Phenotypic Outcomes of Imprinted Gene Models in Mice: Elucidation of Pre- and Postnatal Functions of Imprinted Genes

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Abstract

Genomic imprinting is an epigenetic process causing expression of a subset of genes in a parent-of-origin-specific manner. Among vertebrates, only therian mammals have been demonstrated to imprint, indicating that placentation and imprinting arose at similar time points in evolution and that imprinting may be involved in key mammal-specific processes. However, although several theories have been posited to explain the evolution of imprinting, each has shortcomings and none fully explains the wide variety of genes regulated by imprinting. In this review, we catalog the phenotypes associated with genetic mutation and overexpression at particular imprinted loci in order to consider the wide impact of imprinted genes on development. In addition to the well-described roles of imprinted genes in prenatal growth and placentation, more recent data emphasize that imprinted genes are critical for specific aspects of postnatal mammalian development involving adaptive processes, metabolism, and behavior.

INTRODUCTION

Genomic imprinting: an epigenetic process whereby genes are expressed in a parent-of-origin-specific manner

Imprinting control region (ICR): a differentially methylated region established in the germline that controls the imprinted expression of all genes within an imprinted cluster

Differentially methylated region (DMR): a region of DNA that is hypermethylated on one chromosome and unmethylated on the other

Beckwith–Wiedemann syndrome: an imprinting disorder with symptoms that include overgrowth, omphalocele, macroglossia, increased risk of childhood cancers, and hypoglycemia

Silver–Russell syndrome: an imprinting disorder with symptoms that include intrauterine growth restriction, feeding problems resulting in hypoglycemia, and short stature

Genomic imprinting is an epigenetic process that causes a subset of genes to be monoallelically expressed in a parent-of-origin-specific manner. Approximately 80–100 imprinted genes have been identified in mice (232). The majority of these are located in genomic clusters, each of which is controlled by an epigenetically regulated imprinting control region (ICR) (59). ICRs are differentially methylated regions (DMRs) that, at least in rodents, acquire their methylation imprint in either the male or female germline during development. Three regions—*Igf2/H19*, *Dlk1/Dio3*, and *Rasgrf1*—acquire methylation in the male germline and are unmethylated in the female germline; all other ICRs acquire methylation during oogenesis.

The imprinting process effectively causes functional haploidy, and imprinted genes therefore lose the protective advantage of diploidy, rendering the regions potentially vulnerable, as is seen in imprinting disorders. Failure to correctly imprint genes in humans is associated with multiple diseases, including Beckwith–Wiedemann syndrome, Silver–Russell syndrome, Prader–Willi syndrome (PWS), Angelman syndrome (AS), uniparental disomy 14 (UPD14) syndromes, and transient neonatal diabetes mellitus (for recent reviews, see 94, 242). In mice, complementation studies of maternal and paternal duplications of various sections of the genome have identified regions where biparental inheritance is necessary for normal development (59). UPDs at 12 murine genomic regions are associated with visible phenotypes (232) (**Supplemental Table 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). However, UPDs at only 3 of these regions are associated with embryonic lethality: MatDp(prox6), which includes the *Peg10* domain; Mat/PatDp(dist7), which includes both the *Igf2* and *Kcnq1* clusters; and Mat/PatDp(dist12), which includes the *Dlk1/Dio3* domain. These UPDs represent the most severe outcomes associated with single-chromosome parent-of-origin effects.

