted (IUGR) conceptuses, hypo-vascular placentae, and reduced total mass of extraembryonic tissues, while the androgenotes yielded hydatiform moles — a trophoblastic neoplasia with a very small embryo component and a very large abnormal placenta. This work formed the basis for the description of the maternal and paternal genome as being non-equivalent, unique and non-interchangeable. Further understanding of this genomic non-equivalence phenomenon came from studies using mice with maternal or paternal uniparental disomies (UPD). Mice carrying heterozygous translocations were crossed to each other to test for non-complementation of parental alleles. This resulted in the mapping of imprinted regions to mouse chromosomes 2, 8 and 17 (Cattanach and Kirk, 1985). Through these studies, the first reciprocal set of imprinted genes in mice were identified: maternal specific expression of *Igf2r* and *H19* on chromosome 17 (Barlow et al., 1991), and paternal specific expression of *Igf2* on chromosome 7 (DeChiara et al., 1991; Ferguson-Smith et al., 1991). Since then, these three genes have played, and continue to play, a key role in our understanding of the mechanisms regulating genomic imprinting.

Interestingly, placental mammals (Hore et al., 2007) and flowering plants (Huh et al., 2008) exhibit genomic imprinting, whereas reptiles and birds do not. Even if a small rudimentary placenta is present, such as that seen in marsupials, evidence for genomic imprinting can be found (Renfree et al., 2009). Such close correlation between the presence of genomic imprinting and the presence of the placenta, an organ that controls the flow of nutrients from the mother to the fetus, has resulted in several hypotheses explaining the evolutionary pressure leading to the appearance of imprinting. The most accepted hypothesis is the kinship or parental conflict hypothesis (Moore and Haig, 1991), which predicts that paternally expressed genes promote extraction of resources from the mother to enhance fetal and postnatal growth, while maternally expressed genes act to restrain fetal and postnatal growth to conserve maternal resources. In essence a “conflict” over the control of resources with maternally imprinted genes acting to counter-balance the paternally imprinted genes. In a normal pregnancy the two opposing forces balance each other and fetal growth is normal. However, external factors can disrupt this balance and lead to either restricted (intra uterine

growth restriction [IUGR] or small for gestational age [SGA]) or enhanced growth (large for gestational age [LGA]). Key questions regarding genomic imprinting still remain. What mechanisms regulate the differential expression of the maternal and paternal alleles, and what physiological roles do they have that affect fetal growth?

Function of imprinted genes

At present, more than one hundred imprinted genes have been cataloged, and these represent a broad class of gene types, including protein and RNA coding genes (<http://igc.otago.ac.nz/home.html> (Morison et al., 2005). It is important to note two reports describing over 1,000 imprinted genes in the brain (Gregg et al., 2010a; Gregg et al., 2010b), which dampens the notion of a small number of imprinted genes having a large phenotypic effect. If similar results are found in other organs such as the placenta, our notion of the imprinted gene family will have to be drastically reconsidered. Until then, the great majority of the work to date by numerous laboratories including ours (Bischoff et al., 2009) suggests that imprinted genes in non-neural tissues number in the hundreds not the thousands.

What then is the known function of imprinted genes? To help address this question, all genes where there is strong evidence of imprinting in human are shown in Table 1. For each gene, its function, and specifically its effect on fetal growth or behavior are described. Several factors are striking when examining Table 1 including:

1. The relatively large number of imprinted genes that are non-coding RNAs. In a few cases, the function of the non-coding RNA is known, but for the many of them their mechanism of action or function is unknown (Hung and Chang, 2010).
2. The large number of oncogenes and tumor suppressor genes that are imprinted. They are represented in both the maternally and the paternally expressed genes.
3. The large number of imprinted genes that are uniquely imprinted in the brain (*CALCR*, *BLCAP*, *GRB10*) or that affect neural development and behavior (paternally expressed *MEST*, *NDN*, *NNAT*, *PEG3*; maternally expressed *ATP10A*, *KCNQ1*, *TP73*, *PPP1R9a*). For instance, loss of paternal *Mest* expression in mice leads to abnormal maternal behaviors such as the lack of maternal care (e.g., nurturing) and absent placentophagia. The pups themselves are not affected as they develop normally when placed with a wild type foster mother. A similar phenotype is seen in *Peg3* deficient mice, where the mother fails to care for the young. This is compounded by a reduced milk ejection response likely due to reduced levels of oxytocin receptors (Champagne et al., 2009). Other genes implicated in behavior include *UBE3A*, *MAGEL2* (Schaller et al., 2010), and genes within the Prader-Willi/Angelman syndrome imprinted domain (PWS/AS) (Davies et al., 2007) for comprehensive review).
4. The number of imprinted genes that affect metabolic parameters such as thyroid function, insulin, and glycogen metabolism. These include the paternally expressed *DIO3*, *HYMAI*, *MAGEL2*, *NNAT*, and the maternally expressed *GRB10*, *H19*, *KCNQ1*, *PHLDA2* genes.
5. The large number of imprinted genes known to affect fetal and placental growth. They include over half of the paternally expressed genes including *DIO3*, *DLK1*, *HYMAI*, *IGF2*, *MAGEL2*, *MEST*, *PEG10*, *PEG3*, *PLAGL1*, *SFRP2* and the maternally expressed *GRB10*, *PHLDA2*, *CDKN1C* and *RB1* genes.

Collectively, the function of these imprinted genes strongly supports the parental conflict hypothesis. This hypothesis predicts that maternally expressed genes are involved in resource conservation (i.e., less flow to the fetus) and paternally expressed genes in resource

extraction (i.e., more energy to fetus). Thus mutations in maternally and paternally imprinted (i.e., silenced) genes would lead to a larger and smaller conceptus, respectively. This is supported by phenotypes manifested in animal models deficient in the maternally expressed genes, *Cdkn1c*, *Grb10*, *H19*, and *Phlda2*, and the paternally expressed genes, *Dio3*, *Dlk1*, *Hymal*, *Igf2*, *Magel2*, *Mest*, *Peg3*, *Peg10*, and *Plagl1* (see Table 1 for details and references). In cases where no fetal growth phenotype has been described, the effects on specific cell types are the opposite. For instance, inactivation of the maternally expressed *Rb1* leads to excessive trophoblast proliferation, while inactivation of the paternally expressed *Sfrp2* leads to reduced extra villous trophoblasts migration. Moreover, if one were to summarize how allocation of resources between a mother and a fetus/offspring could be regulated at the cellular/behavioral level, the list would include placental surface area, placental and uterine vascular density, transport mechanisms, and factors affecting metabolic rates and glucose utilization. After birth, they would include nurturing behaviors, suckling behavior, milk ejection, and metabolic rate. Table 1 indicates that every imprinted gene for which a function is known falls into one of the above categories. This strongly supports the evolution of the imprinted genes family as a mechanism for resource allocation. Although the function of each gene is well understood, the function as a collective group is not. A simple question that has not been addressed adequately is how the pattern of expression of imprinted genes differs between tissues.

Expression patterns of imprinted genes in different human tissues

In order to determine the expression patterns of imprinted genes in human placenta and their relative expression levels when compared to all other genes (i.e., non-imprinted genes) a meta-analysis was performed using different human microarray datasets available through the Gene Expression Omnibus (GEO). Datasets used included: 1) GSE24129, representing eight normal term placentas; 2) GSE18887, representing eighteen human fetuses at Carnegie stages 9-14; 3) GSE4888, representing twenty-eight uterine samples from non-pregnant women (no uterine tissues from pregnant women were available); 4) GSE12654, representing fifteen adult human brains; and 5) GSE17371, representing twelve muscle samples from diet-resistant and diet-sensitive subjects. Overall, the selected datasets represented tissues interacting with one another (i.e., uterus, placenta, and fetus), and brain and muscle as tissues that play a central role in the so called “brain sparing response”, where brain resources are conserved at the expense of muscle mass (Barker, 1993). As these experiments were carried out independently it was difficult to compare the results without some way of accounting for all the experimental variables. In our case we used a simplified version of the rank aggregation approach (Pihur and Datta, 2008). This method ranks genes on the basis of expression level within each experiment. Once all datasets were independently ranked the data could be compared. This reduced experimental variability as we compared each gene’s behavior against another within each dataset. After ranking the genes using this approach, thirty-seven imprinted genes that were represented on the arrays were extracted from the data. Figure 1 shows how the genes were distributed in order of expression in all the tissues analyzed. As expected a high proportion of the selected genes, but not all, were expressed at very high levels in the placenta. In term placenta 17/37 (46%) of the imprinted genes analyzed were in the top 10th percentile in terms of expression level and 5 were in the 1st percentile. Thus, they were expressed at levels comparable to highly expressed genes such as *ACTIN*, *GAPDH*, *EEF1A1*, and *COL1A2*. The high level of expression was not unique to the placenta, as a small group of imprinted genes, including *GNAS*, *SNRPN*, *BLCAP*, and *CDKN1C*, were expressed at similar levels in all tissues examined. These findings suggest that the genes examined will affect functions that are common to many tissues and that mutations in these genes might lead to complex phenotypes not limited to the placenta. We also observed that genes, such as *PHLDA2*, were highly specific to the placenta where it ranked in the top 6th percentile, but was essentially

