Genomic imprinting is an epigenetic process responsible for the monoallelic expression of a subset of genes in mammals. Imprinted genes have been demonstrated to play important functions prenatally regulating fetal growth and placental development with some functions persisting beyond pregnancy to influence both metabolism and behaviour in adults. This review focuses on the function of imprinted genes in regulating placental hormones, and the probability that these functions manifest their impact beyond pregnancy.

Key words: Genomic imprinting; placental hormones.
Central to the reproductive success of mammals is the \textit{in utero} provision of nutrients to their young via specialised extraembryonic lineages \cite{1}. All extant mammals (monotremes, marsupials and eutherians) rely initially on a yolk sac placenta with some marsupials and all eutherian mammals switching to a more substantial chorioallantoic placenta as gestation proceeds. The mammalian mother continues providing nutrients to their young after birth in the form of milk secretions from mammary patches (monotremes) or more complex mammary glands. High quality maternal care is another vitally important factor in newborn survival with mothers providing body warmth, protection against the environment and potentially fending off predators at risk to her personal safety. The provision of nutrients and care is not simply a passive process but requires substantial physiological and behavioural changes in the mother most of which take place during pregnancy and some of which only become apparent after birth. The provision of resources primarily or exclusively by the mother in mammals suggests a conflict between the mother’s genes and the father’s genes \cite{2, 3}. Simply, it would be advantageous for the paternal genome to extract nourishment from the mother as there is not cost but the maternal genome must ensure a more equitably allocation of maternal resources across numerous pregnancies.

Imprinted genes, expressed from a single parental allele as a consequence of germline epigenetic events \cite{4}, are thought to be the physical embodiment of this conflict. In support of this hypothesis, the number of genes subject to genomic imprinting correlates well with the progressive trend toward internal development and viviparity in mammals with marsupials possessing fewer imprinted genes than the well studied Eutherian mammals, represented by mice and humans \cite{5, 6}. Moreover, numerous studies in genetically altered mice have identified imprinted genes that influence fetal weight, placental development, maternal behaviour, lactation and thermogenesis \cite{7-9} in a manner generally consistent with the conflict theory.
A major function of imprinted genes in the placenta is the regulation of nutrient transport primarily inferred from studies on the paternally expressed/maternally silenced insulin-like growth receptor 2 gene, and extensively reviewed [10]. Nutrient transport is determined by the demand requirements of the fetus and placenta, which both place a substantial burden on maternal resources during pregnancy. The mammalian mother also exclusively supplies nutrients, in the form of milk, after birth. The supply of nutrients prenatally and in the immediate postnatal period requires significant adaptations to maternal physiology, which must be carefully balanced for a successful pregnancy. Effectively, the mother must be able to supply all the nutritional requirements of her developing young while maintaining her own health and welfare. To achieve nutrient supply in pregnancy, maternal food intake increases, peripheral insulin resistance increases and there is an up-regulation of maternal pancreatic islet function alongside a lowered threshold for glucose stimulated insulin all of which channel maternal nutrients to the fetus [11]. During pregnancy maternal fat depots are also laid down, and changes take place in the mammary gland in preparation for the supply of milk in the postnatal period. Placental hormones are key to the induction and maintenance of these changes in maternal physiology during pregnancy [12].

Perhaps the most well studied placental hormones belong to the somatotropin/prolactin family. These belong to a complex family of hormones related to prolactin (expressed in the pituitary) that originated from a common ancestral gene. This same ancestral gene also gave rise to pituitary expressed growth hormone and, in some mammalian species, further duplication to produce placental-specific growth hormone. Together, all these hormones could be viewed as one gene family encompassing both prolactin- and growth hormone-like activities, distinguished by their interaction with their cognate receptors, the growth hormones receptor and the prolactin receptor [13]. In mice, there are 22 prolactin
family members expressed almost exclusively in the placenta and no equivalent for placental growth hormone gene [14]. However, only a small subset of these hormones possess the capacity to activate the prolactin receptor [15]. In humans there are two functional prolactin family members expressed in the placenta (chorionic somatomammotropin hormone 1 and 2) encoding placental lactogen, and one placental growth hormone gene [16]. One of the key changes in pregnancy thought to be induced by a growth hormone-like activity is the increase maternal insulin resistance required to channel maternal glucose to the fetus [17]. Glucose is transported via a passive process that requires a higher concentration in the maternal circulation than in the fetus. Insulin resistance contributes to this gradient but must be balanced by the ability to respond rapidly to meals in order to avoid dangerously high levels of blood glucose. This balance is achieved, in part, through the prolactin-like function of this gene family up-regulating maternal islet function and accommodating the increased demand for insulin during pregnancy [18, 19]. Placental lactogens also play an important role in preparing the new mother to provide nutrients after birth by priming the mammary gland for lactation [20]. Placental lactogens may additionally be involved in the induction of maternal care. Both pituitary prolactin and placental lactogen have been shown to stimulate maternal care in non-pregnant rodents likely influencing maternal care through their interaction with the maternal prolactin receptor, most clearly demonstrated in rodents [21].

