

# GENOMIC IMPRINTING AND KINSHIP: How Good is the Evidence?

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■ **Abstract** The kinship theory of genomic imprinting proposes that parent-specific gene expression evolves at a locus because a gene's level of expression in one individual has fitness effects on other individuals who have different probabilities of carrying the maternal and paternal alleles of the individual in which the gene is expressed. Therefore, natural selection favors different levels of expression depending on an allele's sex-of-origin in the previous generation. This review considers the strength of evidence in support of this hypothesis for imprinted genes in four "clusters," associated with the imprinted loci *Igf2*, *Igf2r*, *callipyge*, and *Gnas*. The clusters associated with *Igf2* and *Igf2r* both contain paternally expressed transcripts that act as enhancers of prenatal growth and maternally expressed transcripts that act as inhibitors of prenatal growth. This is consistent with predictions of the kinship theory. However, the clusters also contain imprinted genes whose phenotypes as yet remain unexplained by the theory. The principal effects of imprinted genes in the *callipyge* and *Gnas* clusters appear to involve lipid and energy metabolism. The kinship theory predicts that maternally expressed transcripts will favor higher levels of nonshivering thermogenesis (NST) in brown adipose tissue (BAT) of animals that huddle for warmth as offspring. The phenotypes of reciprocal heterozygotes for *Gnas* knockouts provide provisional support for this hypothesis, as does some evidence from other imprinted genes (albeit more tentatively). The diverse effects of imprinted genes on the development of white adipose tissue (WAT) have so far defied a unifying hypothesis in terms of the kinship theory.

## CONTENTS

INTRODUCTION .....	554
SCOPE OF THE THEORY .....	555
BECKWITH-WIEDEMANN CLUSTER .....	557
Uniparental Disomies .....	557
Imprinted Genes .....	557
Overview .....	561
<i>Tme</i> CLUSTER .....	562
<i>Callipyge</i> CLUSTER .....	563
Uniparental Disomies .....	564
Imprinted Genes .....	564

<i>GNAS</i> COMPLEX LOCUS .....	566
Human Clinical Data .....	566
<i>Gnas</i> Knockouts .....	568
Evolutionary Conjectures .....	568
CONCLUSIONS .....	570

## INTRODUCTION

It is 15 years since Haig & Westoby proposed that evolutionary conflict between genes of maternal and paternal origin would favor the evolution of “parent-specific gene expression” (65) and 13 years since the description of paternal-specific expression of *Igf2* (30) and maternal-specific expression of *Igf2r* (5) provided apparent support for their hypothesis (64). Since then, much additional information has accumulated about genes with parent-specific expression and it has been claimed that much of this evidence does not support, or even contradicts, the hypothesis (82–84). Therefore, this seems an opportune time to review the current state of empirical support (or lack of support) for the hypothesis.

In its most general form, the hypothesis proposes that parent-specific expression is favored at a locus when two conditions are satisfied (62, 63). First, changes in the aggregate level of expression of the two alleles at the locus influence a trade-off between the fitnesses of related individuals. For example, the fitness of one individual (say a fetus) could be enhanced by increases in expression at the expense of a related individual (say its mother). Second, one or more of the affected individuals must have different probabilities of inheriting a copy of the maternally and paternally derived alleles of the individual in which the gene is expressed (strictly, the theory requires that the ratio of these probabilities differs for one or more of the affected individuals). For example, a mother definitely possesses a copy of the maternally derived allele of a fetus but usually does not carry an identical-by-descent copy of the paternally derived allele. I adopt Trivers & Burt’s suggestion that this hypothesis be known as the kinship theory of genomic imprinting (175), rather than the more common conflict theory, because it is the appeal to interactions among kin that is the theory’s most distinctive feature.

The kinship theory can be considered to have a weak and a strong version (197). The weak version is a logical statement about how natural selection must act if genes have parent-specific expression in one individual and this expression has fitness consequences for other individuals that have different probabilities of carrying the first individual’s maternal and paternal alleles (so-called asymmetric kin). Specifically, alleles at maternally silent loci are subject to selection on their effects on patrilineal kin, whereas alleles at paternally silent loci are subject to selection on their effects on matrilineal kin (62). The logical status of the weak version of the kinship theory, and of its theoretical extensions, has recently been reviewed (63, 197).

The strong version of the kinship theory is the claim that fitness effects on asymmetric kin have been the principal factors responsible for the evolution and

maintenance of parent-specific expression at most imprinted loci. It would be theoretically possible for the weak version of the theory to be correct, but for the strong version to be false because most examples of parent-specific expression have evolved for reasons that do not involve fitness consequences for asymmetric kin.

The current review assesses the status of empirical support for the strong version of the kinship theory. Therefore, the review emphasizes the phenotypic effects of imprinted genes, rather than molecular mechanisms, because it is a gene's phenotypic effects that influence whether or not the gene will be successfully transmitted to the next generation. My review therefore covers some of the same ground as an excellent recent review of the physiological functions of imprinted genes (179).

The number of known imprinted genes is steadily increasing, but little is known about the physiological functions of many of these genes. Therefore, a review that attempted to cover all imprinted genes would be tedious and largely inconclusive, for most loci. Instead, my approach is to pick four clusters of imprinted genes and consider whether the functions of genes in these clusters support the kinship theory's predictions.

The first two clusters were chosen because they contain the *IGF2* and *IGF2R* loci. These are the cluster associated with Beckwith-Wiedmann syndrome in humans and the cluster associated with the *T-associated maternal effect (Tme)* in mice. The contrast between paternal-specific expression of *Igf2* and maternal-specific expression of *Igf2r* is often considered to provide the best support for the kinship theory, and the choice of these clusters might be considered as "stacking the deck" in favor of the theory. Therefore, the remaining two clusters were picked blind, before I had given much thought to their evolutionary interpretation. These are the cluster associated with the *callipyge* mutation in sheep and the cluster of transcripts associated with the *GNAS* complex locus. An obvious genomic region to have considered would have been the cluster of imprinted genes at human 15q11–q13 associated with Prader-Willi and Angelman syndromes (137). However, I have recently attempted an evolutionary interpretation of the phenotype of Prader-Willi syndrome (66) and prefer not to repeat myself.

## SCOPE OF THE THEORY

Before considering the empirical evidence, it is important to be clear about what the kinship theory does, and does not, purport to explain. The theory proposes an adaptive explanation for parent-specific gene expression. That is, the theory proposes reasons why alleles with parent-specific expression are selectively favored over alleles whose expression is independent of parental origin (whether this expression is biallelic or randomly monoallelic). But the theory does not address the mechanisms of parent-specific expression, nor does it address the mechanisms and functions of parent-specific modifications of DNA that are not associated with differences of expression. The theory assumes that mechanisms of parent-specific

modification exist, otherwise there would be nothing on which natural selection could act, but does not explain the origin of these mechanisms.

