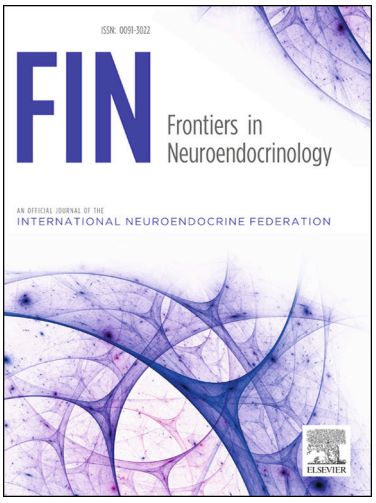

Imprinted genes influencing the quality of maternal care

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Imprinted genes influencing the quality of maternal care.

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Abstract

In mammals successful rearing imposes a cost on later reproductive fitness specifically on the mother creating the potential for parental conflict. Loss of function of three imprinted genes in the dam result in deficits in maternal care suggesting that, like maternal nutrients, maternal care is a resource over which the parental genomes are in conflict. However, the induction of maternal care is a complex and highly regulated process. Unsurprisingly many gene disruptions, as well as adverse environmental exposures in pregnancy, result in maternal care deficits. Recent compelling evidence for a more purposeful imprinting phenomenon comes from studying the impact of two imprinted genes, *Phlda2* and *Peg3,* expressed in the placenta on the mother's behaviour. The explicit demonstration that imprinted genes expressed in the offspring influence maternal behaviour lends significant weight to the hypothesis that maternal care is a resource that has been manipulated by the paternal genome.

Key words: genomic imprinting; maternal care; placenta

Genomic imprinting

Genomic imprinting is the term used to describe an epigenetic process whereby the parental alleles are marked in the germline to be differentially expressed in the offspring [1]. In modern mammals, this remarkable process had resulted in more than 100 protein coding genes and numerous noncoding RNAs being expressed predominantly from a single parental allele. Some imprinted genes are tightly regulated and globally monoallelically expressed, some show tissue and/or temporal specificities in their imprinted expression while others are paternally or maternally expressed in different tissues through the differential use of imprinted promoters. The existence of imprinted genes was essentially predicted prior to their physical discovery through the recognition of a conflict between parent and offspring [2], in particular those imposed by pregnancy [3]. Pregnancy represents a unique and challenging dilemma for mammals as considerable and almost exclusively maternal resources are required to produce offspring but the mother must, at the same time, ensure her own survival for the production of future offspring. Resource allocation has traditionally referred to nutrient allocation both during pregnancy and postnatally (lactation) but the finding in 1998 that an imprinted gene, called *Paternally expressed gene 1* (*Peg1*) influenced maternal behaviour [4] suggested that maternal care provision is another resource which can potentially be manipulated by the parental genomes.

Maternal behaviour is broadly defined as “the pattern of a mother's behaviour that appears to enhance her offspring's survival and reproductive success” [5]. The capacity to respond in a maternal manner is present in most, but not all, female mammals. The natural process of transitioning from a nulliparous female to a mother culminates in the initiation of enhanced maternal care towards new born offspring. These changes irreversibly alter the new mother’s motivational and behavioural repertoire driving an increased interest in sensory modalities associated with young. In humans, a failure to make the appropriate transition into motherhood can have lasting effects upon maternal health in the weeks, months and years after birth. Unsurprisingly, early social experiences gained through the relationship between the new mother and her offspring also significantly affects the developmental trajectory of her offspring [6; 7]. These early life interactions are associated with fundamental changes in the brain and behaviour that persist right through into adulthood. Therefore understanding how maternal responses are primed prenatally and further stimulated from birth is important not only for maternal mental health, but also for the health of future generations.

Over the past two decades, there has been considerable progress in the foundational aspects of mammalian reproduction, including the processes involved in parturition, lactation,

behaviour and stress response. These discoveries have predominately been made in rodents. While rodents do not recapitulate all the sophisticated aspects of humans, mice and rats have been used as a model system to study maternal behaviour because they display behaviours that are predictable and testable, making them the ideal choice of animal model. Mice, and more recently rats, are also genetically modifiable allowing functional assessment of specific genes and, with conditional models, the function of specific genes with a spatial and/or temporal focus. Rodents give birth to relative immature young (altricial) with limited mobility, incapable of fending for themselves or maintaining their own body temperature. Consequently, the new mother must exhibit maternal behaviour at parturition to ensure their immediate survival. While both virgin females and males can respond to pups, both require several days of exposure to the pups before parental behaviours emerge [8].