non-expressed in uterus, fetus and muscle with intermediate expression in the brain (top 29st percentile). *TFPI2* had an unusual pattern with very high levels of expression in placenta and uterus, but low levels of expression in the other tissues, suggesting that this gene may play an important role in placental uterine interactions. Conversely, *NDN* was highly expressed in all tissues tested except the placenta. Overall, this analysis shows that most imprinted genes are not placental-specific, with the exception of *PHLDA2*, suggesting that they affect energy flow through multiple mechanisms specific to a particular tissue type.

Imprinted genes in fetal growth abnormalities

As reviewed in Table 1 there is direct evidence from mouse models that inactivation of a single imprinted gene can lead to IUGR and placental deficiencies. However, what impact would dysregulation of multiple imprinted genes have on fetal growth? The answer to this question lies in the human syndromes, such as Prader-Willi (PWS), Angelman (AS), Silver-Russell (SRS), and Beckwith-Wiedemann (BWS) syndromes. In syndromes such as BWS and SSR there is unbalanced expression between maternal and paternally expressed genes that can result from either uniparental deletion or uniparental disomy (duplication) of the regions of interest. In each case, the end result is unbalanced expression with respect to the imprinted genes located within the affected chromosomal regions (Figure 2).

Prader-Willi and Angelman Syndromes

PWS and AS are caused by paternal and maternal deletions of human chromosome 15q11-q13, paternal uniparental disomy (UPD) of chromosome 15, genomic imprinting defects of 15q11-q13, or loss of function of the HBII-85 snoRNA (PWS) and the ubiquitin-protein ligase E3A (*UBE3A*) gene (AS). Chromosome 15q11-13 encompasses a cluster of imprinted genes including the *SNRPN/SNURF/SNORDs* complex, *MKRN3*, *MAGEL2*, *UBE3A* and *NDN* (Williams et al., 2010). PWS patients exhibit mild prenatal growth retardation, failure to thrive, and poor feeding ability. This is in addition to a broad range of motor and psychological abnormalities later in life. As many candidates in this region have been implicated in the phenotypes seen in PWS (see Table 1) it has been difficult to determine which gene is responsible for the different features of PWS. In humans, micro deletions of *MKRN3*, *MAGEL2*, and *NDN* result in obesity and mental retardation but none of the other PWS phenotypes (Kanber et al., 2009). Similarly in both mice and humans deletion of the region encompassing the members of the SNORD complex, HBII-85 and HBII-438, result in neonatal lethality (Ding et al., 2005). Deletion of just MBII-85, however, results in postnatal growth retardation (Sahoo et al., 2008; Skryabin et al., 2007). Angelman syndrome has also been mapped to this region. The majority of Angelman cases have been implicated with 15q11-q13 maternal deletions (70%), with fewer cases implicated by uniparental disomy, imprinting disorders or mutations in *UBE3A* (Williams et al., 2010).

Silver-Russell (SRS) and Beckwith-Wiedemann (BWS) Syndromes

The molecular basis for SRS and BWS appear to be somewhat more complicated as two different chromosomal regions, 7p11.2p13 and 11p15 are implicated (Eggermann et al., 2008). Phenotypically, SRS is characterized by severe intrauterine and postnatal growth retardation, while BWS is characterized by overgrowth in late pregnancy and childhood, and an increased risk of childhood tumors. Imprinted genes that have been associated with both SRS and BWS include the *H19-IGF2*, *CDKN1C* and *KCNQ1* genes (11p15 region), and the *GRB10* gene, located in 7p11.2p13 (Choufani et al., 2010). In general, it is believed that the overgrowth phenotype seen in BWS is mostly caused by overexpression of *IGF2*. This is supported by cytogenetic studies showing BWS is characterized by paternal duplication of that region (Choufani et al., 2010). As depicted in Figure 2 this would result in a double dose of paternally expressed genes in that region including *IGF2*. More direct evidence that