Many criticisms of the kinship theory are the result of a perfunctory reading of the theoretical literature. I address two common misunderstandings only: the claims that the theory applies only to interactions between mothers and offspring and only to loci that affect preweaning growth (83).

The kinship theory applies to all of an individual's interactions with asymmetric kin, not just its interactions with its mother and sibs (62, 63). Therefore, the demonstration that some imprinted genes lack effects on an individual's immediate family would not automatically contradict the theory. However, the theory's predictions have been most fully developed for mother-offspring relations because it is here that the selective forces favoring parent-specific expression are likely to be strongest, for two interrelated reasons. First, an offspring's interaction with its mother is an important part of every mammal's life, with the potential for strong effects on each other's fitness. Second, the asymmetry of selective forces acting on alleles of different parental origin is large when gene expression in an offspring has fitness consequences for its mother. This is because an offspring's maternally derived alleles are necessarily present in its mother ( $m = 1$ ), whereas its paternally derived alleles are necessarily absent ( $p = 0$ , where  $m$  and  $p$  are probabilities of identity by recent common descent calculated for an outbreeding species).

If maternal investment is defined as "any investment by [a mother] in an individual offspring that increases the offspring's chances of surviving (and hence reproductive success) at the cost of the [mother's] ability to invest in other offspring" (173, 174), then the kinship theory predicts that paternally expressed genes in a fetus will be selected to favor higher levels of maternal investment than will unimprinted genes, which will in turn be selected to favor higher levels of maternal investment than maternally expressed genes (60). The preceding is often expressed as a prediction that paternally derived genes should favor increased growth and maternally derived genes should favor reduced growth. However, the theory applies to any gene expression in an offspring that enhances the offspring's own fitness at a cost to its mother's reproduction via other offspring. Paternally derived alleles favor greater maternal investment whether or not this investment occurs before or after weaning. In particular, maternal care in humans usually extends well beyond weaning. Therefore, the paternal genes of human children have additional opportunities for shifting costs to the children's mothers (66) that are not present in most other mammals, including mice.

An individual's mother is asymmetric kin, but so too is the individual's mother's half-sister's daughter (a first half-cousin). Therefore, the kinship theory applies to the latter relationship as well as the former. However, the selective forces associated with interactions between first half-cousins will usually be weak. Fitness effects of gene expression in one individual on the individual's half-cousins will often be small, because this is an insignificant relationship in the lives of many mammals. Moreover, when fitness effects occur, the associated asymmetry of relatedness will be relatively weak ( $m = 0.25$ ,  $p = 0$ ).

## BECKWITH-WIEDEMANN CLUSTER

A major cluster of imprinted genes occurs at human chromosome 11p15.5 and at the homologous region of distal chromosome 7 in mice (37, 142, 145). This cluster can be divided into two subdomains with distinct imprint control regions (183, 192). At one end of the cluster are two genes, *IGF2* and *H19*, that are reciprocally imprinted and share important regulatory elements (4). At the other end of the cluster are a number of genes that are under the control of *KvDMR1*, a differentially methylated CpG island (42, 80). Genes from both subdomains are implicated in Beckwith-Wiedemann syndrome (BWS) of humans. Evidence from uniparental disomies (UPDs) will be reviewed before discussing the functions of individual imprinted genes from this cluster. A number of genes that have been reported to be imprinted, but for which there are few meaningful data on phenotypic effects, will not be considered.

### Uniparental Disomies

Paternal UPD for 11p15.5 is found in 10–20% of BWS patients (76, 192). This syndrome is associated with prenatal overgrowth and enlarged placentas (100, 123). By contrast to the live birth of humans with paternal UPD 11p15.5, mouse embryos with paternal UPD of distal chromosome 7 (PatDup.d7) die in mid-gestation, apparently from placental dysfunction (126). The placentas of these embryos are characterized by an absence of spongiotrophoblast and by excess trophoblast giant cells. A similar phenotype is observed in embryos with an inactivated maternal allele of *Mash2* (see below). Chimeric mouse embryos carrying a proportion of PatDup.d7 cells show many of the overgrowth features typical of BWS (127). This suggests that PatDup.d7 would be growth-enhancing in mice, as is paternal UPD 11p15.5 in humans, if not for the specific defect in the development of spongiotrophoblast.

Maternal UPD for 11p15.5 has not been reported, possibly because it is incompatible with live birth. However, three cases of digynic trisomy 11p15.5 have been described, all associated with severe intrauterine growth retardation (41). Mice with maternal UPD of distal chromosome 7 die in late gestation. These fetuses are markedly growth-retarded with a roughly 50% reduction in placental weight (156).

### Imprinted Genes

**INSULIN-LIKE GROWTH FACTOR 2 (IGF-II)** Inactivation of the paternal copy of *Igf2* results in mice that are 60% normal size at birth but otherwise normally proportioned. This growth disadvantage is maintained through weaning and into adult life. By contrast, growth is normal when the same mutation is inherited maternally (30). A recent knockout of a placenta-specific promoter of *Igf2* is of particular interest. Newborn mice with a paternal copy of this mutation express similar growth

retardation to mice with a deletion of the *Igf2* coding sequence, but then display catch-up growth to become normal-sized adults (27). This indicates that *Igf2* expression in the mouse placenta is principally responsible for prenatal growth retardation, but that the absence of imprinted transcripts from other promoters is responsible for the failure to achieve catch-up growth in the full gene knockout.

Loss-of-imprinting (biallelic expression) of *IGF2* is common in humans with BWS (192) and has been reported from children with somatic overgrowth, but without other BWS features (133). Circulating levels of IGF-II were significantly elevated in a group of prepubertal children who were more than two standard deviations above mean height for age (48).

*IGF2* has been shown to be imprinted in humans (140), mice (30), rats (143), deer mice (185), sheep (125), pigs (180), and opossums (141), but is biallelically expressed in monotremes (95) and chickens (138, 141, 207). These data suggest that imprinting of *IGF2* arose in a common ancestor of metatherian (marsupial) and eutherian mammals, but was absent from the common ancestor of therian mammals and monotremes.

***H19*** *H19* encodes a highly expressed noncoding RNA. Transcripts are expressed when *H19* is inherited maternally but not when it is inherited paternally (6, 214). A recent review of the outcomes of 30 deletions, insertions, and transgenes involving the *H19-Igf2* region of mice reveals a complex pattern of interdependent regulation of the two genes, some of the details of which remain unclear (4). The *H19* transcript exhibits conservation of secondary structures such as hairpin loops (89, 171) and the gene exhibits faster evolution of intronic than of exonic sequences (85). Both observations suggest that the RNA is functional. A suggestion that *H19* has growth-inhibitory effects has come from a deletion of a silencer element that caused derepression of the paternal allele of *H19* without detectable changes in the expression of *Igf2*. Newborn mice that inherited this deletion from their father were 12% smaller than their wild-type littermates (34). However, the replacement of the *H19* sequence with a reporter gene under control of the *H19* promoter had no readily discernible phenotype (86a).

