1    **Special Edition Review: Lumps and Bumps meeting.**

2

3    **Imprinted genes and the regulation of placental endocrine function: Pregnancy and**

4    **beyond**

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9    **Genomic imprinting is an epigenetic process responsible for the monoallelic**

10   **expression of a subset of genes in mammals. Imprinted genes have been**

11   **demonstrated to play important functions prenatally regulating fetal growth and**

12   **placental development with some functions persisting beyond pregnancy to**

13   **influence both metabolism and behaviour in adults. This review focuses on the**

14   **function of imprinted genes in regulating placental hormones, and the probability**

15   **that these functions manifest their impact beyond pregnancy.**

16

17   Key words: Genomic imprinting; placental hormones.

Central to the reproductive success of mammals is the *in utero* provision of nutrients to their young via specialised extraembryonic lineages [1]. All extant mammals (monotremes, marsupials and eutherians) rely initially on a yolk sac placenta with some marsupials and all eutherians 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 [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 [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 [5, 6]. Moreover, numerous studies in genetically altered mice have identified imprinted genes that influence fetal weight, placental development, maternal behaviour, lactation and thermogenesis [7-9] in a manner generally consistent with the conflict theory.

44  A major function of imprinted genes in the placenta is the regulation of nutrient transport

45  primarily inferred from studies on the paternally expressed/maternally silenced *insulin-like*

46  *growth receptor 2* gene, and extensively reviewed [10]. Nutrient transport is determined by

47  the demand requirements of the fetus and placenta, which both place a substantial burden

48  on maternal resources during pregnancy. The mammalian mother also exclusively

49  supplies nutrients, in the form of milk, after birth. The supply of nutrients prenatally and in

50  the immediate postnatal period requires significant adaptations to maternal physiology,

51  which must be carefully balanced for a successful pregnancy. Effectively, the mother must

52  be able to supply all the nutritional requirements of her developing young while

53  maintaining her own health and welfare. To achieve nutrient supply in pregnancy, maternal

54  food intake increases, peripheral insulin resistance increases and there is an up-regulation

55  of maternal pancreatic islet function alongside a lowered threshold for glucose stimulated

56  insulin all of which channel maternal nutrients to the fetus [11]. During pregnancy maternal

57  fat depots are also laid down, and changes take place in the mammary gland in

58  preparation for the supply of milk in the postnatal period. Placental hormones are key to

59  the induction and maintenance of these changes in maternal physiology during pregnancy

60  [12].

61

62  Perhaps the most well studied placental hormones belong to the somatotropin/prolactin

63  family. These belong to a complex family of hormones related to prolactin (expressed in

64  the pituitary) that originated from a common ancestral gene. This same ancestral gene

65  also gave rise to pituitary expressed growth hormone and, in some mammalian species,

66  further duplication to produce placental-specific growth hormone. Together, all these

67  hormones could be viewed as one gene family encompassing both prolactin- and growth

68  hormone-like activities, distinguished by their interaction with their cognate receptors, the

69  growth hormones receptor and the prolactin receptor [13]. In mice, there are 22 prolactin

70   family members expressed almost exclusively in the placenta and no equivalent for

71   placental growth hormone gene [14]. However, only a small subset of these hormones

72   possess the capacity to activate the prolactin receptor [15]. In humans there are two

73   functional prolactin family members expressed in the placenta (*chorionic*

74   *somatomammotropin hormone 1* and *2*) encoding placental lactogen, and one placental

75   growth hormone gene [16]. One of the key changes in pregnancy thought to be induced by

76   a growth hormone-like activity is the increase maternal insulin resistance required to

77   channel maternal glucose to the fetus [17]. Glucose is transported via a passive process

78   that requires a higher concentration in the maternal circulation than in the fetus. Insulin

79   resistance contributes to this gradient but must be balanced by the ability to respond

80   rapidly to meals in order to avoid dangerously high levels of blood glucose. This balance is

81   achieved, in part, through the prolactin-like function of this gene family up-regulating

82   maternal islet function and accommodating the increased demand for insulin during

83   pregnancy [18, 19]. Placental lactogens also play an important role in preparing the new

84   mother to provide nutrients after birth by priming the mammary gland for lactation [20].

85   Placental lactogens may additionally be involved in the induction of maternal care. Both

86   pituitary prolactin and placental lactogen have been shown to stimulate maternal care in

87   non-pregnant rodents likely influencing maternal care through their interaction with the

88   maternal prolactin receptor, most clearly demonstrated in rodents [21].