there is an epigenetic involvement in BWS and SRS comes from studies showing that methylation abnormalities in two imprinting control regions located near the *IGF2* region cause BWS and SRS. Specifically, hypermethylation of imprinting control region 1 (ICR1) and hypomethylation of ICR2 result in BWS while conversely, hypomethylation of ICR1 and hypermethylation of ICR2, result in SRS (Blick et al., 2006).

Assisted Reproductive technologies and fetal growth abnormalities

Assisted reproductive technology (ART) involves manipulating gametes through *in vitro* methods to achieve pregnancy when pregnancy is not possible by natural fertilization. ART is performed during critical periods of epigenetic reprogramming when epigenetic modifications, including DNA methylation and modifications of histones, are being erased and re-established (Figure 3). Clearly, epigenetic modifications must be reset at each generation otherwise meiotic recombination would give rise to gametes with chromosomes of mixed maternal and paternal origin. In addition, the genome is constantly accumulating changes in DNA methylation and chromatin modifications as a result of environmental factors such as diet, smoking, sun exposure, aging, alcohol, and other toxicants (Aguilera et al., 2010; Gronniger et al., 2010). If these changes were not cleared between generations the genome would gradually accumulate a large number of “epimutations”. Thus, this process of erasing and re-establishing epigenetic modifications is not only a procedure for eliminating errors between generations, it is key to the long-term survival of the species.

The erasure of epigenetic modifications, including those involved with genomic imprinting, and the re-establishment of those modifications have been described in detail elsewhere (Mayer et al., 2000; Santos et al., 2002). Briefly, the maternal and paternal “imprints” (i.e., differential DNA and histone modifications regulating genomic imprinting) as well as all other epigenetic modifications are removed during fetal development but only in the primordial germ cells (PGCs Figure 3). The PGC genome is gradually reprogrammed, thus removing the existing modifications, or “marks”, present on DNA and histones. Following the removal of the “old” markings, new markings are added. These new marks, particularly those at DMRs and ICRs, represent the sex of the fetus. For instance, oogenesis in females will give rise to unique, female markings, whereas spermatogenesis in males will give rise to unique, male markings. The result is differential epigenetic markings that distinguish the maternal and paternal derived chromosomes. These differential markings constitute DMRs and ICRs. Within hours after fertilization though, the paternal genome is rapidly demethylated by an unknown mechanism, while the maternal genome is somehow protected. The maternal genome is gradually demethylated through subsequent cell divisions of the embryo proper leading up to the morulae stage at which point the two parental genomes are remethylated again. Despite this global wave of demethylation, the markings present at ICRs and DMRs are not erased and are stably propagated throughout early embryogenesis and fetal development. What is unusual about this remethylation is that the fetus proper and the placenta are methylated to a different degree. As shown in Figure 3, the placenta is hypomethylated compared to the fetus proper. And this differential methylation level is conserved in mammals as it has been described in multiple species including humans (Novakovic et al., 2010), mice (Santos et al., 2002) and cattle (Dindot et al., 2004) among others. Thus, from an epigenetic standpoint the placenta is unique and particularly susceptible to certain ART as discussed below.

In the last few years, multiple groups have reported increases in the incidence of BWS and AS in response to ART. Although these reports are highly controversial, there is substantial data that indicates a small but significant increase in imprinting disorders in response to ART (Owen and Segars, 2009). One of the main concerns when analyzing data from human ART studies relates to the multitude of variables involved, as different laboratories may use