Some data suggest that maternally expressed *H19* RNA may have a role in regulating the level of paternally expressed *IGF2* mRNA. For example, the levels of *H19* RNA and *IGF2* mRNA associated with polysomes are inversely correlated (108). Similarly, transfection with antisense *H19* has been reported to increase levels of *IGF2* mRNA whereas transfection with sense *H19* reduces the expression of a reporter gene driven by an *IGF2* promoter (195). The possibility of posttranscriptional interactions between RNA products of *IGF2* and *H19* is also suggested by the fact that *H19* RNA and *IGF2* mRNAs bind to a shared RNA-trafficking protein (151). *H19* has been reported to have tumor-suppressing activity in some cell lines (68) but to have tumorigenic properties in others (114).

**KCNQ1 OVERLAPPING TRANSCRIPT 1 (*KCNQ1OT1*; ALSO KNOWN AS *LIT1*)** *KvDMR1* is a differentially methylated region located roughly 500 kb from *H19* on 11p15.5

in intron 10 of *KCNQ1* (161). The maternal copy of *KvDMR1* is methylated but the paternal copy is unmethylated in both the mouse and human genome (161, 205). *KvDMR1* contains the promoter for *KCNQ1OT1*, a large noncoding DNA that is widely expressed and transcribed exclusively from its paternal allele (106, 121, 129, 161).

*KvDMR1* appears to act as the imprinting control region for nearby maternally expressed genes. In mice, inheritance of a deleted paternal allele of *KvDMR1* causes derepression of normally silent paternal alleles of *Kcnq1*, *Cdkn1c*, *Tssc3*, *Tssc4*, *Slc22a11*, and *Mash2* (42). These mice are 20–25% smaller than wild-type littermates, both as fetuses and adults. Mice with a deleted maternal copy of *KvDMR1* are normal sized and do not show activation of paternal alleles at the imprinted loci (42). Deletion of the paternal copy of *KvDMR1* results in the loss of *Kcnq1ot1* transcripts (183). It is currently unclear whether it is the absence of *Kcnq1ot1* transcripts that causes growth retardation and loss-of-imprinting of neighboring genes or whether these effects can be more directly ascribed to the absence of a paternal copy of *KvDMR1* (183).

Deletion of human *KvDMR1* in somatic cell hybrids results in the activation of paternal alleles of *KCNQ1* and *CDKN1C* (80). Biallelic expression of *KCNQ1OT1*, and an associated loss of methylation of paternal *KvDMR1*, is observed in roughly half of all cases of BWS (106, 129, 161, 192).

MAMMALIAN ACHAETE-SCUTE HOMOLOGOUS PROTEIN-2 (*Mash2*; ALSO KNOWN AS *ASCL2*) Expression of *Mash2* in mice is restricted to extraembryonic tissues (59, 164), where the gene is expressed predominantly from its maternal allele (59). Placentas of 10.5-day embryos with an inactivated maternal allele exhibit increased numbers of trophoblast giant cells but a complete lack of spongiotrophoblast and an underdeveloped labyrinthine trophoblast (58, 59). This phenotype resembles that of mice with paternal UPD for distal chromosome 7 (126). Conversely, overexpression of *Mash2* causes an absence of trophoblast giant cells (155). Thus, *Mash2* appears to act by inhibiting differentiation of giant cells. If so, spongiotrophoblast may be absent in *Mash2*-null placentas because its precursors have all differentiated down the giant-cell pathway. *ASCL2*, the human ortholog of *Mash2*, does not appear to be imprinted (130, 194). This difference between species may explain why paternal UPD for the Beckwith-Wiedemann cluster is compatible with survival to birth in humans but not in mice.

The phenotype of *Mash2*-null mice has been interpreted as contrary to predictions of the kinship theory because the absence of a maternally expressed gene results in a failure of placental development (83). However, knockouts are an extreme perturbation and maternal-specific expression of *Mash2* would be explicable in terms of the theory if a partial reallocation of cells from spongiotrophoblast to giant cells would enhance fetal growth (62). Giant cells are the site of production of a number of placental hormones, including placental lactogen-1 (115, 150). Such hormones have been proposed to manipulate maternal physiology for fetal benefit, and thus increase the mother's costs of pregnancy (61).

CYCLIN-DEPENDENT KINASE INHIBITOR 1C *CDKN1C* encodes the cyclin-dependent kinase inhibitor p57<sup>KIP2</sup>. This gene shows preferential expression of the maternal allele in humans (122) and mice (69). A subset of patients with BWS have inactivating mutations of the maternal allele of *CDKN1C* (11, 70, 101). Moreover, BWS patients with loss of methylation at *KvDMR1* show greatly reduced expression of *CDKN1C* (32). By contrast, mice with inactivating mutations of maternal *Cdkn1c* do not exhibit somatic overgrowth although they exhibit other features of BWS (167, 203, 212). Some data suggest that maternally expressed *CDKN1C* and paternally expressed *IGF2* have antagonistic effects on cell proliferation (20, 57). Such antagonism could help to explain how mutations of *CDKN1C* and paternal UPD for 11p15.5 both cause BWS.

Mouse embryos with knockouts of the maternal allele of *Cdkn1c* have enlarged placentas (93, 170, 213). In these placentas, proliferation of labyrinthine trophoblast is abnormally maintained into the latter stages of pregnancy (213). Curiously, mothers pregnant with p57<sup>KIP2</sup>-deficient embryos develop preeclampsia-like symptoms (93). Induction of *Cdkn1c* in cells of the mouse ectoplacental cone is coincident with cessation of proliferation and differentiation into trophoblast giant cells. Thereafter, cyclical degradation of p57<sup>KIP2</sup> is required for entry into each cycle of endoreduplication in the highly polyploid giant cells (71).

IMPRINTED IN PLACENTA AND LIVER (ALSO KNOWN AS *PHLDA2* OR *TSSC3*) *IPL* encodes a small protein of unknown function. The gene is highly expressed in human and mouse placenta, with preferential expression from the maternal allele (35, 43, 104, 148, 153). Inactivation of the maternal allele in mice results in generalized overgrowth of the placenta, but without associated overgrowth of the fetus (44).

POTASSIUM VOLTAGE-GATED CHANNEL, KQT-LIKE SUBFAMILY, MEMBER 1 In human fetuses, *KCNQ1* exhibits monoallelic maternal expression in most tissues except heart, where expression is biallelic (105). In mice, *Kcnq1* exhibits preferential maternal expression in most fetal tissues (including heart) but is biallelically expressed in juvenile and adult tissues (56).