Maternal behaviours can be categorised as direct and pup-orientated or indirect and non-pup orientated. Pup oriented behaviours are displayed as discrete acts of maternal care present from parturition. These include retrieving, grouping, crouching over pups to encourage suckling, and licking/grooming each individual pup to stimulate urination and heighten sensory stimuli developed only through nursing contact [9]. Non-pup orientated behaviours encompass everything from finding and remembering food stores and water sources, identifying danger zones to building nests [9]. Nests, even if already built, are re-constructed in a more elaborate and functional way for the young, to provide protection and warmth [10]. Late in pregnancy the female's aggressive behaviour increases, important for defending resources [11]. Postpartum dams show heightened aggression towards intruders stimulated by the presence of the suckling pups, and reduced anxiety thought to be important for pup focused behaviour [12]. Pregnant females have increased appetite throughout pregnancy to support the developing fetus and to generate surplus energy stores (fat) for lactation with further increases in feeding and drinking postpartum [13]. Alterations in behaviour cannot come at the cost of maternal wellbeing. Postpartum the dam must continue to maintain her wellbeing through self-grooming, enhanced feeding and drinking to ensure survival of the litter and her future reproductive health. Taken together these behaviours are necessary to maximise the level of care and fitness she can bestow upon both herself and her young.

Hormones and maternal behaviour

Maternal care is primed in pregnancy through the direct action of hormones on the maternal brain, both those produced by the mother and also those that originate from the fetally-derived placenta [8; 13; 14]. A number of hormones are produced by the maternal brain and function locally in the induction and maintenance of maternal care including prolactin [15;

16], serotonin [17], dopamine [18; 19; 20], oxytocin [21; 22; 23] and vasopressin [24; 25]. However, initial external stimuli are required to initiate the cascade of changes that occur to the maternal brain. In mice, prolactin secretion from the pituitary is initially stimulated by mating [26] and then secreted in surges in the afternoon and night until mid-gestation [14; 27]. Prolactin has been shown to suppress the stress response in part through inhibiting the hypothalamic-pituitary-adrenal axis [28; 29], to induce changes in activity of oxytocin neurons [30], to stimulate neurogenesis [26], to suppresses ovulation and to regulate its own secretion [31]. At mid gestation, prolactin secretion is suppressed and superseded by the placental lactogens (Prls) which are lactogenic hormones related to prolactin but produced by the fetally-derived placenta. Prls predominate for the rest of pregnancy until, the night before parturition, prolactin secretion again surges in response to progesterone withdrawal [14; 27]. Prolactin stimulates maternal care [15; 16] and prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behaviour and maternal neurogenesis [26] while low levels of prolactin are associated with increased postpartum anxiety and decreased pup retrieval, alongside a reduction in pregnancy-induced neurogenesis [32]. Although the data is less clear, prolactin may also influence maternal neurogenesis in the sub granular zone of the hippocampus, important in learning and memory [33; 34]. Prolactin likely functions entirely via the prolactin receptor (Prlr) as loss of function of this receptor in nulliparous females, either homozygously or heterozygously, results in a defect in foster pup–induced maternal behaviour [35] and pregnant *Prlr*$^{-/+}$ (heterozygous) dams display a 50% reduction in forebrain neurogenesis in the subventricular zone [26]. The fact that animals with a disrupted prolactin gene still exhibit some maternal behaviour [36], combined with the knowledge that Prl3d1 (PL-I) and Prl3b1 (PL-II) are known to bind and activate the Prlr receptor [37] suggests that these Prls contribute to the induction of maternal care, although this has only been shown indirectly through the direct infusion of placental lactogen into the medial preoptic area of nulliparous rats [38].

Lactogenic hormones are not solely responsible for initiating changes in the maternal brain. Steroid hormones (progesterone and oestradiol) produced primarily from the ovary diffuse across the blood brain barrier to act directly on the maternal brain [14]. Progesterone steadily increases during the pregnancy priming the female brain to respond to an acute increase in oestradiol that occurs at parturition while a fall in progesterone secretion at the end of pregnancy synchronises the onset of maternal behaviour with parturition [14; 39; 40]. Steroid hormones are produced from the ovary partly controlled by lactogenic hormones [40] but, while the placenta expresses some stereogenic enzymes, it does not appear to play a role in *de novo* steroid synthesis, at least in rodents [41]. Similarly, the placenta expresses

components of the serotonin, dopamine, oxytocin and vasopressin synthesis pathways but expressed at such low levels [42] that their site of action, if any, is likely to be fetal rather than maternal, as shown for serotonin [43]. The mouse placenta manufactures other protein hormones including secretin and galanin at more functionally convincing levels [44; 45]. Secretin is required for hippocampal synaptic plasticity and *secretin receptor* mutant mice display abnormal social and cognitive behaviours [46; 47] while galanin-expressing neurons in the maternal hypothalamus are important for maternal behaviour with activation inducing pup grooming in virgin females and ablation of these neurons resulted in postpartum females attacking rather than caring for pups [45].

