1   Genomic imprinting and mammalian reproduction

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8    Abstract

9    Amongst animals, genomic imprinting is a uniquely mammalian phenomenon in which

10   certain genes are monoallelically expressed according to their parent-of-origin. This silencing

11   of certain alleles often involves differential methylation at regulatory regions associated with

12   imprinted genes and must be recapitulated at every generation with the erasure and

13   reapplication of these epigenetic marks in the germline. Imprinted genes encode regulatory

14   proteins that play key roles in fetal growth and development, but they also exert wider effects

15   on mammalian reproduction. Genetic knockout experiments have shown that certain

16   paternally expressed imprinted genes regulate post-natal behavior in offspring as well as

17   reproductive behaviors in males and females. These deficits involve changes in

18   hypothalamic function affecting multiple areas and different neurochemical pathways.

19   Paternally expressed genes are highly expressed in the hypothalamus which regulates

20   growth, metabolism and reproduction and so are well placed to influence all aspects of

21   reproduction from adults to the resultant offspring. Coadaptation between offspring and

22   mother appears to have played an important role in the evolution of some paternally-

23   expressed genes, but the influence of these genes on male reproductive behavior also

24   suggests that they have evolved to regulate their own transmission to successive

25   generations via the male germline.

26

27   Keywords: genomic imprinting; imprinted genes; reproduction; sexual behavior; maternal

28   behavior;    hypothalamus;    paternally    expressed    genes;    coadaptation;    conflict

**Introduction**

In the early nineteen-eighties it became clear that the parental genomes in mammals were functionally non-equivalent (McGrath and Solter, 1984; Surani et al., 1984), a phenomenon termed 'genomic imprinting'. A subset of autosomal genes is expressed not in accordance with classical Mendelian laws of inheritance, but according to the sex of the parent from which they are inherited. Imprinted genes are thus not expressed biallelically but in non-stochastic, monoallelic fashion from either the maternally donated allele or the paternally donated allele. This parent-of-origin silencing of specific alleles is an important example of stable epigenetic regulation of gene expression and of crucial importance in mammalian development. Silencing at imprinted genes involves both DNA methylation and chromatin modification (Delaval and Feil, 2004) and imprinting research has revealed much about mechanisms of epigenetic regulation.

The original experiments by McGrath and Solter (1984) and Surani et al (1984) demonstrated that mouse embryos created with two male pronuclei (androgenetic – 'AG') or two female pronuclei (gynogenetic – 'GG') failed to reach term. The absence or doubling of expression of imprinted genes resulted in lethal phenotypes very early in development. Later research in which such AG or GG embryos were rescued by fusing them with wild-type blastocysts revealed the different developmental roles played by the two parental genomes. AG chimeras were much larger at birth than wild-type embryos while GG chimeras were much smaller (Allen et al., 1995; Barton et al., 1991), suggesting that the paternal and maternal genomes regulate offspring growth in opposite directions. Analysis of the fate of AG and GG cells in brains of these chimeras showed that GG cells segregated almost exclusively to the cortex and striatum, while AG cells were only found in the hypothalamus (Allen et al., 1995; Keverne et al., 1996). The differential fate of these AG and GG cells suggested that paternally and maternally expressed genes regulate the development and function of different brain areas. In recent years imprinted genes have been shown to have important roles in brain function and in behavior (Davies et al., 2008; Isles and Wilkinson, 2000; Wilkinson et al., 2007).

57    Imprinted genes present a particularly interesting genetic conundrum, as their haploid

58    expression results in the loss of protection from mutation which diploidy confers (Orr, 1995)

59    and so would seem to be a non-adaptive evolutionary step. The debate about how and why

60    such a mechanism has evolved has been ongoing since genomic imprinting was first

61    discovered (Hurst and McVean, 1997; Hurst and McVean, 1998; Moore and Haig, 1991;

62    Moore and Mills, 2008) but the evolution of placentation and viviparity in mammals appears

63    to coincide with the acquisition of genomic imprinting (Kaneko-Ishino et al., 2003). There are

64    approximately 80 imprinted genes in eutherian mammals (Morison et al., 2005), of which

65    only a subset are imprinted in marsupials (Renfree et al., 2008), and none in monotremes

66    (Hore et al., 2007; Killian et al., 2001). The taxonomic distribution of imprinting coincides with

67    the relative placental complexity and the development of viviparity in these different

68    mammalian groups. Eutherians have elaborate placentas and prolonged gestation,

69    marsupials have more rudimentary placentas and give birth to very altricial young, while

70    monotremes are egg-laying mammals in which there is only a brief period of maternal

71    nutrient supply after fertilization (Renfree et al., 2009). Moreover, almost all imprinted genes

72    are expressed in the placenta (Bressan et al., 2009; Coan et al., 2005) and imprinted genes

73    are also strongly expressed in the developing embryo. The importance of imprinted genes in

74    development is indicated by the proteins they encode, which include growth factors,

75    transcription regulators, apoptotic proteins and regulatory non-coding RNAs (Morison et al.,

76    2005). The taxonomic patterns of genomic imprinting show that this regulatory mechanism

77    has evolved in step with modes of reproduction in mammals, while the expression and

78    functions of imprinted genes show that they play important developmental roles in

79    reproduction. However experimental evidence has been also been accumulating that

80    suggests that imprinted genes are involved in mammalian reproduction beyond just fetal

81    development and placental functions. The influence of imprinted genes on brain and

82    behavior extends to post-natal offspring behavior as well as adult reproductive behaviors.

83    These findings raise new questions about the roles that imprinted genes play in behavior

84    and consequently about the selective pressures which drove their evolution.