Mutations of *KCNQ1* in humans have been associated with autosomal dominant Romano-Ward syndrome (RWS) and autosomal recessive Jervell and Lange-Nielsen syndrome (JLNS) (136, 186): RWS is characterized by heart arrhythmias associated with a long QT interval; JLNS is characterized by a long QT interval and deafness (172). Prior to the identification of *KCNQ1* as a gene mutated in some cases of RWS, Hashiba (68a) reported maternal transmission in 22 of 30 RWS sibships and speculated that genomic imprinting might be involved. However, pedigrees with known mutations in *KCNQ1* show both maternal and paternal transmission of long QT interval (186, 187). The possibility of subtle parent-of-origin effects in RWS does not appear to have been systematically studied. Mice that are homozygous for inactivating mutations of *Kcnq1* exhibit developmental defects in the inner ear, among other anomalies (18, 107), but parent-of-origin effects have not been reported in heterozygotes.

The absence of clear parent-of-origin-dependent phenotypes associated with mutations in *KCNQ1* makes it tempting to suggest that the stage-specific imprinting observed for this gene may not have been selected per se. Rather, maternal-specific expression of *KCNQ1* may be a side effect of selection for imprinting of nearby genes, in particular selection for paternal-specific expression of *KCNQ1OT1*, a gene that is transcribed from the opposite strand to *KCNQ1* and that largely overlaps its sequence.

Two other genes in the Beckwith-Wiedemann cluster have also been reported to show stage-specific imprinting but to lack a heterozygous phenotype in mouse knockouts. *Ins2* shows biallelic expression in most tissues of the mouse embryo, including the yolk sac of 12.5-day-old embryos, but shows paternal-specific expression in the yolk sac of 14.5-day-old embryos (31). However, no heterozygous phenotype has been reported in *Ins2* knockouts (36). Expression of *CD81* shows a strong maternal bias in early mouse embryos, but expression is biallelic by embryonic day 8.5 (19). Reports of inactivating mutations of *CD81* do not mention a heterozygous phenotype (118, 131, 178).

## Overview

The phenotypes of paternal UPDs suggest that a paternal copy of the Beckwith-Wiedemann cluster enhances prenatal growth. Moreover, the cluster has been shown to contain at least two paternally expressed enhancers of intrauterine growth, namely *IGF2* and *KCNQ1OT1*. The growth-enhancing effects of *KCNQ1OT1* are probably indirect, mediated by the inactivation of the paternal alleles of maternally expressed genes. These observations all support the prediction of the kinship theory that paternally expressed genes should enhance fetal growth.

Evidence from maternally expressed genes generally supports the prediction that such genes should act as growth inhibitors. However, support for the kinship theory from maternally expressed genes is somewhat more equivocal than that provided by paternally expressed genes. Some data suggest that *H19* has antagonistic effects to *IGF2*, and would thus act as a growth inhibitor. Similarly, mice with an inactivated maternal allele of *Cdkn1c* and *Ipl* have large placentas, suggesting that the products of these genes inhibit placental growth. However, in neither case is placental overgrowth associated with an enlarged fetus. By contrast, mice with an inactivated maternal allele of *Mash2* have small placentas, because of a specific defect in the development of spongiotrophoblast. This phenotype has been interpreted as contradicting predictions of the kinship theory. If *Mash2* is to support the theory, small decreases in its expression must be shown to increase maternal investment.

Other imprinted genes within the cluster have phenotypes that are presently difficult to interpret in terms of the kinship theory. Perhaps some of these genes are mere “innocent bystanders” swept up in the evolution of imprinting at neighboring loci (181). However, it is hard to exclude the possibility that imprinting is maintained at some loci because of phenotypic effects that are sufficiently subtle to have escaped the attention of researchers looking for major effects.

## *Tme* CLUSTER

Mice that are heterozygous for a small deletion of proximal chromosome 17 die in utero if the deletion is inherited from their mother but survive if the deletion is inherited from their father (87, 124). Therefore, this chromosome region was inferred to contain a genetic element labeled *T-associated maternal effect* (*Tme*). The *Tme* phenotype is now known to be caused by the absence of expression of *Insulin-like growth factor 2 receptor* (*Igf2r*), a gene that is expressed when maternally derived but silent when paternally derived (5). *Tme* deletions also contain a paternally expressed noncoding transcript, *Air* (*Antisense Igf2r RNA*) (117), and two maternally expressed organic cation transporters, *Slc22a2* and *Slc22a3* (215).

The ancestral function of IGF2R is the targeting of mannose 6-phosphate-labeled ligands to lysosomes (28, 53). In this guise, the receptor is known as the cation-independent mannose 6-phosphate receptor (CI-MPR). In marsupial and eutherian mammals, the CI-MPR has acquired a distinct binding site for IGF-II (94, 204). This binding site is absent from the CI-MPR of monotremes, birds, and amphibians (25a, 94). Most evidence suggests that binding of IGF-II to IGF2R does not initiate signal transduction, but rather targets IGF-II to lysosomes for degradation (28).

*IGF2R* is imprinted in marsupials, rodents, and artiodactyls (94, 96), but the orthologous *CI-MPR* of monotremes and chickens is not imprinted (94, 138). The correlation between imprinting and possession of an IGF-II-binding site is not perfect however, because the *IGF2R* of primates, colugos, and tree shrews is biallelically expressed (96). This phylogenetic distribution is consistent with a single origin of imprinting in an ancestor of marsupial and eutherian mammals and a subsequent loss of imprinting in an ancestor of the Euarchonta (primates and their relatives).

There is strong evidence that *Igf2r* functions as a maternally expressed inhibitor of prenatal growth in mice. Inactivation of the maternal copy of *Igf2r* causes a roughly 30% increase in fetal size (102, 188) and is associated with elevated levels of circulating IGF-II (102, 116). Perinatal lethality associated with inactivating mutations of the maternal copy of *Igf2r* is prevented if mice also inherit an inactivating mutation of the paternal copy of *Igf2* (38, 188). This suggests that perinatal lethality and fetal overgrowth are both caused by excess IGF-II, secondary to inactivation of *Igf2r*.

The second intron of *Igf2r* contains Region2, a CpG island that is more heavily methylated when maternally derived than when paternally derived (165). Region2 contains the promoter for *Air*, a paternally expressed noncoding RNA (117). Deletion of the paternally derived Region2 causes the suppression of *Air* transcripts and reactivation of the normally silent paternal allele of *Igf2r* (199, 200). These mice have reduced birthweight, with reduced size maintained into adulthood (200). Mice with a truncated *Air* transcript also exhibit biallelic expression of *Igf2r* and reduced birthweight, despite normal methylation and imprinted expression from the *Air* promoter (158). This indicates that a paternally expressed *Air* transcript is

required for silencing of the paternal allele of *Igf2r*. Taken together, these results suggest that *Igf2r* functions as a maternally expressed inhibitor of fetal growth in mice, whereas *Air* functions as a paternally expressed growth enhancer. *IGF2R* also functions as a maternally expressed growth inhibitor in fetal sheep (208, 209).