The complexity of changes to the maternal brain both during pregnancy and postpartum are such that it is not surprising that a vast number of genetic modification to the mother result in deficits in maternal behaviour. Indeed, when we surveyed the literature on genetic mutations leading to alterations in maternal behaviour, we found 64 examples reporting a deficit in maternal care and none that enhanced this behaviour [42]. The caveat is, of course, that it is much easier to spot a deficit in maternal care, particularly when there is reduced pup survival, than it is to detect improved maternal care. However, the potential for hormones manufactured by the placenta to influence the programming of the maternal brain in pregnancy suggests that placental endocrine changes driven by genetic alterations may also impact maternal behaviour, as now demonstrated for the first time by our lab [42].

Disruptions of imprinted genes in the dam resulting in maternal care deficits

***1) Paternally expressed gene 1* (Peg1)**

Peg1 (aka *Mest*) was the first imprinted gene linked to maternal care in mice [4]. *Peg1* was initially identified as an imprinted gene through subtractive hybridization between cDNAs from normal and parthenogenetic embryos, and found to map to the proximal region of mouse chromosome 6 [48]. The maternal promoter and part of exon 1 acquire DNA methylation during oocyte maturation [49] which is present in fetal tissues [50]. *Peg1* is a member of the divergent α-β hydrolase protein family where 8 β-sheets are connected by α-helices in the core of the protein [51]. During fetal development *Peg1* is predominantly expressed in mesodermal tissues including heart, lung, cartilage, skeletal muscle and tongue, and also within the remnants of Rathke's pouch, the amygdala, ventral hippocampus, main and accessory olfactory bulbs, cortex, dorsal hippocampus and striatum and the choroid plexus [4; 48]. Expression in the developing mouse midbrain overlaps with mesodiencephalic dopaminergic neurons before becoming restricted to part of the substantia nigra in adults [52].

Loss-of-function of *Peg1* (paternal inheritance of the targeted allele) was found to result in a ~15% proportionate growth restriction of the fetus and placenta at E18.5 when studied on the 129Sv strain background [4]. In this study, less than 50% of *Peg1* mutants survived to adulthood and those that survived remain small with no evidence for catch-up growth. When *Peg1* mutant females (paternal inheritance of targeted allele) were mated with wild type males, they underwent a normal first pregnancy (although this was not assessed in great detail) and delivered at term. However, *Peg1* mutant dams frequently failed to remove extraembryonic membranes, eat the placenta or nurse their pups and nearly 90% of newborn died shortly after birth. In a pup retrieval task, mutant dams were found to sniff their pups indicating an intact olfactory response but did not retrieve them, and were disinterested in nest building. Neonates cross fostered to wild type foster mothers survived attributing the deficit to mutant dam rather than her offspring.

2) Paternally expressed gene 3 (*Peg3*)

Peg3 was the second imprinted gene linked to maternal care in mice [53]. *Peg3* was identified with *Peg1* [48] and in a second screen for novel imprinted genes and maps to the proximal mouse chromosome 7 [54]. Like *Peg1*, the maternal promoter region of *Peg3* inherits a DNA methylation mark from the oocyte [49]. *Peg3* encodes a Kruppel C2H2-type zinc finger protein [54] which function to repress gene transcription [55; 56; 57]. Expression is predominantly from the paternal allele although maternal allele expression has been reported in the adult brain [58]. During fetal development *Peg3* expression overlaps substantially with *Peg1* [4; 53].

Loss-of-function of *Peg3* (paternal inheritance of the targeted allele) results in fetal and placental growth restriction [53; 57; 59]. Mice are born small and remained small framed but deposit excess fat despite consuming less food [60]. Adult male and female mutant mice display altered behaviour with males failing to respond normally to sexually receptive females [61] and females exhibiting deficits in classic tests of maternal behaviours in their first pregnancy [53]. *Peg3* mutant dams sniff their pups in the pup retrieval task but fail to return them to the nest in a timely manner, are slow to crouch over them and are poor nest builders. These original studies were made on the 129Sv stain background but despite naturally occurring differences in maternal behaviour [62], poor quality maternal care was also apparent when this same modification was examined on a C57BL/6J strain background [63], although not with a different modification [59]. When presented with wild type pups, parturient and nonparturient multiparous dams as well as virgin females all show impaired maternal behaviour [63], again attributing the deficit to mutant female. These deficits may in

part be explained by a reduction in oxytocin-positive neurons in the hypothalamus compared to wild-type females [53; 63].

3) Type 3 deiodinase (Dio3)

Loss-of-function of *Dio3* (paternal inheritance of the targeted allele) has more recently been linked to a deficit in maternal care [64]. *Dio3* was identified as an imprinted gene in part due to its proximity to an imprinted gene cluster on chromosome 12 spanning *Dlk1* and *Gtl2* [65; 66; 67]. Preferential allelic expression of *Dio3* from the paternal allele occurs in most but not all fetal tissues [68] and the promoter lacks direct DNA methylation [66]. During development *Dio3* is expressed in the fetal brain, eye, palate, tongue, skeletal muscle around the digits, mesenchyme of the frontal region, upper lip, and lower jaw, liver, gut mesentery, testes and in the labyrinthine trophoblast of the placenta. *Dio3* is highly expressed in mature brain [65] where expression is reportedly biallelic [68].