85

**Genomic imprinting mechanisms and reproduction**

The majority of imprinted genes are organized into clusters where gene expression is regulated from imprinting control regions (ICRs) associated with these clusters (Williamson et al., 2006). The clustering of imprinted genes and the shared ICRs suggest that imprinting has evolved as a regulatory mechanism to which genes are recruited, rather than evolving independently at each imprinted gene. ICRs contain differentially methylated regions (DMRs) rich in CpG repeats which are methylated on one of the parental alleles and not the other, and which determine the monoallelic expression of genes in the imprinted cluster. While other epigenetic mechanisms are also involved, such as histone modification and non-coding microRNAs, DNA methylation is the main mechanism by which imprinted gene expression is regulated (Delaval and Feil, 2004; Kacem and Feil, 2009). The imprints at each DMR must be recapitulated within the germline of each generation to reflect the gender of the individual and ensure the correct complement of imprinted genes in offspring. This is achieved in the germ line when imprints are erased and re-established in a sex specific manner during gametogenesis in the early embryo. In both males and females, primordial germ cells (PGCs) migrate to the genital ridge where they undergo widespread epigenetic modification including active demethylation of parental imprints (between embryonic days 10.5 and 12.5 in the mouse) (Sasaki and Matsui, 2008). After this demethylation of DMRs there is subsequent sexual dimorphism in imprint application. In females, DNA methylation at ICRs occurs after birth as the post-meiotic oocytes grow and mature (Hiura et al., 2006), while in males the paternal imprints are applied in the pre-meiotic prospermatogonia before birth. Moreover, the vast majority of imprinted ICRs are methylated during female gamete formation and only 3 DMRs have been identified which are methylated during spermatogenesis in males (Sasaki and Matsui, 2008). This asymmetry indicates that the mechanism of imprinting is primarily under maternal control and involves active silencing of maternal alleles in the female germ line.

112  As with other forms of epigenetic regulation, the methylation of imprinted DMRs during

113  gametogenesis is mediated by DNA methyltransferases (DNMTs). DNMT3A mediates *de*

114  *novo* methylation in both male and female gametes, although in males a second

115  methyltransferase, DNMT3B, is also involved in methylation at the *Rasgrf1* imprinted locus

116  (Kato et al., 2007). Furthermore, the methylation of all imprinted DMRs is dependent on

117  DNMT3L, which is non-enzymatic but plays a crucial role in methylation at imprinted loci

118  (Kaneda et al., 2004). DNMT3L appears to form a complex with DNMT3A/DNMT3B and

119  interprets an existing mark (possibly histone-based) which is then converted into gender-

120  specific DNA methylation at DMRs. The importance of appropriate levels of imprinting in

121  mammalian gametogenesis is illustrated by the phenotype of DNMT3L mutant mice.

122  Deleting DNMT3L and thus disrupting methylation at DMRs causes gross reproductive

123  deficits in both sexes such that males are azoospermic and infertile, while females are

124  unable to produce viable offspring (Schaefer et al., 2007). DNMT3L's important role in

125  imprinting mechanisms is also evident from phylogenetic comparisons of the mammalian

126  taxa: while DNMT3L is found in both eutherians and marsupials, there appears to be no

127  ortholog in the monotremes which lack imprinted genes (Renfree et al., 2009; Yokomine et

128  al., 2006).

129

130  **Imprinting and reproduction in mammals**

131  Almost all imprinted genes are expressed in either the embryo, the placenta or both and

132  have been shown to have important regulatory roles in development. Imprinted genes are

133  explicitly involved in mammalian reproduction through their influence on fetal development

134  (Constancia et al., 2002) and placental function (Charalambous et al., 2010), however they

135  also influence reproduction in other ways. Much of the research into the roles and functions

136  of imprinted genes has involved the use of imprinted gene-knockout mice and while such

137  studies have only been conducted in one experimental model, they indicate that imprinted

138  genes are important regulators of reproductive behavior in the mouse and potentially also in

139  other mammals. Due to monoallelic expression of imprinted genes, these knockouts also

140  provide unique opportunities to study imprinted genes independently in mother and offspring

141  (fig. 1). Such knockout mouse studies have demonstrated that imprinted genes have

142  important effects on brain function (Wilkinson et al., 2007) and behavior (Isles and Wilkinson,

143  2000) which include post-natal offspring behavior as well as reproductive behavior in both

144  males and females. While direct effects on reproductive potential of global disruption of

145  imprinting are evident in the infertility of DNMT3L-knockout mice in which appropriate

146  imprinting is not established in the germline, behavioral effects suggest that imprinted genes

147  also regulate different aspects of mammalian reproduction through effects on the brain (table

148  1). Such effects are particularly evident in knockouts of paternally expressed genes, perhaps

149  due to their high levels of expression in the hypothalamus and related structures which

150  mediate reproductive behaviors in mammals.

151  ***Imprinted gene effects in offspring***

152  The first mouse knockouts of the imprinted genes *Igf2, Igf2r* and *H19* demonstrated that

153  imprinted genes are involved in placentation and embryonic development (Barlow et al.,

154  1991; Bartolomei et al., 1991; DeChiara et al., 1991), and subsequent studies also showed

155  imprinted gene effects on post-natal behavior in offspring. One of the first such studies

156  involved the paternally expressed gene *Peg1/Mest* on mouse chromosome 6. Although a

157  paternally inherited mutation in this gene results in reduced weight at birth, this deficit is

158  subsequently exacerbated by reduced post-natal growth rate relative to wild-type littermates

159  (Lefebvre et al., 1998). This suggested that imprinted genes not only regulate resource

160  extraction during gestation, but could also influence offspring physiology or behavior post-

161  natally.