In mice and humans, placental lactogens are expressed by derivatives of the trophoblast lineage, a specialised cell type that emerges from the first differentiation event in development [22]. Early in pregnancy, trophoblast cells contribute to the choriovitelline (yolk sac) placenta, a structure that is replaced as gestation proceeds by the chorioallantoic (mature) placenta. In mice, there are four main region of the chorioallantoic placenta: the maternally-derived decidua, a single layer of cells with giant nuclei called the
secondary parietal trophoblast giant cells (TGCs), the junctional zone and the labyrinth
[23]. The junctional zone contains two distinct lineages, the spongiotrophoblast and the
glycogen cells, while the bulk of the labyrinth is composed of fetal endothelium surrounded
by a trilaminar layer of trophoblast-derived cells consisting of a single mononuclear TGC
layer (sinusoidal; previously called trophoblast layer I) and two multinucleated
syncytiotrophoblast layers that function in nutrient transport (I and II; previously called
layers II and II). Three other distinct TGC placental lineages have been classified. The
spiral artery (SpA) TGCs line the maternal blood system on entry to the placenta, the canal
(C-) TGCs line the maternal blood canals in the junctional zone and the channel (Ch-)
TGCs line the maternal blood spaces located just beneath the decidua where maternal
blood leaves the placenta [14, 23-25]. Placental lactogens are expressed by all TGC
subtypes, the spongiotrophoblast and the glycogens cells. Moreover, the large nuclei of
the TGCs result from endoreduplication with specific parts of their genome further over
replicated including regions encoding placental lactogens [26]. Along with their close
proximity to the maternal circulation, these gene amplification events suggest TGCs as a
major source of placental lactogens in mice. In contrast, the human placenta appears to
possess a single major cell type manufacturing hormones termed the syncytiotrophoblast.
These multinucleated layer of cells is generated from the fusion of cytotrophoblast cells
lying beneath, and both cell types overlie a core of mesenchymal cells that make up the
numerous chorionic villi of the human placenta [13].

The role of placental hormones in manipulating the mother to provide resources to her
offspring both \textit{in utero} and in the immediate postnatal period suggested placental
hormones as candidates for the expression of parent-offspring conflict [27, 28]. Apart from
one rare example in the new world mouse, \textit{Peromyscus} [29], there is no evidence that
placental hormones or their maternal receptors are directly subject to genomic imprinting.
However, a fetally-derived product of the paternally-expressed imprinted \textit{delta-like homolog 1} gene (\textit{Dlk1}) has recently been shown to reach the maternal circulation and influence maternal metabolism [30], and it is possible that the \textit{Igf2} gene product has a similar function [31]. We hypothesised an alternative mechanism whereby imprinting could influence placental hormone production - by regulating the placental lineages that express these hormones [32]. This hypothesis was based initially on our studies on one imprinted gene, \textit{Pleckstrin homology-like domain family a member 2} (\textit{Phlda2}). \textit{Phlda2} is a maternally expressed imprinted gene that encodes a PH domain-only protein expressed most highly in the ectoplacental cone and the visceral endoderm of the yolk sac [33-35]. Our studies on \textit{Phlda2} revealed a precise function for this gene in negatively regulating the size of spongiotrophoblast compartment, without altering the gross contribution of other placental lineages [7, 36-39]. Loss-of-function of \textit{Phlda2} resulted in a much larger spongiotrophoblast, approximately twice the volume normally present. Conversely, a two-fold gain in expression of \textit{Phlda2} (modeling loss-of-imprinting) reduced the size of this compartment by 50%. The spongiotrophoblast is a key site for the production of placental lactogens, pregnancy-specific glycoproteins and a number of other hormones important in pregnancy [14, 37]. Using the same dosage interrogating approach applied to \textit{Phlda2}, we have recently shown that overexpression of a second maternally expressed imprinted gene, \textit{Achaete-scute complex homolog 2} (\textit{Ascl2}), repressed both the spongiotrophoblast and the parietal TGCs [40]. Although mouse models with increased dosage have not been reported, loss-of-function of studies suggest that \textit{Paternally expressed gene 3} (\textit{Peg3}), \textit{Paternally expressed gene 10} (\textit{Peg10}), \textit{Cyclin-dependent kinase inhibitor 1c} (\textit{Cdkn1c}) and several non-classically imprinted genes located on the X chromosome also regulate the placental endocrine lineages positively or negatively in a manner generally consistent with parental conflict [32]. As a consequence, imprinted genes indirectly modulate the expression of placental hormones by regulating the size of the placental endocrine
compartment. This is illustrated most elegantly with the \textit{Phlda2} gene where loss of expression resulted in a 2-fold increase in expression of the placental lactogens expressed in the spongiotrophoblast while a double dose of \textit{Phlda2} resulted in a 50\% decrease in their expression \cite{41}.