Genomic imprinting is the reason that parthenogenetic (derived solely from maternal genomes) and androgenetic (derived only from paternal genomes) conceptuses are nonviable in mammalian systems (11, 135, 198). Early embryo manipulations that generated zygotes containing two maternal or two paternal pronuclei showed that parthenogenetic/gynogenetic mouse embryos die at midgestation, have embryonic growth defects, and have abnormal extraembryonic derivatives (197, 198). Androgenetic conceptuses fail much earlier in development, with delayed or absent embryonic components but extensive extraembryonic development reminiscent of complete hydatidiform molar pregnancies in humans, which also derive solely from paternally inherited chromosomes (11, 63). With their total imbalance in the parental origin of their chromosomes, these diploid conceptuses represent the most extreme phenotypes associated with genomic imprinting. Interestingly, genetic manipulation of imprinting control at both the *Igf2/H19* locus and the *Dlk1/Dio3* locus allows parthenogenetically activated embryos to survive to adulthood, indicating that these two loci represent the sole imprinted barriers to a viable, fertile mammalian bimaternal adult (103).

To date, the only vertebrates in which genomic imprinting has been observed are the therian mammals (marsupials and eutherians), but only limited imprinting has been observed in marsupials. The *Igf2*, *H19*, *Ins*, *Igf2r*, and *Peg10* genes are all imprinted in marsupials; other, larger clusters, such as *Dlk1/Dio3*, *Snrpn/Ube3a*, and *Kcnq1*, are specific to eutherians. This suggests that imprinting has evolved progressively alongside the evolution of a more invasive, longer-lived placenta and a longer gestation.

In this review, we systematically catalog the phenotypes associated with genetic mutation and overexpression at particular imprinted loci in order to consider the impact of imprinted genes on development and disease and to perhaps contribute to ideas surrounding the evolution of the

Supplemental Material

imprinting process (**Supplemental Table 2**). Consideration of imprinting function and evolution is better served by the assessment of models in which imprinted genes are overexpressed, representing the situation when dosage is not reduced by monoallelic imprinted expression. Regardless, gene knockout models are more prevalent and can provide a phenotypic understanding of the developmental roles of imprinted genes rather than insights into selective pressures acting to control their dosage by imprinting. We review current mouse models with an altered dosage of imprinted genes, explore the functions of these genes, and identify recurrent themes that enable us to consider the evolution of genomic imprinting and the advantages associated with its establishment and maintenance in mammals. The nomenclature for each model is taken from the Mouse Genome Database (16), available at the Mouse Genome Informatics website (**http://www.informatics.jax.org**). The increase in the number of available models has, in some cases, enabled the ascertainment of individual genes responsible for the gross phenotypes in UPD models with perturbed expression of multiple imprinted genes. Moreover, in addition to the previously well-described roles of imprinted genes in prenatal growth and placentation, more recent data emphasize that imprinted genes are critical for specific features of postnatal mammalian development involving adaptive processes, metabolism, and behavior.

Prader–Willi syndrome (PWS): an imprinting disorder with symptoms that include hypotonia, poor growth and developmental delay in infants, and hyperphagia and obesity in adults

Angelman syndrome (AS): an imprinting disorder with symptoms that include mental retardation, developmental delay, frequent laughter, and ataxia

Transient neonatal diabetes mellitus: neonatal hyperglycemia caused by reduced insulin levels, with symptoms resolving within the first 18 months after birth

ncRNA: noncoding RNA

Supplemental Material

Igf2/H19: MOUSE CHROMOSOME 7 (HUMAN CHROMOSOME 11)

The *Igf2/H19* region plays an important role in prenatal growth and placentation and in humans is associated with Beckwith–Wiedemann syndrome and increased incidence of tumors. The *Igf2/H19* cluster [imprinted cluster 1 (IC1)] contains two paternally expressed protein-coding genes, *Igf2* and *Ins2*; a paternally expressed noncoding RNA (ncRNA) gene, *Igf2as*; and one maternally expressed ncRNA gene, *H19* (10, 47, 60, 77). This cluster lies adjacent to a second imprinted domain, the multigene *Kcnq1* locus [imprinted cluster 2 (IC2)] on mouse distal chromosome 7. The ICR is the intergenic *H19* DMR located between *Igf2* and *H19*. MatDp(dist7) embryos are growth retarded and die at approximately embryonic day 15.5 (~E15.5), whereas PatDp(dist7) embryos die earlier, at ~E10.5 (178, 232). However, these disomies contain both the IC1 and IC2 domains and hence involve the altered dosage of at least 12 imprinted genes. Many mouse models have been generated to study the *Igf2/H19* region, with most of them created to study the imprinting mechanism itself (for a review, see 8).