*IGF2R* is biallelically expressed in humans (92) and other primates (96). On theoretical grounds, a change in mating system toward greater monandry could have favored the loss of imprinting of *IGF2R* (196), but it is hard to envisage how one could ever test such a hypothesis for an unknown ancestor living in the distant past. However, if imprinting of *IGF2R* was indeed lost in an ancestor of the modern Euarchonta, then the gene's phenotypic effects will have been subject to selection when inherited both maternally and paternally for at least 75 Myr (96). If so, the growth-inhibitory effects of *IGF2R* should be greatly attenuated in primates relative to mice and sheep (where the gene's effects have been subject to selection only when inherited maternally). This prediction remains to be tested, although it is worth noting that reduced expression of *IGF2R* has not yet been found to be a cause of human overgrowth (54).

Deletion of the paternal copy of *Region2*, or expression of a truncated *Air* transcript, both cause biallelic expression of *Slc22a2* and *Slc22a3* (158, 215). Thus, the *Air* transcript controls the maternal-specific expression of these nearby genes. *Slc22a3* is abundantly expressed in labyrinthine trophoblast (182) and therefore may be involved in maternal-fetal relations. However, mice with inactivated maternal copies of either *Slc22a2* or *Slc22a3* lack an obvious phenotype (88, 216). Thus, an explanation of the maternal-specific expression of these organic cation transporters is a challenge that is yet to be met by the kinship theory.

The phenotypic effects of *Igf2r* and *Air* in mice strongly support the kinship theory. The lack of imprinting of *IGF2R* in primates leads to the prediction that the growth-inhibitory effects of the receptor should be attenuated in primates. Imprinted expression of *Slc22a2* and *Slc22a3* in mice is yet to be satisfactorily explained by the kinship theory.

## *Callipyge* CLUSTER

A cluster of imprinted genes resides on sheep chromosome 18, human chromosome 14q, and mouse distal chromosome 12 (24, 109, 146). Early evidence for imprinting in this genomic region was provided by the unusual inheritance of the *callipyge* phenotype in sheep. Heterozygotes that inherit the *callipyge* mutation from their fathers have increased muscle mass in their hindquarters, but this phenotype is absent in heterozygotes that inherit the mutation from their mothers and in homozygotes that inherit the mutation from both parents (26).

The *callipyge* mutation is now known to be caused by a single-base substitution that causes dysregulation of multiple imprinted genes within the cluster (45, 162). The paternally expressed imprinted genes *DLK1* and *PEG11* are highly expressed in skeletal muscle when the *callipyge* mutation is paternally inherited whereas the

maternally expressed imprinted genes *GTL2* and *MEG8* are highly expressed in skeletal muscle when the *callipyge* mutation is maternally inherited. Thus, muscle hypertrophy is conjectured to be caused by the combination of high expression of *DLK1* and *PEG11* with low expression of *GTL2* and *MEG8* (14, 25). These genes are expressed at much lower levels in skeletal muscle of wild-type homozygotes. The muscle hypertrophy of *callipyge* sheep is not directly relevant to understanding the normal function of imprinted genes in the cluster because the *callipyge* mutation causes inappropriate expression in skeletal muscle. However, the normal appearance of *callipyge* homozygotes suggests that the maternally expressed and paternally expressed genes of the cluster have antagonistic effects.

Functional information is largely restricted to two of the imprinted genes in the *callipyge* cluster: *DLK1* and *DIO3*. The known functions of these genes are discussed after a presentation of UPD phenotypes in mice and humans.

## Uniparental Disomies

Both maternal and paternal UPDs for chromosome 14 have been reported from liveborn humans. Maternal UPD has a distinctive phenotype that includes low birth weight, postnatal growth deficiency, and premature puberty (166). By contrast, digynic trisomy for the imprinted region of chromosome 14 does not have an obvious phenotype (128, 167). Therefore, the phenotype of maternal UPD is most probably due to the absence of the products of one or more paternally expressed genes. Paternal UPD of chromosome 14 is associated with low birth weight, skeletal defects of the rib cage and respiratory difficulties (99).

Hurst & McVean (83) interpret the phenotypes of chromosome 14 UPDs as “strongly contradictory” of the kinship theory, particularly the prenatal growth retardation of paternal UPDs and the presence of postweaning effects on growth in maternal UPDs. Poor growth is likely to be a correlate of many major perturbations of development. Therefore, cases of poor growth associated with paternal UPD provide a less compelling challenge to the kinship theory than would a single case of maternal UPD associated with overgrowth. Moreover, the kinship theory is not automatically contradicted by postweaning effects, especially since parental care in humans extends well beyond weaning (66).

Mice with maternal UPD of chromosome 12 are growth retarded with small placentas and die in the perinatal period, whereas mice with paternal UPD have an enlarged placenta, defects of the rib cage, and die late in gestation (49). Paternal UPD 12 is associated with excess placental glycogen cells (50) and with defects of the rib cage and heart (184).

## Imprinted Genes

DELTA-LIKE 1 (ALSO KNOWN AS PREADIPOCYTE FACTOR-1 = PREF-1) *DLK1* is paternally expressed in mice (154, 168), sheep (24, 25), and humans (201). Mice with an inactivated paternal allele of *Dlk1* have reduced weight at birth and at weaning (132). The reduced growth of *Dlk1*-null mice cannot be interpreted as evidence in

favor of the kinship theory, because mice expressing increased levels of *Dlk1* from a transgene also exhibit decreased prenatal and postnatal growth (103).

*DLK1* is expressed in multiple embryonic and adult tissues. In adipose tissue, *Dlk1* inhibits the differentiation of preadipocytes into adipocytes (159, 160). Consistent with this function, increased adiposity is observed in *Dlk1*-knockout mice (132) and reduced adiposity in mice expressing *Dlk1* transgenes (103). Thus, the kinship theory needs to explain why the inhibition of adipogenesis benefits patrilineal interests.

Elsewhere, I have argued that paternally expressed genes of humans may have favored lesser development of fat reserves if human mothers compensated for reduced adiposity of their children by increased provisioning in times of famine (66). However, this argument is unlikely to apply to mice. Another possibility is that mouse pups gain a competitive growth advantage in sib-competition by limiting the diversion of lipids to storage in white adipose tissue. Any such interpretation, however, is complicated by the observation that knockouts of the paternal allele of *Gnas* are associated with decreased adiposity, whereas knockouts of the maternal allele have increased adiposity (see below).

Expression of *Dlk1* transgenes appears to inhibit the differentiation of brown preadipocytes as well as that of white preadipocytes (103). I argue below that paternally expressed genes are predicted to favor reduced recruitment, and reduced thermogenic activity, of brown adipocytes.