Functional data demonstrating that imprinted genes regulate placental hormone lineages in species other than the mouse is sparse. Reduced expression of \textit{PHLDA2} is a common feature of bovine cloning associated with overgrowth of both the fetus and placenta but not the altered expression of placental hormones \cite{42, 43}. Elevated placental \textit{PHLDA2} has been reported in a number of studies on human fetal growth restriction, fetal death and low birth weight \cite{44}. In our recent study on women with a perception of reduced fetal movements (RFM), we found placental \textit{PHLDA2} expression was 2.3 fold higher in RFM pregnancies resulting in delivery of a growth restricted infant compared with a normal birth weight infant \cite{45}. Importantly, we found a significant inverse association between placental \textit{PHLDA2} levels and maternal serum placental lactogen (hPL) levels suggesting that \textit{PHLDA2} may regulate the production of placental hormones in human pregnancies.

In another study focusing on prenatal depression, we examined placental expression levels of four genes, \textit{PHLDA2}, \textit{CDKN1C}, \textit{PEG3} and \textit{PEG10}, based on the conserved imprinting status between mouse and human and their predicted role in regulating production of placental hormones \cite{32}. In women with clinically diagnosed depression during pregnancy, we observed significantly lower expression of placental \textit{PEG3}. We also found low placental \textit{PEG3} in pregnancies where women reported a depressed mood assessed using two self-rating psychometric questionnaires: Edinburgh Postnatal Depression Scale (EPDS), used as a measure of maternal prenatal depressive symptoms \cite{46} and the Spielberger State-Trait Anxiety Inventory (STAI), used as a measure of anxiety symptoms \cite{47}. Both diagnosed and self-reported symptoms of depression were
also significantly associated with low expression of hPL. Critically, we found a positive correlation between placental PEG3 and hPL expression. In mice, loss-of-function of Peg3 has been reported to result in changes in the expression of a number of placental lactogens [48, 49] although a specific endocrine lineage analysis has not been performed. Together, these data are consistent with a role for PEG3 in regulating hPL expression in humans. Peg3 and another paternally expressed imprinted gene, Peg1, have previously been linked to maternal care in rodents [50, 51]. In both these reports the dam carried the inactivating mutation for the respective gene, with considerable impact on a number of physiological as well as neurological processes. Moreover, a recent study did not find a maternal care deficit when a second Peg3 targeted allele was generated, and examined on a different strain background [52]. A role for loss of Peg3 in the placenta influencing any aspect of maternal behaviour in rodents has yet to be reported.

In human pregnancies, a number of complications can commonly co-occur. Women with prenatal depression are three times more likely to have a low birth weight baby [53]. Maternal depression in the first and second trimester is associated with an increased risk of gestational diabetes while women with gestation diabetes have a >4-fold risk of postnatal depression with elevated depressive symptoms particularly high among non-obese women [54]. A recent systematic review reported that women with symptoms of postnatal depression were less likely to breastfeed exclusively and more likely to terminate breastfeeding earlier [55]. This could be interpreted to mean that postpartum depression negatively impacts maternal breast feeding behaviour or that pressures around breast feeding increase the risk of depression. However, given the potential for placental hormones to influence both lactation and maternal behaviour, it is possible that difficulties with breast feeding and postnatal depression are manifestations of the same underlying problem, placental endocrine dysfunction (Figure 1). Similarly, prenatal depression
accompanied by low birth weight could be indicative of placental endocrine dysfunction (Figure 1). As well as these manifestations of maladapted pregnancy, a transient increase in the risk of breast cancer diagnosis has been reported for first time mothers likely linked to the pregnancy induced changes in mammary development [56]. It is therefore possible that placental hormone dysfunction could influence both short term and long term risk of breast cancer. Measuring placental hormones in maternal blood is already an important diagnostic tool early in pregnancy and the use of DLK1 assays in characterising types of fetal growth restriction holds great promise [30]. New techniques such as multiplexed quantification of fetal RNAs circulating in maternal blood may provide even more accurate tools, and for a variety of conditions [57].

In summary, current data supports a conserved function in mammals for imprinted genes in regulating placental hormones via regulating the size of the placental endocrine compartment. These data essentially support the prediction by David Haig more than 20 years ago that imprinted genes would regulate signaling between the mother and her fetus. Importantly for human pregnancies, placental hormones play key roles in driving the physiological and, potentially, behavioural adaptations required to support optimal fetal growth and postpartum care. It is therefore plausible that aberrant imprinting in the placenta contributes to the common co-occurrence of a number of complications of pregnancy including low birth weight, maternal mood disorders, gestational diabetes and poor breast feeding. Finally, given the link between all these complications and poor long term outcomes for children, the detrimental consequences of placental endocrine function may influence offspring wellbeing considerably beyond the period of pregnancy (Figure 1).
References


Figure 1. Aberrant imprinting and placental endocrine dysfunction.