89

90   In mice and humans, placental lactogens are expressed by derivatives of the trophoblast

91   lineage, a specialised cell type that emerges from the first differentiation event in

92   development [22]. Early in pregnancy, trophoblast cells contribute to the choriovitelline

93   (yolk sac) placenta, a structure that is replaced as gestation proceeds by the

94   chorioallantoic (mature) placenta. In mice, there are four main region of the chorioallantoic

95   placenta: the maternally-derived decidua, a single layer of cells with giant nuclei called the

96 secondary parietal trophoblast giant cells (TGCs), the junctional zone and the labyrinth

97 [23]. The junctional zone contains two distinct lineages, the spongiotrophoblast and the

98 glycogen cells, while the bulk of the labyrinth is composed of fetal endothelium surrounded

99 by a trilaminar layer of trophoblast-derived cells consisting of a single mononuclear TGC

100 layer (sinusoidal; previously called trophoblast layer I) and two multinucleated

101 syncytiotrophoblast layers that function in nutrient transport (I and II; previously called

102 layers II and II). Three other distinct TGC placental lineages have been classified. The

103 spiral artery (SpA) TGCs line the maternal blood system on entry to the placenta, the canal

104 (C-) TGCs line the maternal blood canals in the junctional zone and the channel (Ch-)

105 TGCs line the maternal blood spaces located just beneath the decidua where maternal

106 blood leaves the placenta [14, 23-25]. Placental lactogens are expressed by all TGC

107 subtypes, the spongiotrophoblast and the glycogens cells. Moreover, the large nuclei of

108 the TGCs result from endoreduplication with specific parts of their genome further over

109 replicated including regions encoding placental lactogens [26]. Along with their close

110 proximity to the maternal circulation, these gene amplification events suggest TGCs as a

111 major source of placental lactogens in mice. In contrast, the human placenta appears to

112 possess a single major cell type manufacturing hormones termed the syncytiotrophoblast.

113 These multinucleated layer of cells is generated from the fusion of cytotrophoblast cells

114 lying beneath, and both cell types overlie a core of mesenchynmal cells that make up the

115 numerous chorionic villi of the human placenta [13].

116

117 The role of placental hormones in manipulating the mother to provide resources to her

118 offspring both *in utero* and in the immediate postnatal period suggested placental

119 hormones as candidates for the expression of parent-offspring conflict [27, 28]. Apart from

120 one rare example in the new world mouse, *Peromyscus* [29], there is no evidence that

121 placental hormones or their maternal receptors are directly subject to genomic imprinting.

122 However, a fetally-derived product of the paternally-expressed imprinted *delta-like*

123 *homolog 1* gene (*Dlk1*) has recently been shown to reach the maternal circulation and

124 influence maternal metabolism [30], and it is possible that the *Igf2* gene product has a

125 similar function [31]. We hypothesised an alternative mechanism whereby imprinting could

126 influence placental hormone production - by regulating the placental lineages that express

127 these hormones [32]. This hypothesis was based initially on our studies on one imprinted

128 gene, *Pleckstrin homology-like domain family a member 2* (*Phlda2*). *Phlda2* is a maternally

129 expressed imprinted gene that encodes a PH domain-only protein expressed most highly

130 in the ectoplacental cone and the visceral endoderm of the yolk sac [33-35]. Our studies

131 on *Phlda2* revealed a precise function for this gene in negatively regulating the size of

132 spongiotrophoblast compartment, without altering the gross contribution of other placental

133 lineages [7, 36-39]. Loss-of-function of *Phlda2* resulted in a much larger

134 spongiotrophoblast, approximately twice the volume normally present. Conversely, a two-

135 fold gain in expression of *Phlda2* (modeling loss-of-imprinting) reduced the size of this

136 compartment by 50%. The spongiotrophoblast is a key site for the production of placental

137 lactogens, pregnancy-specific glycoproteins and a number of other hormones important in

138 pregnancy [14, 37]. Using the same dosage interrogating approach applied to *Phlda2*, we

139 have recently shown that overexpression of a second maternally expressed imprinted

140 gene, *Achaete-scute complex homolog 2* (*Ascl2*), repressed both the spongiotrophoblast

141 and the parietal TGCs [40]. Although mouse models with increased dosage have not been

142 reported, loss-of-function of studies suggest that *Paternally expressed gene 3* (*Peg3*),

143 *Paternally expressed gene 10* (*Peg10*), *Cyclin-dependent kinase inhibitor 1c* (*Cdkn1c*) and

144 several non-classically imprinted genes located on the X chromosome also regulate the

145 placental endocrine lineages positively or negatively in a manner generally consistent with

146 parental conflict [32]. As a consequence, imprinted genes indirectly modulate the

147 expression of placental hormones by regulating the size of the placental endocrine

6

148 compartment. This is illustrated most elegantly with the *Phlda2* gene where loss of

149 expression resulted in a 2-fold increase in expression of the placental lactogens expressed

150 in the spongiotrophoblast while a double dose of *Phlda2* resulted in a 50% decrease in

151 their expression [41].