DEIODINASE 3 *DIO3* encodes D3, the major enzyme responsible for inactivation of thyroid hormones (13). D3 is the predominant deiodinase expressed in most tissues of the fetal rat (9) and is highly expressed in the placentas of humans (22, 81) and mice (78). *DIO3* is paternally expressed in sheep (109) and mice (79, 176), although imprinting is incomplete in the mouse placenta, with substantial expression of the maternal allele (206). D3 activity in the placenta creates a barrier to the transfer of maternal thyroid hormones to the fetus (134, 150a). The high levels of D3 activity in the placenta and fetal tissues probably contribute to the low levels of triiodothyronine observed in serum of human fetuses (149, 152).

The evolutionary reason for preferential paternal expression of *DIO3* is unclear, but some conjectures are possible. Among other effects, thyroid hormone acts to increase oxygen consumption and heat production. Therefore, suppression of thyroid hormone during fetal development could off-load the metabolic costs of keeping the fetus warm onto the mother and thereby conserve substrates for enhanced growth. After birth, brown adipose tissue (BAT) is largely responsible for nonshivering thermogenesis (NST) induced by cold exposure. Thyroid hormone is necessary for adaptation to cold because NST is impaired in mice without functional thyroid hormone receptors (55). D3 activity can be induced in cell cultures derived from neonatal brown fat of rats (77). Perhaps, expression of *Dio3* in BAT reduces NST, with paternal-specific expression favored because reduced NST off-loads an offspring's heating costs onto other members of the litter, or onto its

mother. An inactivating mutation of *Dio3* has been reported but few details were provided about the phenotypes of heterozygous mice (79).

The evidence for antagonistic effects of maternally-expressed genes and paternally-expressed genes in determination of the *callipyge* phenotype of sheep is consistent with a general prediction of the kinship theory. However, the known functions of imprinted genes in this cluster do not at present provide compelling support for the kinship theory.

## GNAS COMPLEX LOCUS

*GNAS* is perhaps the most complex of imprinted loci (189, 190). Four alternative first exons are spliced onto 12 downstream exons: Exon 1 contains the transcription start site for  $G_s\alpha$ , the heterotrimeric G-protein stimulatory  $\alpha$ -subunit (stop codon located in exon 13); exon 1A lacks a transcription start site and its transcripts appear not to encode a protein product (111); exon XL contains transcription start sites for two protein products, one of which (*XL $\alpha$ s*) shares the same open reading frame and stop codon as  $G_s\alpha$ , whereas the other (*ALEX*) is encoded in an overlapping reading frame with a stop codon located precisely at the end of the exon (97); exon NESP55 contains the entire coding sequence of NESP55, a neuroendocrine secretory protein (40, 86). Alternative splicing has also been reported for downstream exons (40, 98a, 144). To add further complexity, an antisense transcript (*Nespas*) overlaps the NESP55 coding sequence (72, 198).

The pattern of imprinting is equally complex: *NESP55* transcripts are expressed only from the maternally derived allele (74, 147), whereas *Nespas* (72, 198), *XL $\alpha$ s* (73, 147), and exon 1A (7, 110, 111) transcripts are expressed only from the paternally derived allele. Exon 1 transcripts are biallelically expressed in most tissues but, in some cell types, are expressed only from the paternally derived allele (75, 210).

## Human Clinical Data

$G_s\alpha$  relays messages from G-protein coupled receptors (GPCRs) at the cell surface to the cAMP-signaling pathway within the cell. In humans, inactivating mutations of  $G_s\alpha$  are associated with a constellation of symptoms—obesity, short stature, brachydactyly, and subcutaneous ossification—known as Albright's hereditary osteodystrophy (AHO). Significantly, AHO can be caused by mutations in exon 1, the exon that is unique to  $G_s\alpha$  (3) and its symptoms are present whether an inactivating mutation is present on the maternal or paternal allele. Therefore, AHO appears to be caused by haploinsufficiency for  $G_s\alpha$  and its symptoms do not involve imprinting. These symptoms will not concern us here, except to note that, under the kinship theory, dosage-sensitivity of effects is a prerequisite for the evolution of parental-origin-specific silencing at a locus (62).

Although AHO does not involve imprinting, there is accumulating evidence that parent-of-origin influences  $G_s\alpha$ -mediated signaling from a number of GPCRs. Mutations of  $G_s\alpha$  are associated with renal resistance to parathyroid hormone

(PTH) when the mutation is present on an individual's maternal allele but PTH-resistance is absent if the mutation is present on the individual's paternal allele. AHO with PTH-resistance is known as pseudohypoparathyroidism type Ia (PHP-Ia), whereas AHO without PTH-resistance is known as pseudopseudohypoparathyroidism (PPHP) (29, 189). PHP-Ia and PPHP can occur within the same family and be caused by the same mutation in the coding region of  $G_s\alpha$  (39). Not all clinical data support such a neat story (1, 90). Some inactivating mutations of  $G_s\alpha$  are not associated with AHO, and a family has been reported in which paternal transmission of an inactivating mutation is associated with progressive osseous heteroplasia (POH), whereas maternal transmission is associated with apparent PPHP (157).

Rare individuals with PTH-resistance, but without AHO, are described as having pseudohypoparathyroidism type Ib (PHP-Ib). In one particularly illuminating case, PHP-Ib was caused by paternal UPD for the *GNAS* region (8). Thus, the effects of haploinsufficiency for  $G_s\alpha$  (AHO) were absent, but phenotypes associated with the absence of imprinted maternal expression were fully expressed (PTH-resistance). In familial cases, PHP-Ib has been mapped at or close to *GNAS*, with affected carriers inheriting the mutant haplotype from their mother but unaffected carriers inheriting the mutant haplotype from their fathers (91). Most PHP-Ib patients show biallelic expression of transcripts from exon 1A, rather than the usual monoallelic paternal expression (110). This observation could be explained if transcription from exon 1A inhibits the production of functional  $G_s\alpha$  mRNA in *cis*, but this cannot be the whole story because exon 1A mRNA is expressed in many tissues in which  $G_s\alpha$  mRNA is biallelically expressed (110). In one family, PHP-Ib was caused by a single amino-acid deletion in the carboxyl terminus of  $G_s\alpha$ . The deletion prevented functional interaction of  $G_s\alpha$  with the PTH receptor. Three affected brothers inherited the mutant allele from their unaffected mother who inherited the allele from her father (198a).

PTH-resistance is present when inactivating mutations are inherited from an individual's mother (PHP-Ia), but not when these mutations are inherited from fathers (PPHP) (189). This suggests that  $G_s\alpha$  is preferentially translated from maternal transcripts in at least some cell types. Resistance to PTH in PHP-Ia appears to be limited to proximal renal tubules, however, because distal tubules (202) and the skeleton (135) maintain their responsiveness to PTH.