162  More detailed evidence for such post-natal effects came from studies of the paternally

163  expressed gene *Peg3*, which encodes a large  zinc-finger protein on mouse proximal

164  chromosome 7 (Kuroiwa et al., 1996). Paternally-inherited deletions of *Peg3* cause deficits

165  not only before birth but also post-natally, affecting growth, suckling and thermoregulation

166  (Curley et al., 2004). *Peg3* mutant animals have small placentas and low birth weight which

167  they fail to make up after birth. These pups remain smaller than their wild-type littermates

168  throughout post-natal nursing due to a suckling deficit which reduces their ability to take on

169  milk. The *Peg3* mutation also affects thermoregulation as mutant pups are unable to

170  maintain body temperature in response to maternal separation. Metabolism, appetite and

171  thermoregulation are all mediated by the hypothalamus, an area where there is high *Peg3*

172  expression during development (Li et al., 1999). The *Peg3* protein is involved in p53-

173  mediated apoptosis (Deng and Wu, 2000) and these mutant animals show alterations in

174  postnatal hypothalamic apoptosis (Broad et al., 2009), which suggests that aberrant

175  neuronal pruning during development may disrupt normal functioning of the hypothalamus.

176  Further disruption to hypothalamically-mediated behavior is seen in adolescence when *Peg3*

177  females enter puberty later than their wild-type littermates (Curley et al., 2005).

178  The *GNAS* locus on mouse distal chromosome 2 encodes multiple transcripts with different

179  parent-of-origin expression (Peters et al., 1999), including paternally expressed *Gnasxl* and

180  *Nespas*, maternally expressed *Nesp,* and maternally expressed *Gnas*, which is only

181  imprinted in adipose and endocrine tissues (Peters and Williamson, 2007). The relationship

182  between these different transcripts is complex, however a lack of the protein XLαs, the

183  *Gnasxl* product, causes deficits in post-natal behavior that bear some similarities to those

184  seen in *Peg3* mutant pups (Plagge et al., 2004). Mice inheriting a paternal deletion of *Gnasxl*

185  suffer post-natal growth retardation caused by metabolic deficits and an inability to suckle

186  properly resulting in death no later than post-natal day 9. *Gnasxl* is expressed in the

187  hypothalamus and pituitary that regulate energy homeostasis and in pontine and medullary

188  nuclei which innervate the facial and jaw muscles involved in suckling. *Gnasxl* appears to be

189  directly involved in the neural and endocrine control of post-natal suckling behavior and the

190  similar phenotypes seen in the Peg1, Peg3 and Gnasxl mutants suggest that these

191  paternally expressed genes are all involved in the ability of mouse pups to thrive post-

192  natally.

193  ***Imprinted gene effects in females***

194  As well as affecting offspring, deletion of paternally expressed *Peg1/Mest* also affects

195  female maternal care (Lefebvre et al., 1998). Pups born to mutant mothers suffer elevated

196  post-natal mortality, even if they are wild-type offspring with a normal paternal *Peg1*/*Mest*

197  allele. The deletion affects multiple maternal behaviors and mutant females do not engage in

198  placentophagia after birth and exhibit reduced levels of pup investigation, pup retrieval to the

199  nest, nest building and post-natal nursing.

200  Similarly, the *Peg3* deletion also results in an adult phenotype which includes effects on

201  maternal care. Li et al (1999) noted very high levels of pre-weaning mortality in the offspring

202  of heterozygous *Peg3* mutant females despite these pups being wild-type, having been

203  fathered by wild-type males with normal *Peg3* expression. The elevated mortality thus could

204  not have been due to direct genetic effects in the wild-type offspring but rather to the

205  maternal responses of the females. Behavioral phenotyping of the *Peg3* mutant mothers

206  revealed a wide-ranging deficit affecting nest building, retrieval of scattered pups to the nest

207  and milk letdown. More detailed characterization of maternal behavior showed that overall

208  nursing levels are lower and that licking and grooming of pups is also reduced in these

209  females (Champagne et al., 2009). Licking and grooming is an important modulator of

210  offspring behavioral phenotypes (Francis et al., 1999) and it causes stable epigenetic

211  changes in gene expression (Weaver et al., 2004). The offspring and even grand-offspring of

212  these *Peg3* mutant mothers also display lower levels of maternal care, despite being wild-

213  type themselves (Curley et al., 2008). *Peg3* thus appears to exert an influence on offspring

214  behavioral phenotypes through its effects on maternal care. The *Peg3* mutation appears to

215  involve disruption of the oxytocin circuitry in the hypothalamus where *Peg3* is expressed.

216  Oxytocin is an important regulatory neuropeptide in maternal behavior and milk production

217  and the maternal effects of the knockout appear to be mediated by a reduction in the

218  number of oxytocinergic neurons in the paraventricular nucleus (Li et al., 1992) and a

219  reduction in oxytocin receptor density in the medial pre-optic area (Champagne et al., 2009).

220  *Peg3*'s apoptotic function suggests that developmental disruption in the hypothalamus leads

221  to these functional changes and then to the maternal behavior deficits. Indeed, *Peg3* mutant

222  mice have elevated levels of apoptosis in the medial preoptic area at postnatal days 4 and 6.

223  No changes are seen in paraventricular apoptosis at these time points, however this does

224  not discount the possibility of perturbations during embryonic development (Broad et al.,
225  2009).