Dio3 encodes an enzyme which inactivates the hormones thyroxine and 3,5,3′-triiodothyronine [69]. *Dio3*-deficiency leads to brain thyroid hormone excess. Female mice show normal social interest in unfamiliar female mice outside their home cage but in their home cage they are aggressive towards unfamiliar females [64]. *Dio3* mutant dams make flatter nests and show a deficiency in pup retrieval in their first but not their second pregnancy. They spend less time interacting with their pups and there is a high rate of pup mortality. *Dio3* mutant females also show a mild olfactory impairment. How much of this behaviour is really specific to pregnancy is unclear as non-pregnant females exhibit increased activity and decreased anxiety/depression-like behaviour [64] alongside many other significant impairments [70].

Given the extensive and complex changes that occur in the maternal brain during pregnancy and postpartum [14; 71], it is perhaps not surprising that the genetic disruption of a number of imprinted genes in the dam result in deficits in maternal behaviour. A number of other imprinted genes are required for the normal function of the postnatal brain [72] and may also be important for maternal care. However, any finding based on global targeted deletions must be interpreted cautiously as loss-of-function of essential genes will have a catastrophic impact on many aspects of physiology and behaviour. Determining how these findings are to studies on imprinting will require both dose- and brain-specific manipulations.

Postnatal influence of imprinted genes expressed in the offspring on maternal care.

The presence of pups is clearly important for the manifestation of maternal behaviour and any mutation impacting pup characteristics has the potential to result in a secondary effect

on maternal behaviour. From birth pups begin communicating to their mothers using clicks and whistles. These ultrasonic vocalizations (USVs) increase in intensity and frequency when pups are separated from their mothers - hence the alternative and more forlorn term - “whistles of loneliness” [73]. USVs are known to induce maternal behaviours such as nest building, pup retrieval and nursing [74; 75; 76; 77]. Loss of several genes within the *Ubiquitin-protein ligase E3A* (*Ube3a*) region [78] increase pup USVs while increased expression of *Ube3a* impairs these vocal communications [79]. Stimulation of dams by the act of suckling (but not lactation *per se*) initiates and maintains maternal aggression postpartum by stimulating oxytocin release from the maternal hypothalamus [80; 81]. Loss of function *MAGE Family Member L2* (*Magel2*) [82], *Peg3* [83] and *Growth factor receptor-bound substrate 10* (*Grb10*) [84] have all been reported to impact the ability of the pups to suckle with the potential of impacting maternal aggression. Even the pup’s ability to maintain their own body temperature could influence maternal behaviour requiring increased maternal 'crouching', elevating demand on maternal resources. Several imprinted genes have been reported to play a role regulating brown adipogenesis impacting thermogenesis (body warmth) including *Delta Like Non-Canonical Notch Ligand 1* (*Dlk1*)-*Dio3* domain [85], *cyclin dependent kinase inhibitor 1C* (*Cdkn1c*) [86] and *G protein alpha-subunit* (*Gnas*) [87]. However, the effect of altered neonatal thermogenesis on wildtype maternal behaviour in the context of these models has yet to be explored.

Prenatal influence of imprinted genes expressed in the offspring on maternal care.

The placenta is predicted to play a role in programming maternal care through the production of hormones directly or indirectly implicated in maternal behaviour, although until recently this has never been formally demonstrated in a physiologically relevant model. The mouse placenta is grossly composed of three histologically distinct regions: the labyrinth where nutrient exchange takes place, the junctional zone which is a major endocrine compartment and the decidua which is the maternally derived component [88]. Seven distinct placental lineages express hormones including five trophoblast giant cell subtypes in close proximity to the maternal circulation, and the spongiotrophoblast and glycogen cell lineages located within the junctional zone [89; 90; 91; 92]. Of these, the spongiotrophoblast is the most substantial lineage in the mature mouse placenta [93]. Endocrine lineages expresses *Prls* [89; 90] and *pregnancy specific glycoproteins* (*Psgs*), a 17 member multigene gene family that modulate the maternal immune system and remodel vasculature [94]. Several maternally expressed imprinted genes have been shown to regulate these lineages [95] including *Pleckstrin homology-like domain family A member 2* (*Phlda2*) [96] and *Peg3* (in press).

***Pleckstrin homology-like domain family A member 2* (*Phlda2*)**

Phlda2 is expressed primarily from the maternal allele only in extraembryonic structures with maternal bias or biallelic expression in the embryo and adult [97]. *Phlda2* is expressed in the ectoplacental cone where the progenitors of many cell types of the mature placenta reside, and in the extraembryonic membranes (the visceral endoderm of the yolk sac) [97; 98]. From E10.5 *Phlda2* expression is restricted to type I syncitiotrophoblast and, at a lower level, type II syncitiotrophoblast cells in the labyrinth zone, and declines in expression from E14.5.