Signaling via the PTH receptor is not the only GPCR pathway to show imprinting effects of *GNAS* mutations. Most  $G_s\alpha$  mRNA in normal human thyroids is transcribed from the maternal allele (51, 112, 120). As a result, most PHP-Ia patients have mild hypothyroidism due to TSH (thyroid stimulating hormone) resistance. Similarly, the paternal allele of  $G_s\alpha$  is normally silent in human pituitaries (120) and most PHP-Ia patients show some degree of impaired growth hormone (GH) release in response to GH-releasing hormone (52, 121). Moreover, about 40% of GH-secreting pituitary adenomas have constitutively active mutations of  $G_s\alpha$ . In almost all cases, the mutation occurs on the maternal allele (75). Finally, olfaction is impaired in PHP-Ia but not in PPHP (33, 191). This suggests preferential expression of maternal  $G_s\alpha$  somewhere within the olfactory system.

Few data are available on the functions of transcripts containing exon XL or exon NESP55. Some individuals who inherit a polymorphic 38-bp insertion in exon XL from their fathers have enhanced trauma-related bleeding and variable psychomotor retardation (46). Co-immunoprecipitation of XL $\alpha$ s and ALEX is almost completely abolished in these patients. ALEX may normally function to sequester XL $\alpha$ s and prevent its functional interaction with GPCRs (47).

## Gnas Knockouts

Heterozygous mice that inherit a targeted disruption of exon 2 of *Gnas* from their mother (maternal knockout = mKO) have increased birth weight, and develop into obese adults, whereas heterozygous mice that inherit the disrupted allele from their father (paternal knockout = pKO) have decreased birth weight, and develop into lean adults. Most pKO mice fail to suckle and die within the first 24 h after birth (210). mKO mice have a lower resting metabolic rate, lower activity levels, and decreased activation of the sympathetic nervous system compared with wild-type littermates. By contrast, pKO mice have a higher resting metabolic rate, higher activity levels, and increased activation of the sympathetic nervous system compared with wild-type littermates. It would be tempting to ascribe the conservative energy metabolism of mKO mice to TSH-resistance (as observed in human PHP-Ia). However, serum levels of TSH, T<sub>3</sub> and T<sub>4</sub> of mKO and pKO mice resemble those of controls (211).

G<sub>s</sub> $\alpha$  mRNA and protein were reduced in brown adipose tissue (BAT) and white adipose tissue (WAT) of mKO mice, but were similar to controls in these tissues of pKO mice (210, 211). BAT of two-day-old mKO mice appeared less active than that of wild-type littermates, whereas the BAT of two-day-old pKO mice appeared more active than that of wild-type littermates. These differences of mKO and pKO mice persisted into adulthood. A difference in activity of G<sub>s</sub> $\alpha$  in BAT was also suggested by lower levels of UCP-1 (uncoupling protein-1) mRNA in mKO BAT and increased levels in pKO BAT (211). Two day-old mKO mice already have increased lipid reserves in WAT compared to wild-type littermates, whereas pKO mice have reduced reserves (211).

Proximal renal tubules of mKO mice exhibit an impaired cAMP response to stimulation with PTH. Moreover, expression of G<sub>s</sub> $\alpha$  protein was reduced in proximal renal tubules of mKO mice but not in renal inner medulla (primarily collecting tubules) nor outer medulla (primarily thick ascending limbs) (210). Thus, with respect to responses to PTH, mKO mice resemble humans with PHP-Ia and pKO mice resemble humans with PPHP.

## Evolutionary Conjectures

Maternally inherited inactivating mutations of G<sub>s</sub> $\alpha$  are associated with PTH resistance in proximal renal tubules of both humans and mice. Elsewhere, I have hypothesized that preferential maternal expression of G<sub>s</sub> $\alpha$  in proximal renal tubules evolved because marginal increases of G<sub>s</sub> $\alpha$  protein increased secretion of

phosphate by the fetal kidney in circumstances of maternal calcium stress (63a). In this scenario, reduced serum phosphate causes reduced fetal bone deposition and thereby reduces the drain of maternal calcium across the placenta. At present, this hypothesis is untested but it does make clear predictions. The calcium content of the neonatal skeleton should be greater in PHP-Ia than in PPHP, and in mKO than in pKO pups. Moreover, maternal bone loss during pregnancy should increase with the number of mKO offspring in a litter, and decrease with the number of pKO offspring, especially for mothers on calcium-poor diets.

Energy metabolism of mKO can be characterized as frugal and that of pKO mice as spendthrift. Why should paternally derived alleles (expressed in mKO mice) have been selected to favor greater economy in energy expenditure, by contrast to maternally derived alleles (expressed in pKO mice) that have been selected to favor greater prodigality? One possibility is that paternally derived genes have been selected to favor greater allocation of resources to growth rather than to other uses. Such a trade-off appears to be acting prenatally because mKO mice are heavier at birth, and pKO mice are lighter, than their wild-type littermates (210). Prenatal competition for maternal investment is also suggested by the observation that wild-type littermates of pKO mice are heavier at birth than wild-type littermates of mKO mice (210).

Mice with an inactivated *Gnas* allele show pronounced parent-of-origin effects on the physiology of BAT. BAT is responsible for nonshivering thermogenesis (NST) in the young of most eutherian mammals. Activation of  $\beta_3$ -adrenergic receptors on the membranes of brown adipocytes results in the release of  $G_s\alpha$ , which induces the mitochondrial degradation of lipids without generation of ATP (17). Lipids that are consumed by NST are necessarily unavailable for storage in WAT or for the generation of chemical energy for growth-related activities.

In animals that produce litters, the costs of NST can be reduced if offspring huddle together to reduce their exposed surface area. The energetic savings from huddling can be substantial. For example, a group of eight five-day-old rat pups was able to reduce oxygen consumption by 32% at 30°C (2). The ability to huddle with a littermate can translate into increased survival in the cold. At 17–22°C, six of ten rabbit pups died by postnatal day 7 when raised alone, but there were no deaths in groups of two or more pups (10).

Within a huddle, pups benefit from heat generation by their sibs. When NST was blocked pharmacologically with chlorisondamine, rat pups were able to maintain higher body temperatures if their huddle contained saline-treated littermates. By contrast, saline-treated pups maintained lower body temperatures if their huddle contained chlorisondamine-treated pups (163). Thus, heat generation within a huddle has aspects of a public good: A pup bears the full metabolic cost of its own thermogenesis, but the benefits are partially shared with its sibs. Provision of this public good would be predicted to be undersupplied if pups could gain a competitive advantage over their littermates by reducing their own contribution to communal heating. On average, matrilineal relatedness within huddles will be higher than patrilineal relatedness because of occasional (or frequent) multiple

paternity. Therefore, maternally derived alleles should be selected to contribute more of the public good and paternally derived alleles to contribute less (67). This provides an appealing evolutionary explanation for why the metabolic activity of BAT is suppressed in mice with an inactivated maternal copy of *Gnas* but is increased in mice with an inactivated paternal copy.