Insulin-like growth factor 2 (IGF2) is both a survival factor and a mitogen. The coding sequence of *Igf2* lies in exons 4–6, with expression driven by three alternative promoters (P1–3). There is also a placenta-specific promoter (P0) that drives an alternative transcript containing two upstream exons (U1–2) (147). Mice with a paternally inherited disruption in exon 4 of the *Igf2* gene ($Igf2^{tm1Rob}$) are ~60% of the size of their wild-type littermates from E16.5 onward (46, 47). Those that survive to adulthood remain small but are fertile (46). Paternal heterozygotes have fewer colon crypts and disorganized pulmonary alveoli (14, 187). Their placentas are small, with fewer glycogen cells and a greater proportion of spongiotrophoblast cells in the junctional zone, which is located between the maternal decidua and the labyrinthine capillary network of maternal–fetal exchange (126).

Mice with *Igf2* exons 4–6 replaced by a *LacZ-neo* cassette ($Igf2^{tm2Wrk}$) recapitulate this embryonic growth phenotype upon paternal inheritance, as do $Igf2^{tm1Kel}$ mice (148, 39) (**Figure 1**). Their placentas are ~60% of the normal size at E18.5, with reduced labyrinthine and junctional zones and a theoretical diffusion capacity reduced to ~24% of normal, which has an impact on nutrient transfer (36). Replacement of the P2 promoter with *LacZ-neo* on the paternal chromosome results in a 40% decrease in *Igf2* expression and a 30% decrease in growth as well as shorter, thinner bones and delayed ossification (84). None of these models show changes in phenotype upon maternal