distinct ovulation induction protocols, different sources of growth factors to culture the embryo, etc. As it is difficult to systematically examine whether ART affects genomic imprinting in humans, animal models can help fill this void. Recent studies in mice have convincingly demonstrated effects of ART on fetal growth and development. One of the most striking observations is the effect of super ovulation on imprinting of both maternally and paternally expressed genes (Market-Velker et al., 2010). More specifically, Market-Velker et al., (2010) reported loss of *Snrpn*, *Peg3* and *Kcnq1ot1* expression, and gain of H19 imprinted methylation in response to super ovulation. Similarly, *in vitro* culture has been associated with changes in imprinted gene expression and long-term behavioral changes (for comprehensive review see Fernandez-Gonzalez et al., 2007). In a more invasive ART process, my group and others have shown that somatic cell nuclear transfer (SCNT) has direct effects on imprinted genes and on methylation in a variety of species (Cui et al., 2009). SCNT in species such as cattle and sheep has drastic effects on placental morphology and function and fetal growth. The combined phenotype is known as the large offspring syndrome (Dindot et al., 2004; Guillomot et al., 2010; Hori et al., 2010; Renard et al., 1999; Suzuki et al., 2009) or abnormal offspring Syndrome (Farin et al., 2006). In essence, offspring generated by SCNT from these two species have an overgrowth phenotype highly reminiscent of BWS. And like BWS, examination of both the placenta and the fetus has shown imprinted gene dysregulation accompanied by methylation defects. However, the effect is greater in the placenta with essentially no changes in methylation levels in the fetus proper but significant levels of hypermethylation in the placenta itself (Dindot et al., 2004; Yang et al., 2007) or with expression levels of imprinted genes affected in the placenta but not the fetus (Inoue et al., 2002). Thus, the evidence from animal models strongly supports epigenetic dysregulation in response to ART.

Role of Imprinted genes in intra uterine growth restriction (IUGR)

Many groups have reported changes in imprinted gene expression and methylation levels in response to IUGR. Recent examples include reports of abnormal patterns of methylation in multiple imprinted loci (Turner et al., 2010), decreased placental methylation in the *IGF2/H19* locus in IUGR but not preeclamptic placentas (Bourque et al., 2010), and differential expression in multiple imprinted genes in IUGR placentas, but the altered expression was not associated with imprinting dysregulation or loss of imprinting (LOI) (Angiolini et al., 2006) (Diplas et al., 2009). A limitation with these studies is that only one or two genes (*IGF2/H19*) or a small subset of genes among the thousands that are expressed in the placenta were examined. More global studies have shown that, at least with respect to methylation changes, the effects are not focused on imprinted genes. Einstein et al. (2010) examined global methylation in CD34 cells obtained from control and IUGR patients and detected significant levels of methylation differences in 56 different loci, none of which represent imprinted genes. Similarly, gene expression analysis using microarrays have indicated that several hundred genes are affected and only few of those are imprinted genes (McCarthy et al., 2007; Struwe et al., 2010). An excellent example of this complexity is represented by the results of McMinn et al. (2006) which showed that a small group of imprinted genes (*PHLDA2*, *MEST*, *MEG3*, *GATM*, *GNAS*, and *PLAGL1*) and an additional 400 genes were affected in IUGR placentas. In an ongoing study similar to the ones described above we identified about 50 differentially expressed genes in IUGR term placentas (at a false discovery rate of $q < 0.05$), of which only two were imprinted genes (*PEG10*, *PEG3*). Thus, at least four independent groups using similar approaches but different source of material found essentially identical results with respect to imprinted genes and IUGR. That is, that while they play a role, that role is shared with many other non-imprinted genes.

How does one then reconcile the very strong experimental evidence for the role in fetal and placental growth and function of individual imprinted genes (Table 1) and imprinting dysregulation on uniparental disomy of regions containing imprinted genes, with the lack of strong evidence for a role of imprinted gene function in IUGR? A simple explanation lies in the highly heterogeneous nature of IUGR. While the definition can be as simple as those births falling below the 8-10 percentile of body weight, that definition also captures births that are part of the normal variation in a population. More stringent criteria is based on both fetal growth curves as well as Doppler velocimetry abnormalities (Ferrazzi et al., 2002). But even with very stringent criteria for selection, IUGR is still an extremely complex phenotype to dissect due to the large number of factors, such as maternal, fetal, placental and environmental, which can affect fetal growth. Yet most gene expression studies are based on small number of samples. It is unrealistic to expect to find one, or a few, genes responsible for causing IUGR using this approach. What we are most likely to find is genes that are dysregulated in **response to** IUGR. That these response genes can be found is supported by reports identifying genes such as corticotropin releasing hormone (CRH), IGF-1, IGFBP-1 and leptin (LEP) as affected in IUGR placentas (McMinn et al., 2006; Struwe et al., 2010).