The problem of free-riding on the heat production of others does not arise in such clear form in animals that produce singletons. If there is to be selection for imprinting in such species, one would need to argue that mothers respond to reduced NST by their offspring by increasing maternal investment. I know of no data concerning NST in human infants with PHP-Ia or PPHP. However, it is not only newborn offspring that huddle for warmth. Adults of many small mammals huddle during cold weather (193). To give just one example, female gray squirrels commonly form communal nests during winter that consist of groups of close matrilineal kin (98). As is the case for young in litters, natural selection may positively favor parent-specific expression of genes that influence NST in adults if there is an asymmetry of patrilineal and matrilineal relatedness within huddles.

Mice with an inactivated *Gnas* allele show pronounced parent-of-origin effects on the physiology of WAT, as well as of BAT. It is possible that the effects of *Gnas* mutations on WAT are secondary to their effects on BAT. That is, mice store more fat if they burn less. Lipid stored in WAT is available for the exclusive use of the individual pup, whereas the benefits of lipid consumed in NST is partially shared with littermates. Therefore, the action of maternally derived *Gnas* alleles favors relatively greater allocation of the lipid in a pup's milk intake to BAT, whereas the action of paternally derived *Gnas* alleles favors relatively greater allocation to WAT. An evolutionary interpretation of the effects of imprinted genes on fat deposition however is fraught with difficulties because a number of imprinted genes affect fat deposition, but these do not show a consistent pattern either with each other, or between mice and humans (see Conclusions).

The *GNAS* complex locus illustrates the potential complexity and specificity of imprinting:  $G_3\alpha$  is translated from both alleles in most tissues, but only from a single allele in some cell types; signaling via the PTH receptor is disrupted in some cell types but not in others. Disruption of *Gnas* in mice provides provisional support for the kinship theory, with increased birth weight when the maternal allele is disrupted but decreased birth weight when the paternal allele is disrupted. The kinship theory is also supported by evidence that the expression of maternally derived alleles favors greater allocation of lipid to use in NST than does the expression of paternally derived alleles.

## CONCLUSIONS

The kinship theory posits that genomic imprinting evolves at a locus when changes in the level of expression in one individual influences a trade-off between the individual's own fitness and the fitness of one or more of the individual's nondescendant kin. This fitness trade-off will be expressed within the individual in which the gene is expressed as a physiological trade-off (e.g., between allocation of lipid to BAT

or WAT) or a developmental trade-off (e.g., between the allocation of cells to spongiotrophoblast or the giant cell layer of the mouse placenta).

The challenge faced by the kinship theory is to identify the physiological and developmental trade-offs that are influenced by expression of an imprinted gene and to relate these to fitness trade-offs among kin. So far, the theory has been most successful at explaining the imprinting of paternally expressed enhancers of prenatal growth (*IGF2*, *KCNQ1OT1*, *Air*) and of maternally expressed inhibitors of prenatal growth (*Igf2r*, *CDKN1C*, *Grb10* (23)). For expression at these loci, a fitness trade-off exists between a fetus's own fitness and its mother's fitness via other offspring. This fitness trade-off is expressed within the mother as a physiological trade-off between resources transferred to the fetus versus resources retained for other uses. It may be expressed within the fetus as a trade-off between allocating resources to the development of placental tissues that directly increase maternal investment versus allocating resources to other tissues. Genes expressed in the fetus that influence these trade-offs will evolve parent-specific expression because the fetus's maternal alleles are more likely than its paternal alleles to be present in the mother and her other offspring.

Despite such successes, a satisfying story cannot yet be told for the majority of imprinted genes. The effects of imprinted loci of the *callipyge* and *GNAS* clusters do not fit neatly into categories of paternally expressed growth enhancers and maternally expressed growth inhibitors. Rather, these loci suggest a number of other physiological trade-offs that are candidates for influencing fitness trade-offs among kin. Both clusters appear to influence multiple aspects of energy metabolism. In particular, genes in both clusters influence the development and activity of white and/or brown adipose tissue.

A plausible hypothesis can be presented to explain effects of imprinted genes on the function of BAT. Animals that huddle to conserve heat benefit from heat production by other members of their huddle. If relatedness within huddles differs for genes of maternal and paternal origin, then the set of genes with the lower relatedness will favor reduced levels of NST in BAT. For mammals that produce litters, this leads to the prediction that maternally expressed genes will favor higher levels of NST by huddling offspring whereas paternally expressed genes will favor lower levels of NST.

The effects of inactivating mutations of *Gnas* conform closely to the above predictions: inactivation of paternal alleles is associated with evidence of increased NST; inactivation of maternal alleles is associated with reduced NST (211). Other genes considered in this review may also influence BAT, although this remains to be demonstrated. Paternally expressed *Dlk1* is believed to inhibit the differentiation of preadipocytes into adipocytes. If *Dlk1* has this effect in BAT, the effect would be to reduce levels of NST, consistent with predictions. NST in BAT is dependent on locally produced triiodothyronine ( $T_3$ ) (12). Therefore, if paternally expressed *Dio3* is expressed in BAT it would function to reduce intracellular  $T_3$  and thus reduce NST. Other imprinted genes not considered in this review may also influence BAT. For example, paternally expressed *necdin* (from the Prader-Willi/Angelman syndrome region) is one of the few genes to show higher expression in brown

preadipocytes than in white preadipocytes (15). There may be systematic reasons why influences of imprinted genes on BAT function have been missed in studies of gene knockouts. BAT is recruited postnatally in response to cold exposure (139). Therefore, the predicted effects of imprinted genes in BAT should be most pronounced in young mice raised at colder temperatures than are typical of those used in most knockout studies.

Multiple imprinted genes also affect the function of WAT, but a unifying hypothesis in terms of the kinship theory remains elusive because of the diversity of gene effects. Lipid reserves in WAT are increased by disruption of the maternal copy of *Gnas* but decreased by disruption of the paternal copy. These changes occur without a change in food intake (211). By contrast, disruption of either the maternal or the paternal copy of human *GNAS* results in increased adiposity (192). A different pattern of associations is observed for genetic deletions of the imprinted cluster at human 15q11–q13 and the homologous region of mouse chromosome 7. Paternal deletions of 15q11–q13 cause the obesity and hyperphagia of Prader-Willi syndrome (16), but equivalent deletions of the homologous cluster in mice do not cause obesity (177). Maternal deletions of 15q11–q13 are the most common cause of Angelman syndrome (AS). The distribution of body mass index is normal in these AS patients. However, most AS patients with paternal UPD 15q11–q13 are obese (113). Paternal UPD for the homologous gene cluster in mice is associated with late-onset obesity (21). Finally, deletion of the paternal copy of *Dlk1* is associated with obesity in mice (132). No clear pattern emerges from these comparisons. This may reflect the complexity of the evolutionary trade-offs that influence fat reserves and the existence of more than one pathway to obesity.

How good then is the evidence for a strong version of the kinship theory? The theory has been able to provide an evolutionary explanation for the imprinting of some loci, but is yet to provide such an explanation for the majority of imprinted genes. Opinions will differ as to whether the glass is better perceived as being half full or half empty. Time will tell whether the glass can be filled.

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