226  Recent studies of the paternally expressed gene *Magel2* have demonstrated that imprinted
227  genes can influence female fertility and reproductive physiology as well as maternal care.
228  The first knockout study of *Magel2* showed that these mutant mice have aberrant circadian
229  rhythms due to abnormalities in the suprachiasmatic nucleus of the hypothalamus (Kozlov et
230  al., 2007). Further phenotyping has shown they have a more wide-ranging deficit in
231  hypothalamic function, with significant effects on female reproduction (Mercer and Wevrick,
232  2009). *Magel2* mutant females enter puberty late, despite similar post-weaning body weights
233  in mutant and normal females. These females also have extended and irregular estrous
234  cycles and enter reproductive decline at an early age, becoming infertile after 24 weeks
235  when corpora lutea are no longer seen in the ovaries, even though mature follicles are
236  present. *Magel2* mutant females were also slower to mate after pairing with males, produced
237  smaller litters and had significantly higher litter mortality rates which were not connected to
238  litter genotype.

239  ***Imprinted gene effects in males***

240  The effects on female behavior described above all involve paternally expressed genes. All
241  are strongly expressed in the hypothalamus in females and males, and thus it is not
242  surprising that the phenotypes of several paternally expressed gene knockouts involve
243  effects on male reproductive behavior too. As well as disrupting female maternal behavior, a
244  paternal deletion of *Peg3* also causes reproductive deficits in males. *Peg3* mutant males are
245  fertile but are unable to improve copulatory ability with sexual experience, unlike wild-type
246  animals. Sexual experience typically results in shorter latencies and increased frequencies
247  of sexual behaviors such as mounting and intromission in wild-type males but these
248  behavioral changes are not seen in *Peg3* mutant males whose behavior once sexually
249  experienced does not differ from that of virgin animals (Swaney et al., 2007). Wild-type male
250  mice also develop preferences for the odors of receptive estrous females once sexually
251  experienced, potentially enabling them to focus reproductive effort towards receptive

252 females. *Peg3* mutant males show no such changes in female-odor sensitivity (Swaney et

253 al., 2008). While such a deficit may appear relatively minor, olfaction is the primary sensory

254 modality in rodents as illustrated by the gross deficits in reproductive behavior which result

255 from ablation of olfactory membranes (Keller et al., 2009). The *Peg3* mutation appears to

256 disrupt plasticity in the main and accessory olfactory systems and in hypothalamic regions

257 which regulate sexual behavior, where significant increases in female odor-elicited neural

258 activity are seen in sexually experienced wild-type males but not in *Peg3* mutant males

259 (Swaney et al., 2007; Swaney et al., 2008).

260 Although *Peg1* mutant mice have not been reported to suffer deficits in male reproductive

261 behavior, studies in humans and mice suggest that this gene is involved in male fertility. The

262 human homolog *PEG1* and its paternally expressed anti-sense transcript are strongly

263 expressed in human testes and studies of male infertility have shown that hypermethylation

264 of the *PEG1* DMR and consequent silencing are strongly associated with different classes of

265 male infertility (Hammoud et al., 2009; Poplinski et al., 2009). In mice, pre-natal

266 administration of the endocrine disruptor vinclozolin produces male offspring with

267 significantly lower sperm counts with adults. Sperm from these males have greatly increased

268 methylation at both the *Peg1* and *Peg3* DMRs suggesting that normal methylation patterns

269 at these imprinted loci are necessary for male fertility (Stouder and Paoloni-Giacobino,

270 2009).

271 The *Magel2* mutants also have deficits in male reproductive behavior (Mercer and Wevrick,

272 2009), with some phenotypic similarities between mutant males and mutant females. Mutant

273 males also become infertile by 24 weeks and take several days to mate after being paired

274 with females. However the male phenotype also involves an olfactory deficit which includes

275 a lack of preference for opposite-sex odors which is not seen in the female mutants. Basal

276 forebrain neurochemistry animals is also affected by *Magel2* deletion which causes

277 reductions in levels of both serotonin and dopamine in the hypothalamus, as well as reduced

278 amygdala and nucleus accumbens volumes (Mercer et al., 2009). In line with the

279 reproductive deficits, *Magel2*-knockout males also have reduced serum testosterone levels.

280    These hypothalamic and endocrine deficits are of interest given the involvement of the

281    human homolog *MAGEL2* in Prader-Willi syndrome (PWS), a multigenic imprinting disorder

282    involving paternal deletion or maternal duplication at chromosome 15q11-q13 region (Lee et

283    al., 2000). PWS symptoms involve hypothalamic dysfunction affecting appetite and

284    metabolism, as well as hypogonadism and infertility (Eiholzer et al., 2006). Aberrant

285    expression of paternally expressed *NDN* is also involved in the etiology of PWS and mouse

286    knockout studies have shown that paternal deletion of homologous *Necdin* disrupts

287    reproductive hormone circuits. Muscatelli et al (2000) reported that *Necdin*-null mice (both

288    male and female) have fewer oxytocin neurons in the paraventricular nucleus and fewer

289    hypothalamic GnRH neurons. This latter deficit has recently been shown to be due to

290    reduced migration of GnRH neurons to the hypothalamus during development (Miller et al.,

291    2009), and lower numbers of both oxytocin and GnRH neurons have been reported in PWS

292    patients. The *Magel2* and *Necdin* mutant phenotypes suggest that disruption of

293    developmental expression contributes to PWS hypogonadism and provides further evidence

294    that paternally expressed genes influence male reproductive behavior.