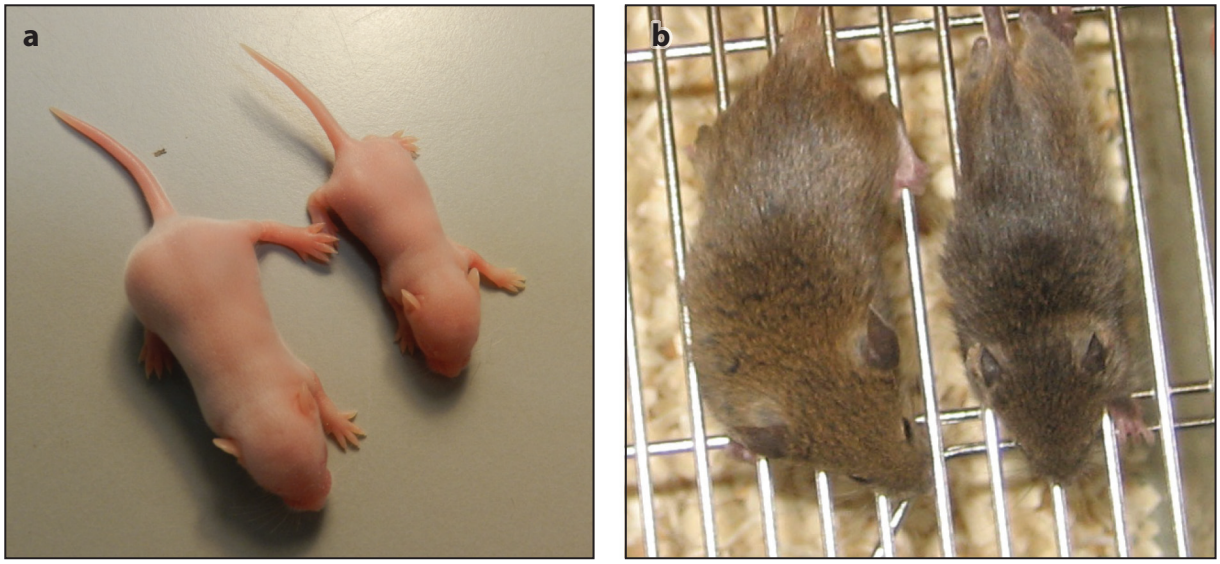

Figure 1

Examples of genetic mouse models of imprinted genes. (*a*) Igf2^{tm1Kel} model on a mixed C57BL/6;CD1 background at postnatal day 6. The paternal heterozygote (*right*) is smaller than its wild-type littermate (*left*). (*b*) Meg3$^{Gt(pGTi)216Gos}$ (Gtl2LacZ) model on a 129Sv/J background at postnatal day 21. The maternal heterozygote (*right*) is smaller than its wild-type littermate (*left*).

transmission, which is perhaps unsurprising given that the maternally inherited allele is normally repressed.

Regulatory mutations have also provided functional insights. Deletion of upstream exon U2, which contains a regulatory DMR, on the maternal chromosome causes *Igf2* to be biallelically expressed in the heart, lungs, and kidneys, but despite the overexpression of this mitogen, this reactivation has no effect on fetal growth (38). Paternal inheritance of the deletion leads to loss of the placenta-specific transcript, with no loss of *Igf2* imprinting in the embryo. Though morphologically normal, the placentas are small, and the fetuses show intrauterine growth retardation but catch up after birth (38, 39). The placentas of paternal knockouts have reduced diffusion and permeability at E15.5 and E18.5; however, this results in a simultaneous adaptive increase in glucose transport that abrogates the detrimental effects of these defects until the last stages of gestation (36, 37, 186).

There are also models in which *Igf2* is upregulated by 1.5–3-fold compared with endogenous levels, depending on the tissue. Mice homozygous for an *Igf2* transgene [Tg(Igf2)2Ddp] are ~14% heavier than wild types and die between E17.5 and shortly after birth (235). Some of these animals have a cleft palate. Heterozygotes are ~11% heavier than wild-type littermates and survive to adulthood, at which time females develop multiple mammary tumors (164, 235). Deletion of the maternal *H19* ICR along with the *H19* gene itself (H19^{tm1Tlg}) reactivates both maternal *Igf2* and *Ins2* and results in mice that are 28% larger (118) and have heavier placentas containing greater numbers of glycogen cells and trophoblast giant cells (57). Furthermore, mice with both a maternal *H19* deletion (H19^{tm1Tlg}), which reactivates maternal *Igf2*, and a paternal *Igf2* deletion (Igf2^{tm1Rob}), which removes the normally active allele, are phenotypically normal, indicating that increased IGF2 levels cause overgrowth and that a lack of *H19* plays no role in this phenotype (118).

The other paternally expressed protein-coding gene in the region is *Ins2*, which encodes insulin. *Ins2* is expressed at low levels and imprinted only until E18.5, after which its pancreatic

enhancer becomes functional, the gene is upregulated, and expression is from both alleles. *Ins2* homozygous knockout mice are viable and fertile, presumably because of functional redundancy between *Ins2* and the nonimprinted *Ins1* (51). Double knockout of these two genes results in the rapid development of diabetes and neonatal death within two days of birth (51, 58). Thus, the key imprinted genes in this cluster are *Igf2* and *H19*, and their correct expression is required for pre- and postnatal growth as well as normal placental function.