152

153 Functional data demonstrating that imprinted genes regulate placental hormone lineages

154 in species other than the mouse is sparse. Reduced expression of *PHLDA2* is a common

155 feature of bovine cloning associated with overgrowth of both the fetus and placenta but not

156 the altered expression of placental hormones [42, 43]. Elevated placental *PHLDA2* has

157 been reported in a number of studies on human fetal growth restriction, fetal death and low

158 birth weight [44]. In our recent study on women with a perception of reduced fetal

159 movements (RFM), we found placental *PHLDA2* expression was 2.3 fold higher in RFM

160 pregnancies resulting in delivery of a growth restricted infant compared with a normal birth

161 weight infant [45]. Importantly, we found a significant inverse association between

162 placental *PHLDA2* levels and maternal serum placental lactogen (hPL) levels suggesting

163 that *PHLDA2* may regulate the production of placental hormones in human pregnancies.

164 In another study focusing on prenatal depression, we examined placental expression

165 levels of four genes, *PHLDA2*, *CDKN1C*, *PEG3* and *PEG10*, based on the conserved

166 imprinting status between mouse and human and their predicted role in regulating

167 production of placental hormones [32]. In women with clinically diagnosed depression

168 during pregnancy, we observed significantly lower expression of placental *PEG3*. We also

169 found low placental *PEG3* in pregnancies where women reported a depressed mood

170 assessed using two self-rating psychometric questionnaires: Edinburgh Postnatal

171 Depression Scale (EPDS), used as a measure of maternal prenatal depressive symptoms

172 [46] and the Spielberger State-Trait Anxiety Inventory (STAI), used as a measure of

173 anxiety symptoms [47]. Both diagnosed and self-reported symptoms of depression were

7

174  also significantly associated with low expression of *hPL* Critically, we found a positive

175  correlation between placental *PEG3* and *hPL* expression. In mice, loss-of-function of *Peg3*

176  has been reported to result in changes in the expression of a number of placental

177  lactogens [48, 49] although a specific endocrine lineage analysis has not been performed.

178  Together, these data are consistent with a role for *PEG3* in regulating *hPL* expression in

179  humans. *Peg3* and another paternally expressed imprinted gene, *Peg1*, have previously

180  been linked to maternal care in rodents [50, 51]. In both these reports the dam carried the

181  inactivating mutation for the respective gene, with considerable impact on a number of

182  physiological as well as neurological processes. Moreover, a recent study did not find a

183  maternal care deficit when a second *Peg3* targeted allele was generated, and examined

184  on a different strain background [52]. A role for loss of *Peg3* in the placenta influencing

185  any aspect of maternal behaviour in rodents has yet to be reported.

186

187  In human pregnancies, a number of complications can commonly co-occur. Women with

188  prenatal depression are three times more likely to have a low birth weight baby [53].

189  Maternal depression in the first and second trimester is associated with an increased risk

190  of gestational diabetes while women with gestation diabetes have a >4-fold risk of

191  postnatal depression with elevated depressive symptoms particularly high among non-

192  obese women [54]. A recent systematic review reported that women with symptoms of

193  postnatal depression were less likely to breastfeed exclusively and more likely to terminate

194  breastfeeding earlier [55]. This could be interpreted to mean that postpartum depression

195  negatively impacts maternal breast feeding behaviour or that pressures around breast

196  feeding increase the risk of depression. However, given the potential for placental

197  hormones to influence both lactation and maternal behaviour, it is possible that difficulties

198  with breast feeding and postnatal depression are manifestations of the same underlying

199  problem, placental endocrine dysfunction (Figure 1). Similarly, prenatal depression

200 accompanied by low birth weight could be indicative of placental endocrine dysfunction

201 (Figure 1). As well as these manifestations of maladapted pregnancy, a transient increase

202 in the risk of breast cancer diagnosis has been reported for first time mothers likely linked

203 to the pregnancy induced changes in mammary development [56]. It is therefore possible

204 that placental hormone dysfunction could influence both short term and long term risk of

205 breast cancer. Measuring placental hormones in maternal blood is already an important

206 diagnostic tool early in pregnancy and the use of DLK1 assays in characterising types of

207 fetal growth restriction holds great promise [30]. New techniques such as multiplexed

208 quantification of fetal RNAs circulating in maternal blood may provide even more accurate

209 tools, and for a variety of conditions [57].