295

296    **Reproduction and the evolution of genomic imprinting**

297    There have been many theories proposed to explain the evolution of imprinting, each of

298    which has different strengths and weaknesses regarding mechanisms of imprinting and

299    imprinted gene-related phenotypes. The most widely cited of these is the conflict or kinship

300    theory for the evolution of imprinting (Haig and Graham, 1991; Moore and Haig, 1991). This

301    predicts that the conceptus (placenta and offspring), which inherits approximately half of its

302    genes from its mother and half from its father, may be a site for potential conflict between the

303    two parental genomes. The paternal genome would favor maximal maternal investment in

304    offspring, to the possible detriment of the mother's long term health and any subsequent

305    offspring conceived with other males. Conversely, the maternal genome would favor

306    balanced investment of resources in all offspring across her entire reproductive career. The

307    placenta is an important endocrine organ that interfaces and communicates with the

308   maternal brain to regulate maternal investment and so is functionally capable of influencing

309   maternal behavior and physiology. The conceptus can thus be viewed as a parasite in which

310   imprinted genes influence resource extraction from the mother in opposite directions.

311   Paternally expressed genes are predicted to be growth-enhancing and maternally expressed

312   genes are predicted to be growth-restricting. The first knockout studies showed that

313   paternally expressed *Igf2* is indeed growth enhancing (DeChiara et al., 1991), while

314   maternally expressed *Igf2r* and *H19* limit fetal growth (Lau et al., 1994; Leighton et al.,

315   1995). While mutations at other imprinted loci also cause growth phenotypes which match

316   the predictions of the conflict theory, there are some that do not (Hurst and McVean, 1997).

317   Furthermore, some of the imprinted gene knockout mice also exhibit phenotypes which

318   extend beyond birth and even into adulthood, where conflict between the parental genomes

319   is less apparent. While it can be argued that post-natal, pre-weaning effects of imprinted

320   genes are in line with the predictions of the conflict hypothesis (Isles and Holland, 2005), the

321   adult phenotypes of some imprinted gene mutants are more difficult to reconcile with it.

322   The parent-of-origin transmission of the *Peg3* transgene allowed the effects of the mutation

323   to be compared independently in mutant pups and mutant mothers. The phenotypes are

324   remarkably complementary, with offspring deficits in pre-natal growth, suckling, and

325   thermoregulation matched by reduced maternal food intake, milk letdown and nesting

326   behavior (Curley et al., 2004). These result in similar outcomes for the offspring whether they

327   or the mother carry the mutation. The different peri-natal behaviors are regulated by an

328   endocrine interplay between the fetal hypothalamus, the maternal hypothalamus and the

329   placenta (Keverne and Curley, 2008), all areas of high *Peg3* expression. *Peg3* appears not

330   to have evolved in response to conflict between parental genomes but under selection

331   pressures that favor coadaptation of behaviors between offspring and mother, leading to

332   increased fitness for both. While no other imprinted gene mutants have such clearly

333   dovetailing phenotypes, the effects of deletions of other paternally expressed genes on

334   maternal behavior and post-natal behavior suggest that coadaptation may have played an

335   important role in the evolution of imprinting at multiple loci. Detailed behavioral phenotyping

336     of other imprinted gene knockouts would help to substantiate the evolutionary significance of

337     coadaptation and clarify whether other imprinted genes may also have evolved under such

338     coadaptive selection pressures. Moreover, data from other species is required to confirm the

339     importance of imprinted genes in mammalian reproduction and whether coadaptation has

340     played a wider role in the evolution of imprinting.

341     Some imprinted genes seem to have evolved to regulate female reproductive behavior

342     despite being paternally expressed. Such effects are not surprising given the high

343     expression of paternally imprinted genes in the hypothalamus which regulates reproductive

344     behavior and maternal care, however any adaptive effects will skip every other generation

345     due to the silencing of paternally expressed genes in the female germline. Most of these

346     genes have also been shown to influence male reproductive behavior to greater or lesser

347     degrees. This is significant, as any effects of paternally expressed genes on male behavior

348     would occur in every generation due to patrilineal transmission of actively expressed alleles

349     at these loci. Any effects on male reproductive success would be magnified by a

350     combination of paternal allele-only expression and reproductive skew, resulting in much

351     faster spread of any paternally expressed alleles that regulate adaptive male reproductive

352     behavior (Keverne, 2009). Paternal expression appears to have allowed the imprinted genes

353     that govern the development and function of the hypothalamus to regulate both male and

354     female reproduction, and so maximize reproductive success in both sexes.

355

356     Allen, N.D., Logan, K., Lally, G., Drage, D.J., Norris, M.L., Keverne, E.B., 1995. Distribution

357         of parthenogenetic cells in the mouse brain and their influence on brain development

358         and behavior. Proc. Natl. Acad. Sci. U.S.A. 92, 10782-6.

359     Barlow, D.P., Stoger, R., Herrmann, B.G., Saito, K., Schweifer, N., 1991. The mouse insulin-

360         like growth factor type-2 receptor is imprinted and closely linked to the Tme locus.

361         Nature. 349, 84.

362     Bartolomei, M.S., Zemel, S., Tilghman, S.M., 1991. Parental imprinting of the mouse H19

363         gene. Nature. 351, 153-5.

364    Barton, S.C., Ferguson-Smith, A.C., Fundele, R., Surani, M.A., 1991. Influence of paternally
365        imprinted genes on development. Development. 113, 679-87.

366    Bressan, F.F., De Bem, T.H.C., Perecin, F., Lopes, F.L., Ambrosio, C.E., Meirelles, F.V.,
367        Miglino, M.A., 2009. Unearthing the roles of imprinted genes in the placenta.
368        Placenta. 30, 823-834.

369    Broad, K.D., Curley, J.P., Keverne, E.B., 2009. Increased apoptosis during neonatal brain
370        development underlies the adult behavioral deficits seen in mice lacking a functional
371        paternally expressed gene 3 (*Peg3*). Dev. Neurobiol. 69, 314-325.