Kcnq1/IC2 DOMAIN: MOUSE CHROMOSOME 7 (HUMAN CHROMOSOME 11)

Adjacent to *Igf2/H19* lies the *Kcnq1* domain (IC2), which also regulates prenatal growth and, like the *Igf2/H19* domain, has been implicated in Beckwith–Wiedemann syndrome. This imprinted cluster contains at least 10 imprinted genes: *Kcnq1*, *Kcnq1ot1*, *Cdkn1c*, *Phlda2*, *Slc22a18*, *Cd81*, *Th*, *Ascl2*, *Tssc6*, and *Tssc4*. The domain contains a single regulatory paternally expressed ncRNA, *Kcnq1ot1* (26), and maternally expressed protein-coding genes, some of which are imprinted only in the mouse placenta (56). Unlike imprinting of the IC1 domain, imprinting of the IC2 domain is eutherian specific (201). The domain ICR is the promoter for the *Kcnq1ot1* ncRNA and is hypermethylated during oogenesis but remains hypomethylated during spermatogenesis (131). Targeted deletion of the entire domain leads to embryonic lethality upon maternal transmission, but upon paternal transmission the pups are viable and fertile, reflecting the maternal allelic expression of the genes in this cluster (155). In contrast, deletion of the paternal ICR results in placental, embryonic, and postnatal growth retardation because *Kcnq1ot1* no longer functions to repress the protein-coding genes on the paternal chromosome, leading to their biallelic expression (64, 173).

Kcnq1 encodes the $K_v7.1$ voltage-gated potassium channel, which is imprinted in embryos but biallelically expressed in juveniles and adults (82). Mutations in *Kcnq1* are associated with atrial fibrillation (54), long QT syndrome 1 (224), Jervell and Lange-Nielsen syndrome (191), and epilepsy (31). Mouse knockouts of *Kcnq1* recapitulate this, resulting in inner-ear defects, a long QT phenotype, gastric hyperplasia, impaired gastric acid secretion, and seizures (18, 21, 55, 80, 116, 170, 216). However, whether these phenotypes can be attributed to imprinted *Kcnq1* expression is unclear. The regulatory *Kcnq1ot1* has numerous knockout mouse models (64, 132, 143, 182); because *Kcnq1ot1* functions to control imprinting across the whole domain, the phenotypes of these models are attributable to altered expression of multiple genes within the cluster.

Erratum

Cdkn1c encodes a cyclin-dependent kinase inhibitor involved in cell cycle control and is expressed exclusively from the maternal allele in mice (86) but only preferentially so in most tissues in humans (34, 134). Loss of maternal *CDKN1C* expression in humans is associated with Beckwith–Wiedemann syndrome (87) and multiple types of sporadic cancer, with gain-of-function mutants causing IMAGe syndrome (intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies) (6). Loss of mouse maternal *Cdkn1c* expression leads to multiple abnormalities, including hyperproliferation in various tissues, placentomegaly, placental defects, embryonic growth retardation, abdominal hernia, defective endochondral ossification, cleft palate, hematological defects, poor suckling, and highly penetrant neonatal lethality (133, 199, 207, 208, 243, 255).

Phlda2 encodes pleckstrin homology-like domain, family A, member 2 (165). Loss of maternal *Phlda2* expression results in late-gestational placental defects, including overgrowth without any corresponding increase in embryonic growth (67). Expression of either double or quadruple doses of *Phlda2* in mice results in identical phenotypes—placental growth retardation, placental defects, and progressive slowdown of embryonic growth (2, 97, 173, 215)—indicating that the function of this gene is not dosage sensitive in mice and that its imprinting may be due to proximity effects

from other, more dosage-sensitive imprinted genes. In humans, however, *PHLDA2* levels correlate with birth weight, suggesting that this may not universally be the case (4, 122).