Studies from the Tycko laboratory and our research group defined a key role for *Phlda2* in the development of the placenta [96; 99; 100; 101; 102]. Loss-of-function (maternal inheritance of targeted allele) resulted in placental overgrowth with a substantial increase in placental glycogen but with a negative impact on late fetal growth [96; 99]. Overexpression of *Phlda2* also resulted in fetal growth restriction which we demonstrated first using at a transgenic mouse model in which *Phlda2* was overexpressed at four-fold the normal level from a bacterial artificial chromosome (BAC) transgene [100]. Overexpression at both four-fold and two-fold the normal level resulted in a similar degree of growth restriction of both the fetus and the placenta [96; 100; 101; 102]. Further assessment of the placental phenotype in the single copy line, effectively modelling loss of imprinting of *Phlda2* (two-fold expression), identified the specific reduction of only one placental lineage, the spongiotrophoblast [96; 101; 102]. We showed that *Phlda2* suppresses the proliferation of the spongiotrophoblast from as early at E10.5 resulting in a 50% loss of this lineage by E14.5. Making use of the *Phlda2* deletion allele, we were able to show that loss of *Phlda2* resulted in a substantial increase in the contribution of the spongiotrophoblast lineage to the mature mouse placenta [96]. This work identified *Phlda2* as a negative rheostat controlling the ultimate contribution of the spongiotrophoblast lineage to the mature mouse placenta. Given the expression of placental hormones from the spongiotrophoblast, this implied that *Phlda2* indirectly regulates a vast array of placental hormones, further supported by RNA expression analysis that identified the spongiotrophoblast transcriptome [96]. Specifically, *Phlda2* regulates the expression of a number of placental lactogens including *Prl3b1*, known to bind the prolactin receptor [37]. Together, this led us to hypothesise that the expression of imprinted genes in the placenta might influence maternal adaptations to pregnancy via placental hormones [95]. Given the known function of prolactin and the prolactin receptor in maternal care, we further hypothesised that placental *Phlda2* might also influence maternal behaviour, which we tested by exposing wild type females to different offspring *Phlda2* gene doses [42].

A key aspect to this study was the maintenance of the dams genetically wild type status in order to isolate the function of *Phlda2* in the offspring, achieved through recipient transfer of

embryos from genetically modified parents into wild type recipients (a mouse version of IVF). Wild type female mice exposed to offspring with three different doses of *Phlda2* – either two active alleles (loss-of-imprint), one active allele (normal imprint) or no active allele (loss of maternal allele) – showed alterations in their hypothalamic and hippocampal transcriptomes during pregnancy, regions important for maternal-care behaviour. Each group showed distinct changes that included alterations in G protein-coupled receptors (GPCR) pathways through which neuropeptides and hormones mediate their action, olfactory transduction pathways important for maternal care [14; 103; 104] and the gonadotropin-releasing hormone signalling pathway, implicated in maternal care and known to respond to prolactin [105; 106]. In contrast to our prediction that increased placental hormone would translate to "better mothers", in the pup retrieval task dams exposed to higher levels of placental hormones in pregnancy (loss of *Phlda2* expression) took significantly longer to retrieve their first pups than either fully wild type dams or those exposed to the lower dose of hormones (loss of *Phlda2* imprint). Similarly, in the nest building task the dams we predicted would better at this task performed very poorly, with only one dam building a nest and putting her pups inside the nest. In stark contrast, the dams we predicted would be “worse mothers” (exposed to less hormones) actually performed better even than the fully wild type controls in nest building. However, in an undisturbed situation, all dams were able to effectively make nests and gather their pups within the nest. Pups gained weight appropriately arguing against a deficit in lactation and vocalisation (USVs) were normal when pups were separated from their mothers. A further exploration of dams’ behaviour in the disturbed situation (nest building task) revealed that dams exposed to higher levels of placental hormones in pregnancy were prioritising caring for their pups (licking and grooming) and themselves (self-grooming, feeding) over the nest building. As a final test, we asked whether dams exposed *in utero* to higher levels of placental hormones maintained their enhanced nurturing behaviour when presented with wild type pups from birth. These exposed dams similarly displayed enhanced nurturing towards the wild type fostered pups despite a considerable disruption imposed by the fostering process, supporting our original hypothesis that the priming of maternal care occurred prenatally. Overall the changes in maternal behaviour we observed were subtle and did not have a negative impact on either maternal or pup welfare, at least in the short term evidenced by appropriate pup weight gain. However, we do not know what the longer term consequence will be either for the dams in their second pregnancy, not for the offspring exposed to less optimal maternal care. Nor have we explored the neural changes or identified the specific placental hormone(s) mediating the relationship between the placenta and the maternal brain. Given the requirement for two copies of a functional *prolactin receptor* gene in maternal care [26; 35], and the knowledge that Prl3b1 binds this receptor [37], this seems a promising avenue to explore. Irrespective

of the gaps still remaining in our knowledge, this work firstly demonstrates the importance of optimal placental function in maternal care, at least in rodents, and highlights a new role for genomic imprinting in influencing maternal care giving before the offspring are born.