What then is the role of imprinted genes in IUGR? In IUGR, imprinted genes can be simplistically separated into two categories: those genes that are participating in enhancing IUGR or reducing fetal growth (negative effector) and those genes that, through a compensatory mechanism, sense the fetus is at risk and act to increase fetal growth (positive effectors). The evidence supporting mutations in imprinted genes as negative effectors has been presented in Table 1. The action of imprinted genes as positive effectors of growth in IUGR is supported by observations such as downregulation of the maternally expressed genes *GATM*, *GNAS* and *MEG3/DLK1* and overexpression of *PEG10* (McMinn et al., 2006; Diplas et al., 2009). In all of these cases the expectation would be the opposite to the one observed, that is, that reduced expression of maternally expressed genes and overexpression of paternally expressed genes would result in increased fetal growth, not IUGR. Our own results (unpublished) confirm the observations of McMinn et al. (2006) and Diplas et al. (2009). Specifically, we observed overexpression of both *PEG3* and *PEG10* in IUGR placentas again suggesting that these imprinted genes are acting in a compensatory manner.

That the fetus can compensate in response to nutrient supply is a well-documented observation. Probably the most striking example of this compensation is the head to body ratios that are seen in asymmetric IUGR patients where limited resources are channeled for maintenance of brain growth at the expense of tissues and organs such as muscle and kidney — the so-called brain-sparing effect. This observation is common in a large number of induced as well as natural animal models (Peebles, 2004; Struwe et al., 2010) and has recently been confirmed in a large human study that examined the relationship between brain, muscle, and body mass in a sample of over 9,000 individuals. The data supported a protective effect in the brain at the expense of muscle in low birth weight patients (Baker et al., 2010). Physiologically this protective effect has been implicated with a redistribution of blood flow to favor the brain in response to hypoxia and/or undernutrition (Malamitsi-Puchner et al., 2006; Peebles, 2004). This physiological reaction is complex and due to various stimuli including low oxygen, and levels of glucose, lactate, amino-acids, and lipids. The key question is whether imprinted genes play a key role in this compensatory response, and if they do, to what stimuli do they respond to.

In short, imprinted genes play a central role in the development and function of the placenta and have been implicated in a variety of disorders affecting fetal growth. Gene inactivation studies in mice and chromosomal rearrangements in humans have demonstrated that many of these imprinted genes play key roles in placental development and function as well as in fetal growth. Those studies have also demonstrated that imprinted genes act in a complex

manner at many levels to affect energy balance between the mother and fetus. More recent studies also support a role of imprinted genes in a compensatory response to reduced fetal growth in humans.

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Figure 1. Expression patterns of imprinted genes in selected human tissues

As described in the text a series of microarray experiments was utilized to determine the transcriptional profile of imprinted genes in different human tissues. Term placentas from normal pregnancies, uterine samples from the mid secretory phase of the estrus cycle, fetal material from Carnegie stage 9 (E20-21d) and adult brain were examined. All data were filtered to extract genes known to be imprinted in humans, and that were represented in the arrays. This resulted in 37 imprinted genes analyzed. The grey boxes represent genes that were expressed in the placenta at the top ten percentile of the whole gene array (highest expressors). The number to the left of each gene name represents the percentile ranking when compared to all expressed genes in the array. Bold genes represent maternally expressed genes; underlined genes represent genes that have both maternal and paternal expressed isoforms or for which limited information is available. NR=Not represented in the array.

	Origin	Expression	Dosage
A)	MAT PAT	MAT: PAT:	1 MAT + 1 PAT
B)	MATdel	MAT: PAT:	0 MAT + 1 PAT
C)	PATdel	MAT: PAT:	1 MAT + 0 PAT
D)	MATdup	MAT: MAT: PAT:	2 MAT + 1 PAT
E)	PATdup	MAT: PAT: PAT:	1 MAT + 2 PAT