372    Champagne, F.A., Curley, J.P., Swaney, W.T., Hasen, N.S., Keverne, E.B., 2009. Paternal
373        influence on female behavior: the role of *Peg3* in exploration, olfaction, and
374        neuroendocrine regulation of maternal behavior of female mice. Behav. Neurosci.
375        123, 469-80.

376    Charalambous, M., Cowley, M., Geoghegan, F., Smith, F.M., Radford, E.J., Marlow, B.P.,
377        Graham, C.F., Hurst, L.D., Ward, A., 2010. Maternally-inherited Grb10 reduces
378        placental size and efficiency. Dev. Biol. 337, 1-8.

379    Coan, P.M., Burton, G.J., Ferguson-Smith, A.C., 2005. Imprinted genes in the placenta - a
380        review. Placenta. 26 Suppl A, S10-20.

381    Constancia, M., Hemberger, M., Hughes, J., Dean, W., Ferguson-Smith, A., Fundele, R.,
382        Stewart, F., Kelsey, G., Fowden, A., Sibley, C., Reik, W., 2002. Placental-specific
383        IGF-II is a major modulator of placental and fetal growth. Nature. 417, 945.

384    Curley, J.P., Barton, S., Surani, A., Keverne, E.B., 2004. Coadaptation in mother and infant
385        regulated by a paternally expressed imprinted gene. Proc. R. Soc. Lond. B Biol. Sci.
386        271, 1303-9.

387    Curley, J.P., Champagne, F.A., Bateson, P., Keverne, E.B., 2008. Transgenerational effects
388        of impaired maternal care on behaviour of offspring and grandoffspring. Anim. Behav.
389        75, 1551-1561.

390     Curley, J.P., Pinnock, S.B., Dickson, S.L., Thresher, R., Miyoshi, N., Surani, M.A., Keverne,

391           E.B., 2005. Increased body fat in mice with a targeted mutation of the paternally

392           expressed imprinted gene Peg3. FASEB J. 19**,** 1302-4.

393     Davies, W., Isles, A.R., Humby, T., Wilkinson, L.S., 2008. What are imprinted genes doing in

394           the brain? Adv. Exp. Med. Biol.**,** 62-70.

395     DeChiara, T.M., Robertson, E.J., Efstratiadis, A., 1991. Parental imprinting of the mouse

396           insulin-like growth factor II gene. Cell. 64**,** 849.

397     Delaval, K. and Feil, R., 2004. Epigenetic regulation of mammalian genomic imprinting. Curr.

398           Opin. Genet. Dev. 14**,** 188-195.

399     Deng, Y.B. and Wu, X.W., 2000. Peg3/Pw1 promotes p53-mediated apoptosis by inducing

400           Bax translocation from cytosol to mitochondria. Proc. Natl. Acad. Sci. U.S.A. 97**,**

401           12050-12055.

402     Eiholzer, U., l'Allemand, D., Rousson, V., Schlumpf, M., Gasser, T., Girard, J., Gruters, A.,

403           Simoni, M., 2006. Hypothalamic and gonadal components of hypogonadism in boys

404           with Prader-Labhart- Willi syndrome. J. Clin. Endocrinol. Metab. 91**,** 892-898.

405     Francis, D., Diorio, J., Liu, D., Meaney, M.J., 1999. Nongenomic Transmission Across

406           Generations of Maternal Behavior and Stress Responses in the Rat. Science. 286**,**

407           1155-1158.

408     Haig, D. and Graham, C., 1991. Genomic imprinting and the strange case of the insulin-like

409           growth factor II receptor. Cell. 64**,** 1045-1046.

410     Hammoud, S.S., Purwar, J., Pflueger, C., Cairns, B.R., Carrell, D.T., 2009. Alterations in

411           sperm DNA methylation patterns at imprinted loci in two classes of infertility. Fertil.

412           Steril.

413     Hiura, H., Obata, Y., Komiyama, J., Shirai, M., Kono, T., 2006. Oocyte growth-dependent

414           progression of maternal imprinting in mice. Genes Cells. 11**,** 353-361.

415     Hore, T.A., Rapkins, R.W., Graves, J.A.M., 2007. Construction and evolution of imprinted

416           loci in mammals. Trends Genet. 23**,** 440-448.

417    Hurst, L.D. and McVean, G.T., 1997. Growth effects of uniparental disomies and the conflict
418         theory of genomic imprinting. Trends Genet. 13**,** 436.

419    Hurst, L.D. and McVean, G.T., 1998. Do we understand the evolution of genomic imprinting?
420         Curr. Opin. Genet. Dev. 8**,** 701-708.

421    Isles, A.R. and Holland, A.J., 2005. Imprinted genes and mother-offspring interactions. Early
422         Hum. Dev. 81**,** 73-7.

423    Isles, A.R. and Wilkinson, L.S., 2000. Imprinted genes, cognition and behaviour. Trends
424         Cogn. Sci. 4**,** 309-318.

425    Kacem, S. and Feil, R., 2009. Chromatin mechanisms in genomic imprinting. Mamm.
426         Genome. 20**,** 544-556.

427    Kaneda, M., Okano, M., Hata, K., Sado, T., Tsujimoto, N., Li, E., Sasaki, H., 2004. Essential
428         role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting.
429         Nature. 429**,** 900-3.

430    Kaneko-Ishino, T., Kohda, T., Ishino, F., 2003. The regulation and biological significance of
431         genomic imprinting in mammals. J. Biochem. 133**,** 699-711.

432    Kato, Y., Kaneda, M., Hata, K., Kumaki, K., Hisano, M., Kohara, Y., Okano, M., Li, E.,
433         Nozaki, M., Sasaki, H., 2007. Role of the Dnmt3 family in de novo methylation of
434         imprinted and repetitive sequences during male germ cell development in the mouse.
435         Hum. Mol. Genet. 16**,** 2272-2280.