Peg3

As has already been discussed, loss-of-function of *Peg3* in adult female mice negatively affects their maternal behaviour. *Peg3* is expressed in both the developing fetus and in the placenta in the junctional zone and trophoblast giant cells [107]. Microarray studies on placental gene expression in response to loss of *Peg3* suggested an impact on placental hormones [57; 108]. Together with our data on *Phlda2*, this led us to speculate that placental *Peg3* may also function prenatally to influence maternal behaviour, potentially in an antagonistic manner to *Phlda2*. To test this hypothesis, we generated females carrying litters of all *Peg3* mutant pups, this time by natural mating of wild type dams with males homozygous for the *Peg3* targeted allele [109]. We observed very subtle changes in maternal novelty reactivity during pregnancy but no significant alterations in gene expression in the prenatal hypothalamus or hippocampus, at least at E16.5. After birth, dams exposed to *Peg3* mutant pups were slower to retrieve but showed no differences in nest building or the care of their pups during the nest building task. However, during the elevated zero maze task, which measures anxiety, the exposed dams showed evidence of increased anxiety. When we measured USVs, we found that the *Peg3* mutant pups displayed fewer calls. Together, these data suggest that *Peg3* predominantly influences maternal care in the post-natal period through influencing direct communication to the mother. A major caveat to this conclusion is that we did not undertake a cross fostering experiment to isolate the pre- and post-natal influence of *Peg3*. A further caveat is our recent finding of a sexually dimorphic placental endocrine phenotype in *Peg3* mutant mice (Tunster, in press). Loss of function of *Peg3* results in a substantial loss of both the spongiotrophoblast and the glycogen cell lineage in male placenta, with female placenta showing an attenuated phenotype. Furthermore, when we examined the expression of placental hormones, female placenta showed very few alterations and some placental hormones were actually expressed at higher levels, despite the loss of spongiotrophoblast cells expressing these hormones. This finding is explained through a second sexually dimorphic function of *Peg3* as a transcriptional repressor of some placental hormone genes [57]. Effectively, loss of *Peg3* results in fewer endocrine cells expressing higher levels of a subset of hormones both positively and negatively regulating the production of placental hormones with different consequences for male and female placenta. This new observation adds a further layer of complexity to our interpretation of the maternal behavioural changes in response to loss of

offspring *Peg3*. Since mouse litters are composed of both male and female foetuses, the presence of female placenta may "rescue" deficits in the mutant male placenta.

Overall, these experiments highlight the bidirectional relationship between the mother and her offspring. *Phlda2*, which has been silenced by the paternal genome, functions normally to repress the production of placental hormones whereas *Peg3*, silenced by the maternal germline, functions to promote the production of placental hormones, and is also required for pups to effectively communicate to their mothers. During the evolution of mammals, paternalisation of *Phlda2* may have boosted maternal care via the increased production of placental hormones whereas maternalisation of *Peg3* may have decreased maternal care by decreasing the production of placental hormone and decreasing the vocal demands from the pup. *Phlda2* is not imprinted in marsupials [110] but it is not known when, during the evolution of mammals, *Peg3* acquired an imprinted status [111]. Nonetheless, this pre- and post-natal sequence of events suggests a continual and subtle rebalancing of maternal care allocation in mammals through the action of imprinted genes. Moreover, based on their function in regulating placental endocrine lineages [95], we predict that a number of other imprinted genes will similarly influence maternal care giving.

Relevance to humans

Studies on the function of imprinted genes in instructing maternal behaviour have been performed solely in mice. However, there is indirect data to suggest that these genes may have some similar functions in other mammals including humans. Higher placental expression of *PHLDA2* has been reported in association with low birth weight in a number of studies [112]. Traditionally, infants are classified as low birth weight if born weighing less than 2.5 kg at any gestational age (United Nations Children's Fund and World Health Organisation 2004). Low birth weight can be a consequence of a premature birth or fetal growth restriction where the baby has not reached its genetic growth potential, or both factors. Mice with two-fold expression of *Phlda2*, similar to the level reported in affected human pregnancies, display a slowdown in fetal growth late in gestation and are born low birth weight supporting a causal role for elevated *PHLDA2* in driving fetal growth restriction and consequent low birth weight in human pregnancies. The inverse correlation between placental *PHLDA2* expression and maternal serum levels of human placental lactogen [113] further suggests that *PHLDA2* negatively regulates the production of placental hormones in humans and well as mice. Although there are substantial differences in the placental structure between man and mouse, and also in the type and function of placental hormones [114], this suggests the possibility that *PHLDA2* may have a role in the induction of maternal care in human pregnancies. Little is known about the quality of care mothers provide to their