210

211 In summary, current data supports a conserved function in mammals for imprinted genes

212 in regulating placental hormones via regulating the size of the placental endocrine

213 compartment. These data essentially support the prediction by David Haig more than 20

214 years ago that imprinted genes would regulate signaling between the mother and her

215 fetus. Importantly for human pregnancies, placental hormones play key roles in driving the

216 physiological and, potentially, behavioural adaptations required to support optimal fetal

217 growth and postpartum care. It is therefore plausible that aberrant imprinting in the

218 placenta contributes to the common co-occurrence of a number of complications of

219 pregnancy including low birth weight, maternal mood disorders, gestational diabetes and

220 poor breast feeding. Finally, given the link between all these complications and poor long

221 term outcomes for children, the detrimental consequences of placental endocrine function

222 may influence offspring wellbeing considerably beyond the period of pregnancy (Figure 1).

223

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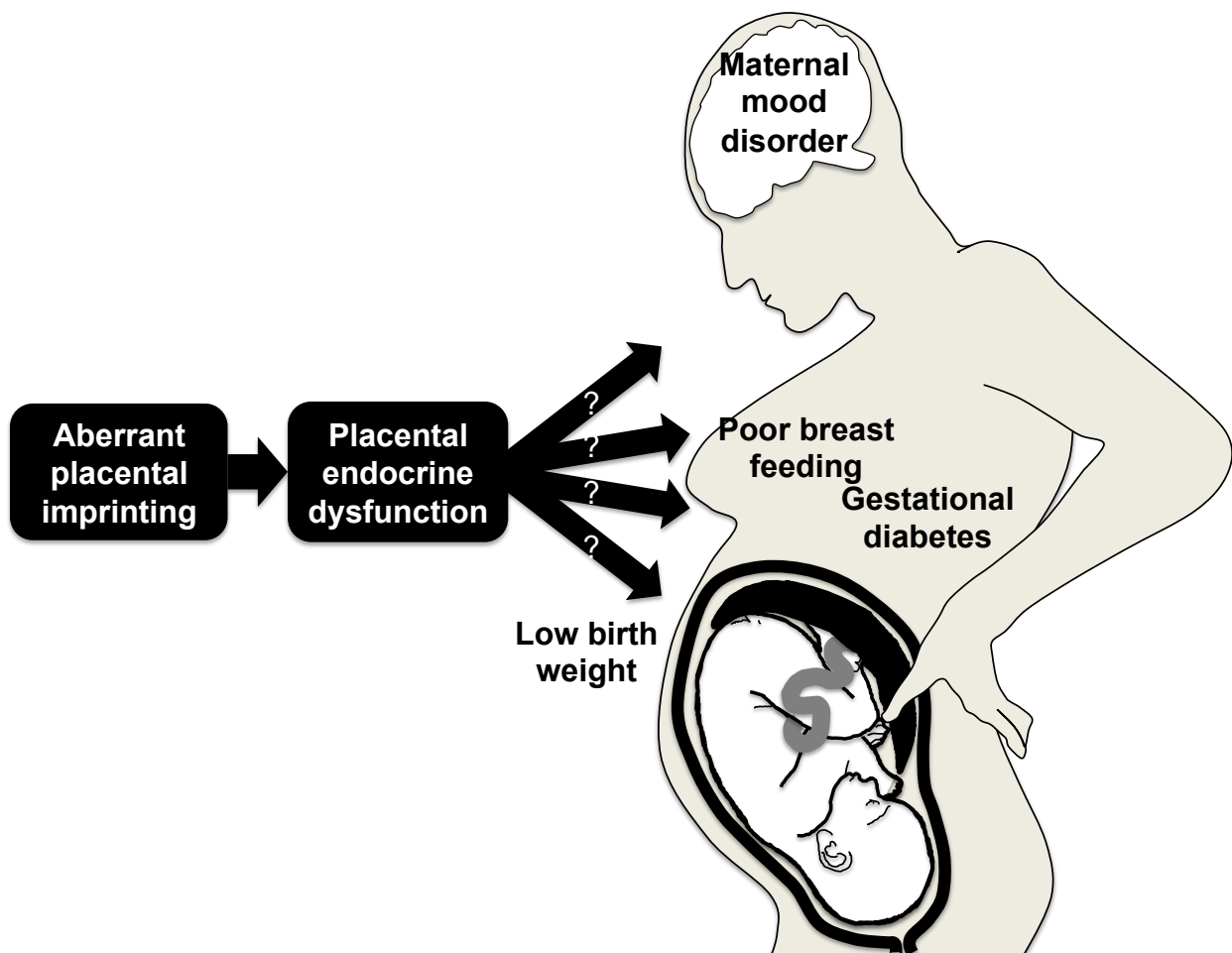
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382   **Figure 1. Aberrant imprinting and placental endocrine dysfunction.**



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