436    Keller, M., Baum, M.J., Brock, O., Brennan, P.A., Bakker, J., 2009. The main and the
437         accessory olfactory systems interact in the control of mate recognition and sexual
438         behavior. Behav. Brain Res. 200**,** 268-76.

439    Keverne, B., 2009. Monoallelic gene expression and mammalian evolution. BioEssays. 31**,**
440         1318-1326.

441    Keverne, E.B. and Curley, J.P., 2008. Epigenetics, brain evolution and behaviour. Front.
442         Neuroendocrinol. 29**,** 398-412.

Keverne, E.B., Fundele, R., Narasimha, M., Barton, S.C., Surani, M.A., 1996. Genomic imprinting and the differential roles of parental genomes in brain development. Dev. Brain Res. 92, 91-100.

Killian, J.K., Nolan, C.M., Stewart, N., Munday, B.L., Andersen, N.A., Nicol, S., Jirtle, R.L., 2001. Monotreme IGF2 expression and ancestral origin of genomic imprinting. J. Exp. Zool. 291, 205-12.

Kozlov, S.V., Bogenpohl, J.W., Howell, M.P., Wevrick, R., Panda, S., Hogenesch, J.B., Muglia, L.J., Van Gelder, R.N., Herzog, E.D., Stewart, C.L., 2007. The imprinted gene Magel2 regulates normal circadian output. Nat. Genet. 39, 1266-72.

Kuroiwa, Y., Kaneko-Ishino, T., Kagitani, F., Kohda, T., Li, L.L., Tada, M., Suzuki, R., Yokoyama, M., Shiroishi, T., Wakana, S., Barton, S.C., Ishino, F., Surani, M.A., 1996. Peg3 imprinted gene on proximal chromosome 7 encodes for a zinc finger protein. Nat. Genet. 12, 186-90.

Lau, M.M., Stewart, C.E., Liu, Z., Bhatt, H., Rotwein, P., Stewart, C.L., 1994. Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. Genes Dev. 8, 2953-2963.

Lee, S., Kozlov, S., Hernandez, L., Chamberlain, S.J., Brannan, C.I., Stewart, C.L., Wevrick, R., 2000. Expression and imprinting of MAGEL2 suggest a role in Prader-willi syndrome and the homologous murine imprinting phenotype. Hum. Mol. Genet. 9, 1813-9.

Lefebvre, L., Viville, S., Barton, S.C., Ishino, F., Keverne, E.B., Surani, M.A., 1998. Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. Nat. Genet. 20, 163-9.

Leighton, P.A., Ingram, R.S., Eggenschwiler, J., Efstratiadis, A., Tilghman, S.M., 1995. Disruption of imprinting caused by deletion of the H19 gene region in mice. Nature. 375, 34-9.

Li, C.S., Kaba, H., Saito, H., Seto, K., 1992. Cholecystokinin - Critical Role in Mediating Olfactory Influences on Reproduction. Neuroscience. 48, 707-713.

Li, L., Keverne, E.B., Aparicio, S.A., Ishino, F., Barton, S.C., Surani, M.A., 1999. Regulation of maternal behavior and offspring growth by paternally expressed Peg3. Science. 284, 330-3.

McGrath, J. and Solter, D., 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell. 37, 179-83.

Mercer, R.E., Kwolek, E.M., Bischof, J.M., van Eede, M., Henkelman, R.M., Wevrick, R., 2009. Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene Magel2. Am. J. Med. Genet. B Neuropsychiatr. Genet. 150B, 1085-99.

Mercer, R.E. and Wevrick, R., 2009. Loss of magel2, a candidate gene for features of Prader-Willi syndrome, impairs reproductive function in mice. PLoS ONE. 4, e4291.

Miller, N.L., Wevrick, R., Mellon, P.L., 2009. Necdin, a Prader-Willi syndrome candidate gene, regulates gonadotropin-releasing hormone neurons during development. Hum. Mol. Genet. 18, 248-60.

Moore, T. and Haig, D., 1991. Genomic imprinting in mammalian development - a parental tug-of-war. Trends Genet. 7, 45-49.

Moore, T. and Mills, W., 2008. Evolutionary theories of imprinting - enough already! Adv. Exp. Med. Biol., 116-22.

Morison, I.M., Ramsay, J.P., Spencer, H.G., 2005. A census of mammalian imprinting. Trends Genet. 21, 457-65.

Muscatelli, F., Abrous, D.N., Massacrier, A., Boccaccio, I., Moal, M.L., Cau, P., Cremer, H., 2000. Disruption of the mouse Necdin gene results in hypothalamic and behavioral alterations reminiscent of the human Prader-Willi syndrome. Hum. Mol. Genet. 9, 3101-3110.

Orr, H.A., 1995. Somatic mutation favors the evolution of diploidy. Genetics. 139, 1441-1447.

Peters, J. and Williamson, C.M., 2007. Control of imprinting at the *Gnas* cluster. Epigenetics. 2, 207-213.

499  Peters, J., Wroe, S.F., Wells, C.A., Miller, H.J., Bodle, D., Beechey, C.V., Williamson, C.M.,
500      Kelsey, G., 1999. A cluster of oppositely imprinted transcripts at the Gnas locus in
501      the distal imprinting region of mouse chromosome 2. Proc Natl Acad Sci U S A. 96,
502      3830-5.

503  Plagge, A., Gordon, E., Dean, W., Boiani, R., Cinti, S., Peters, J., Kelsey, G., 2004. The
504      imprinted signaling protein XLαs is required for postnatal adaptation to feeding. Nat.
505      Genet. 36, 818-26.