low birth weight newborn, and any observations are likely confounded by multiple factors surrounding the birth. However, while maternal care and maternal depression are two very different facets of pregnancy, depression could be a manifestation of miss programmed maternal behaviour in pregnancy. Both preterm birth and low birth weight are more common in pregnancies where mothers report depressive symptoms [115] and mothers of preterm birth and low birth infants are more likely to suffer postpartum depression [116]. In our study of placental gene expression in relation to prenatal clinically diagnosed depression, we did not find any association with *PHLDA2* expression levels in the placenta [117]. However, this was a small number of women and we cannot formally exclude a role for placental *PHLDA2* in maternal mood disorders or postnatal care provision. In this same study we did find markedly reduced expression of *PEG3* in both the placenta from pregnancies where women were clinically diagnosed with depression and also those pregnancies where women self-reported depressive symptoms in pregnancy. This association was only apparent in the placenta from boys exposed to depression and not girls. Given our finding of a sexually dimorphic response of the mouse placenta to loss of Peg3 function (Tunster, in press), this lends support to a causal role for reduced *Peg3* contributing to depression in human pregnancies. While we did not observe indicators of depressive symptoms in our *Peg3* mouse model, we did not use extensive tests for depression due to the pregnant status of the dams but dams were more anxious postpartum. Depression and anxiety are comorbid in human pregnancies and further work will be is essential to distinguish between the prenatal impact of placental dysfunction and the postnatal impact of reduced communication in this model.

In summary, imprinted genes function in many aspects of development and behaviour but the often the catastrophic impact of loss of function can preclude a meaningful interpretation of the function of the imprint. Demonstrating that imprinted genes expressed in the offspring can influence maternal behaviour [42; 109], and more critically, boost the quality of maternal care, lends much greater weight to the idea that maternal care provision is a resource which is manipulated by the parental genomes.

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COMPETING INTERESTS STATEMENT:

Since September 2017, GMN has been employed by Frontiers Media SA. GMN declared her affiliation with Frontiers. RMJ is chief Specialty editor of *Frontiers in Cell and Development Biology*. The other authors declare that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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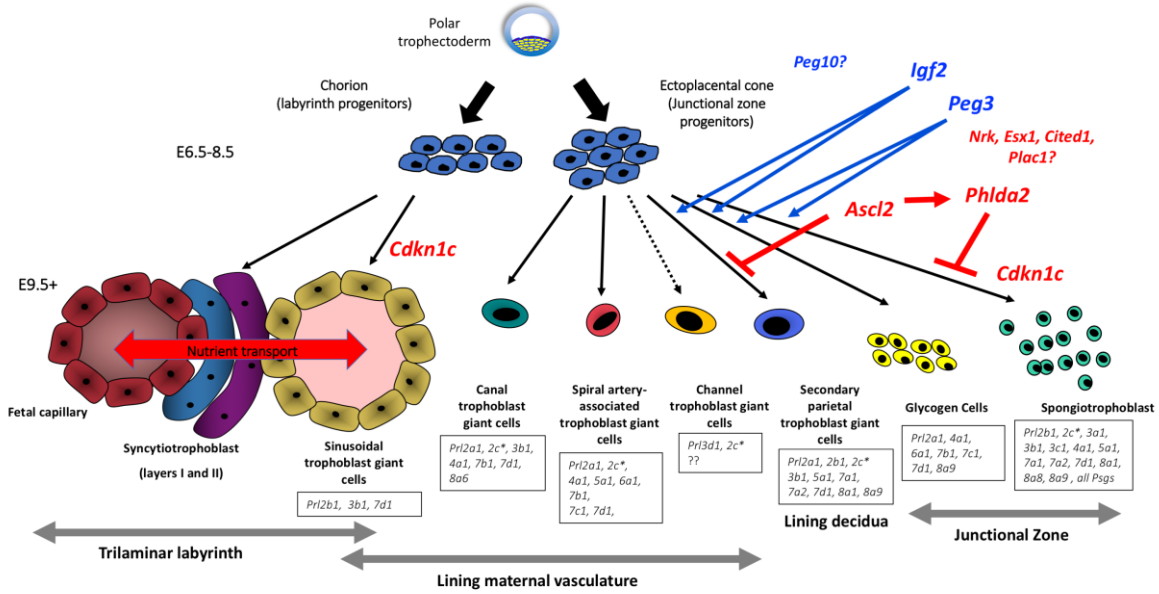
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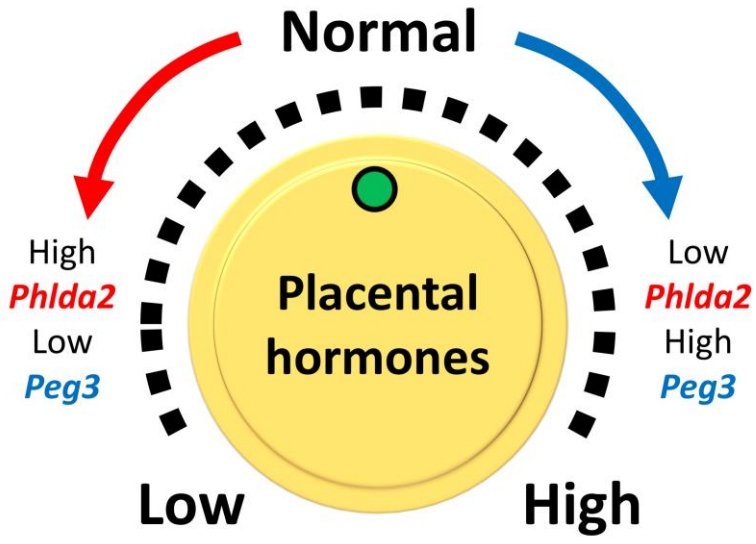
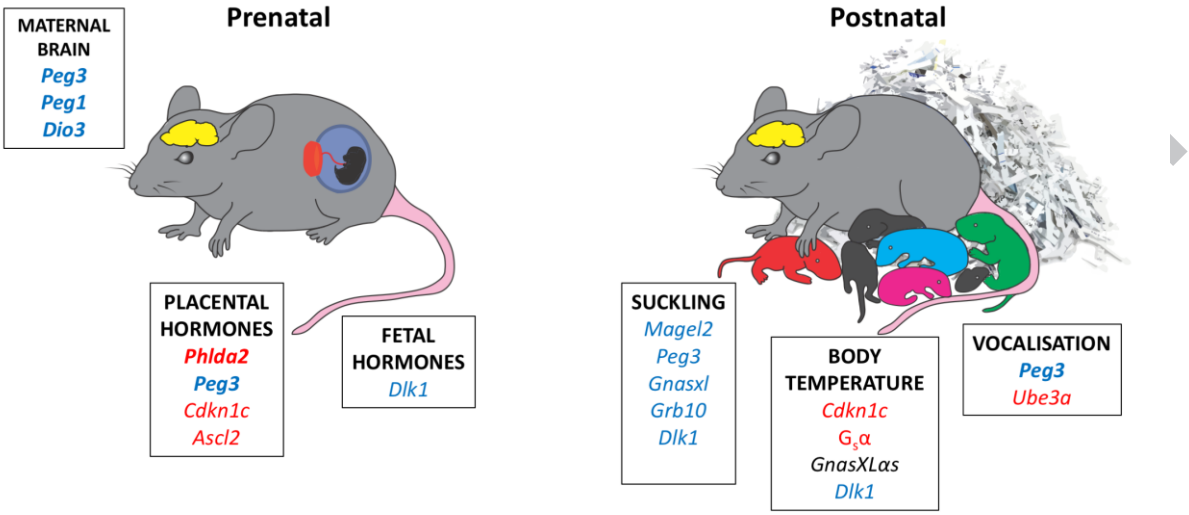
Igf2
Peg3
Nrk, Esx1, Cited1,
Plac1?
Ascl2
Phlda2
Cdkn1c
Cdkn1c
E9.5+
Nutrient transport
Fetal capillary
Syncytiotrophoblast
(layers I and II)
Sinusoidal
trophoblast giant
cells
Prl2b1, 3b1, 7d1
Canal
trophoblast
giant cells
Prl2a1, 2c*, 3b1,
4a1, 7b1, 7d1,
8a6
Spiral artery-
associated
trophoblast giant
cells
Prl2a1, 2c*,
4a1, 5a1, 6a1,
7b1,
7c1, 7d1,
Channel
trophoblast giant
cells
Prl3d1, 2c*
??
Secondary
parietal
trophoblast giant
cells
Prl2a1, 2b1, 2c*
3b1, 5a1, 7a1,
7a2, 7d1, 8a1, 8a9
Glycogen Cells
Prl2a1, 4a1,
6a1, 7b1, 7c1,
7d1, 8a9
Spongiotrophoblast
Prl2b1, 2c*, 3a1,
3b1, 3c1, 4a1, 5a1,
7a1, 7a2, 7d1, 8a1,
8a8, 8a9 , all Psgs
Trilaminar labyrinth
Lining maternal vasculature
Lining decidua
Junctional Zone


Normal
High
Phlda2
Low
Peg3
Low
Phlda2
High
Peg3
Placental
hormones
Low
High



Highlights

- Many gene deletions in the dam, including the imprinted genes Peg1, Peg3 and Dio3, result in maternal care deficits.
- Deletion of the offspring's maternally-expressed *Phlda2* enlarges a placental endocrine lineage boosting maternal care
- Paternally-expressed *Peg3* functions antagonistically to *Phlda2* in the fetally-derived placenta
- Disruption of *Peg3* also limits pup communication (USVs) exposing dams to a two-hit penalty.
- Maternal care is a resource influenced by both parental genomes carried by the offspring