Figure 2. Imprinted gene dosage Silver-Russell/Beckwith-Wiedemann syndromes

A) Wild-type genotype depicting the parent-of-origin of the chromosomal region (in these case 7p11.2p13), and how imprinting in these regions affects the expression of alleles based on parent-of-origin, and the final expression dosage after imprinting is accounted for. In the wild type case there is a balance in dosage with one maternal and one paternal unit of expression. B) Represents a deletion in the region but only in the maternally-derived chromosome. The dosage now lacks any expression from paternally expressed genes and is unbalanced. C) Equivalent change but from the paternally-derived genes. Dosage shows no expression of paternal genes. D) In this case there is a uniparental disomy (UPD) of the maternally-derived chromosome. Dosage is now unbalanced with twice the relative dose of maternally expressed genes as paternal ones. Phenotype should resemble, but not equal, that seen in PATdel as in both cases there is overexpression of maternally expressed genes in relation to paternally-expressed ones E) Equivalent UPD but now the paternally-derived region is duplicated. Dosage is unbalanced as in E. Phenotype should share some characteristics but not be equal to MATdel.

Figure 3. Dynamic changes in DNA methylation levels during development

During fetal development cells destined to become primordial germ cell (PGC) are demethylated. Both global methylation (solid lines) and imprinted gene methylation (broken lines) are affected. This erasure is needed to reset the imprints each generation and to remove any epigenetic DNA methylation changes accumulated in the previous generation. After complete erasure the new imprints are added. The timing of addition of paternal imprints (dark lines) during spermatogenesis differs from maternal imprints (light lines) added during oogenesis. At completion of gametogenesis both global and imprint DNA methylation are at their highest. Following fertilization there is a wave of both passive and active demethylation. This demethylation affects global methylation but does not affect the imprinting markings. Maximal demethylation is reached at the morula stage of embryonic development. Once the blastocyst starts to develop, a new wave of global methylation is initiated. However, the inner cell mass is methylated to a different extent than the trophectoderm. These differences in DNA methylation levels are maintained postimplantation and throughout development with the fetus proper having a higher level of methylation than the placenta itself (adapted from Yang et al., 2007).

Table 1

GENE	KNOWN FUNCTION	KNOWN PHENOTYPIC EFFECTS	REFERENCE
Paternally Expressed Genes			
DIO3	Type 3 deiodinase.	Highly expressed in placenta and fetus. IUGR and hypothyroidism.	(Hernandez et al., 2002; Hernandez et al., 2006)
DLK1	Growth promoter	Expressed in placental villi. Methylation defects associated with IUGR.	(Floridon et al., 2000; Turner et al., 2010; Wylie et al., 2000)
HYMAI	Non-coding RNA	Transient neonatal diabetes and IUGR.	(Inoue et al., 2001; Ma et al., 2004)
IGF2	Growth Factor	Placental and fetal growth restriction.	(Dilworth et al., 2010)
IGF2AS	Non-coding RNA	Implicated in Beckwith-Wiedemann syndrome and Wilms tumor	(Okutsu et al., 2000)
KCNQ1OT1	Non-coding RNA	Control placental-specific imprinting of Kcnq1 domain. Involved in Beckwith-Wiedemann syndrome.	(Beatty et al., 2006; Mohammad et al., 2010)
MAGEL2			(Bischof et al., 2007; Boccaccio et al., 1999)
NDNL1	51% similarity to NDN.	Neonatal growth retardation, altered metabolism	
MEST	Neuronal differentiation	Fetal growth restriction, smaller placentas. Maternal behavior.	(Kobayashi et al., 1997; Lefebvre et al., 1998; McMinin et al., 2006)
MKRN3	E3 ubiquitin ligase	Region implicated in Prader Willi, placental function unknown.	(Jong et al., 1999)
ZNF127			
NDN	Neuronal development, interacts with MAGEL2	Neural deficiencies but no growth abnormalities reported.	(MacDonald and Wevrick, 1997; Muscatelli et al., 2000)
NNAT	Neural development. Ion channel regulator	Obesity. Affects neural development	(Evans et al., 2001; Vrang et al., 2010)
PEG10	Retrotransposon-derived gene	Severe growth retardation, absence of spongio-trophoblast layer, and embryonic lethality.	(Ono et al., 2001; Ono et al., 2006)
PEG3	Inhibits Wnt-signalling	Placental and fetal growth restriction and abnormal maternal behavior.	(Murphy et al., 2001)
PLAGL1		Skeletal defects, neonatal lethality, IUGR, and disrupted transactivation of Igf2	(Varrault et al., 2006)
Zac1	Tumor suppressor		
SFRP2	Wnt signaling	Reduction in vitro of extra villous trophoblast invasion	(Grisaru-Granovsky et al., 2009; Guilleret et al., 2009)
SGCE	Links actin cytoskeleton to extracellular matrix	Myoclonus-dystonia	(Grabowski et al., 2003; Smallwood et al., 2003)
SNRPN			
SNURF	Bicistronic message.	Region implicated in Prader Willi, placental function unknown.	(Nicholls and Knepper, 2001; Skryabin et al., 2007)
HBII-85/PWSer	C/D Box small RNA	Implicated in Prader Willi, Postnatal growth retardation.	(Skryabin et al., 2007)
Maternally Expressed Genes			
APC	Tumor suppressor, Wnt signaling	Intestinal tumors, No known function in placenta	(Guilleret et al., 2009; Hanson and Miller, 2005)
ATP10A	Ubiquitin ligase	Neuronal differentiation. No known uterine/placental function	(Meguro et al., 2001)
CALCr	Calcitonin receptor	Fetal hydropsis with abnormal cardiovascular and vascular development.	(Dackor et al., 2006; Okita et al., 2003)