506  Poplinski, A., Tuttelmann, F., Kanber, D., Horsthemke, B., Gromoll, J., 2009. Idiopathic male
507      infertility is strongly associated with aberrant methylation of MEST and IGF2/H19
508      ICR1. Int. J. Androl. [Epub ahead of print].

509  Renfree, M.B., Ager, E.I., Shaw, G., Pask, A.J., 2008. Genomic imprinting in marsupial
510      placentation. Reproduction. 136, 523-31.

511  Renfree, M.B., Hore, T.A., Shaw, G., Marshall Graves, J.A., Pask, A.J., 2009. Evolution of
512      Genomic Imprinting: Insights from Marsupials and Monotremes. Annu. Rev.
513      Genomics Hum. Genet. 10, 241-262.

514  Sasaki, H. and Matsui, Y., 2008. Epigenetic events in mammalian germ-cell development:
515      reprogramming and beyond. Nat. Rev. Genet. 9, 129-140.

516  Schaefer, C.B., Ooi, S.K., Bestor, T.H., Bourc'his, D., 2007. Epigenetic decisions in
517      mammalian germ cells. Science. 316, 398-9.

518  Stouder, C. and Paoloni-Giacobino, A., 2009. Transgenerational effects of the endocrine
519      disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse
520      sperm. Reproduction. 139, 373-9.

521  Surani, M.A., Barton, S.C., Norris, M.L., 1984. Development of reconstituted mouse eggs
522      suggests imprinting of the genome during gametogenesis. Nature. 308, 548-50.

523  Swaney, W.T., Curley, J.P., Champagne, F.A., Keverne, E.B., 2007. Genomic imprinting
524      mediates sexual experience-dependent olfactory learning in male mice. Proc. Natl.
525      Acad. Sci. U.S.A. 104, 6084-9.

526    Swaney, W.T., Curley, J.P., Champagne, F.A., Keverne, E.B., 2008. The paternally
527        expressed gene Peg3 regulates sexual experience-dependent preferences for
528        estrous odors. Behav. Neurosci. 122**,** 963-73.

529    Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R.,
530        Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal
531        behavior. Nat. Neurosci. 7**,** 847-54.

532    Wilkinson, L.S., Davies, W., Isles, A.R., 2007. Genomic imprinting effects on brain
533        development and function. Nat. Rev. Neurosci. 8**,** 832-843.

534    Williamson, C.M., Turner, M.D., Ball, S.T., Nottingham, W.T., Glenister, P., Fray, M.,
535        Tymowska-Lalanne, Z., Plagge, A., Powles-Glover, N., Kelsey, G., Maconochie, M.,
536        Peters, J., 2006. Identification of an imprinting control region affecting the expression
537        of all transcripts in the Gnas cluster. Nat. Genet. 38**,** 350-355.

538    Yokomine, T., Hata, K., Tsudzuki, M., Sasaki, H., 2006. Evolution of the vertebrate DNMT3
539        gene family: a possible link between existence of DNMT3L and genomic imprinting.
540        Cytogenet. Genome Res. 113**,** 75-80.

541

542 Figure 1. Non-Mendelian parent-of-origin expression allows imprinted genes to be studied

543 independently in mother and offspring using knockout mice. Selective silencing of one

544 parental allele means that heterozygous transgenic mice have either full mutant or full wild-

545 type (WT) phenotypes depending on the imprinting status of the gene and the parent from

546 which the mutation is inherited. By pairing different combinations of mutant-heterozygous,

547 WT-heterozygous and WT individuals, mutant offspring can be born to wild-type mothers

548 and wild-type offspring born to mutant mothers. a) Hypothetical mating combinations to

549 study a paternally expressed gene knockout: i) crossing a WT father with a mutant-

550 heterozygous mother produces WT and WT-heterozygous offspring; ii) crossing a mutant-

551 heterozygous father with a WT mother produces WT and mutant-heterozygous offspring. b)

552 Hypothetical mating combinations to study a maternally expressed gene knockout: i)

553 crossing a WT father with a mutant-heterozygous mother produces WT and mutant-

554 heterozygous offspring; ii) crossing a WT-father with a WT-heterozygous mother produces

555 WT and mutant-heterozygous offspring.

556

557 Table 1. A summary table of the imprinted genes which have been linked to behavioral and

558 neuroendocrine components of reproduction in mammals.

| Gene | Expression | Offspring | Females | Males | References |
|------|-----------|-----------|---------|-------|------------|
| Peg1 | Paternal | Post-natal growth | Maternal behaviors | Male fertility and sperm viability | (Lefebvre et al., 1998; Hammoud et al., 2009; Poplinski et al., 2009) |
| Peg3 | Paternal | Post-natal growth, suckling, thermo-regulation, puberty | Maternal behaviors, milk letdown, hypothalamic oxytocin | Sexual behavior, reproductive olfaction, hypothalamic plasticity | (Li et al., 1992; Curley et al., 2004; Champagne et al., 2009; Swaney et al., 2007; Swaney et al., 2008) |
| Gnasxl | Paternal | Post-natal growth, suckling | - | - | (Plagge et al., 2004) |
| Magel2 | Paternal | - | Reproductive behavior, fertility, gonadal histology hypothalamic serotonin and dopamine, hypogonadism | Reproductive behavior, fertility, reproductive olfaction, hypothalamic serotonin and dopamine, testosterone levels, hypogonadism | (Mercer and Wevrick, 2009; Mercer et al., 2009) |
| Necdin | Paternal | - | Hypothalamic oxytocin and GnRH, hypogonadism | Hypothalamic oxytocin and GnRH, hypogonadism | (Muscatelli et al, 2000; Miller et al., 2009) |