GENE	KNOWN FUNCTION	KNOWN PHENOTYPIC EFFECTS	REFERENCE
CDKN1C	Negative regulator of cell proliferation	Placental overgrowth	(Rajaraman et al., 2010; Wylie et al., 2000)
CPA4	Oncogene.	No expression in placenta.	(Kayashima et al., 2003)
H19	Non-coding RNA. Controls imprinting of IGF2	Highly expressed in placenta. Fetal overgrowth and placentas with larger glycogen stores	(Esquiliano et al., 2009; Zhang and Tycko, 1992)
KCNK9	Potassium channel.	High expression in neural tissues. Knockout mice have no fetal or placental defects	(Mulkey et al., 2007; Ruf et al., 2007)
KCNQ1	Voltage-gated potassium channel	Hypothyroids in mice, deafness, cardiac defects.	(Casimiro et al., 2001; Frohlich et al., 2010; Lee et al., 1997)
KCNQ1DN	No known function	No expression in placenta	(Xin et al., 2000)
KLF14	Transcriptional repressor	No known placental function.	(Djalilian et al., 2006; Truty et al., 2009)
PHLDA2	Cytoplasmic protein with pleckstrin-homology domain	Placental overgrowth; fetal growth remains unchanged.	(Lee and Feinberg, 1998; Tunster et al., 2010)
PPP1R9A neurabin I	Cytoskeletal dynamics Cell morphology	Imprinted in muscle and placenta. Involved in neural development.	(Nakabayashi et al., 2004; Oliver et al., 2002)
SLC22A18 AS	No sequence homology to other known proteins	Expressed in placenta, liver, GI track, and kidney. No known function.	(Bajaj et al., 2004; Cooper et al., 1998)
SLC22A3	Extra neuronal monoamine transporter	Imprinted in first trimester placenta. Knockout mice have reduced monamine transfer from placenta to fetus.	(Monk et al., 2006; Zwart et al., 2001)
TFPI2	Tumor suppressor	Imprinted in first term human placenta. Highly expressed in cytotrophoblasts cells, may be involved in intervillous blood flow.	(Monk et al., 2008; Udagawa et al., 1998)
TP73	Interacts with p53, involved in apoptosis	Impaired neural development, runting, and pheromone unresponsiveness.	(Cai et al., 2000; Yang et al., 2000)
Isoform specific or unknown Maternal/Paternal expression			
BLCAP	Tumor suppressor	Imprinted in brain only. Unknown placental function.	(Schulz et al., 2009)
CD44	Membrane glycoprotein, metastasis	Impaired arteriogenesis,	(Diplas et al., 2009; van Royen et al., 2004)
EPS15	Involved in EGFR and PDGFR receptor signaling	Overexpression leads to cell transformation.	(Diplas et al., 2009; Fazioli et al., 1993)
GRB10	protein kinase phosphorylation	Placental and fetal overgrowth. Increased insulin signaling	(Charalambous et al., 2010; Charalambous et al., 2003)
GNAS	Involved in thyroid function	Complex locus with multiple transcripts and imprinted isoforms.	(Weinstein et al., 2002)
RB1	Tumor suppressor	Inactivation of RB1 in mice leads to excessive proliferation of trophoblasts cells	(Kamber et al., 2009; Wu et al., 2003)