Bacterial artificial chromosome (BAC): a bacterial clone containing a genomic insert of up to 350 kb that can be used to make transgenes

Bacterial artificial chromosome (BAC) transgenes have been elegantly used to tease apart the functions of *Cdkn1c*, *Phlda2*, and their neighboring placental gene, *Slc22a18*. An 85-kb BAC that causes overexpression of *Phlda2* and *Slc22a18* but lacks placental enhancers for *Cdkn1c* leads to placental and early (E13.5) embryonic growth restriction (173, 215). By generating mice with this transgene and a maternally inherited *Phlda2* deletion (thus correcting *Phlda2* levels), Tunster et al. (215) showed that a double dose of *Slc22a18* has no part in the placental growth restriction because the placentas of these mice are phenotypically normal. Moreover, when the 85-kb BAC is engineered to remove all *Cdkn1c* expression from the embryo as well as the placenta, the mice do not exhibit the growth restriction seen at E13.5 in those with the unmodified BAC. This demonstrates that overexpression of *Phlda2* does not cause the growth restriction at E13.5 (which is due to *Cdkn1c* overexpression), although it does result in lower birth weight (173, 215).

Lack of another maternally expressed gene, *Ascl2* (encoding achaete-scute complex homolog 2), results in embryonic lethality between E9.5 and E10.5 (83). This lethality is attributable solely to severe placental defects, including a complete lack of spongiotrophoblast cells (210). However, mice expressing 4.3-fold-greater *Ascl2* mRNA levels have normal placentas (169), suggesting that transcript levels are not functionally relevant.

There are three mouse knockouts of *Cd81* (130, 140, 214). However, *Cd81* is imprinted only in the placenta, and none of the knockouts display placental phenotypes or growth defects in offspring, so the relevance of imprinting to the function of this gene remains unclear.

The *Th* gene (encoding tyrosine hydroxylase) is predominantly maternally expressed in the mouse placenta (175) and E7.5 embryo, but imprinting progressively relaxes during embryonic development (79). Whether *TH* is imprinted in humans is unknown. Heterozygotes lacked a visible phenotype in two of three deletion models (Th^{tm1Rpa} and Th^{tm2Rpa}) (257); in a fourth model (Th^{tm1Tna}), heterozygotes had mild neuropsychological deficits (109). However, this cannot be ascribed to imprinting, because the *Th* gene is biallelically expressed postnatally.

The remaining genes in the IC2 domain lack any mouse models. As with the *Igf2/H19* region, the major roles of the protein-coding genes in the IC2 domain are in embryonic growth and placental function, with failure to correctly imprint the cluster resulting in embryonic lethality. The primary function of the ncRNA *Kcnq1ot1* is to repress the paternal copies of the maternally expressed genes.

Igf2r: MOUSE CHROMOSOME 17 (HUMAN CHROMOSOME 6)

Erratum

In the early 1970s, Johnson (98) identified a parent-of-origin effect on mouse proximal chromosome 17 in hairpin-tail mice [$Del(17)T^{hp}$] that arose through a spontaneous mutation. Maternal inheritance of this 5-Mb deletion is characterized by embryonic lethality; edema; tailbud and/or neural tube defects; and postaxial polydactyly. Upon paternal transmission, mice have short, kinky tails. The *Igf2r* gene was subsequently mapped to this region and shown to be expressed from the maternally inherited chromosome, making it the first endogenous imprinted gene to be identified in mammals (9). Three other imprinted genes reside in this cluster: a paternally expressed ncRNA, *Airn* (179), and two placenta-specific, maternally expressed solute carrier genes, *Slc22a2* and *Slc22a3* (260). Imprinting of *IGF2R* in humans is polymorphic, occurring in only a minority of individuals.

The *Igf2r* gene codes for the IGF2 receptor, which also functions as a cation-independent mannose 6-phosphate receptor. Four targeted deletions of the gene have been generated (114, 128, 226, 238), resulting in consistent phenotypes. All four recapitulate some of the $Del(18)T^{hp}$

phenotype, with an ~30% fetal growth increase, enlarged placentas, kinky tails, and postaxial polydactyly. Three of the models ($Igf2r^{tm1Stw}$, $Igf2r^{tm1Arge}$, and $Igf2r^{tm1Wag}$) die at the perinatal stage, and the fourth ($Igf2r^{tm1Rlj}$) dies in utero (114, 128, 226, 238). The $Igf2r^{tm1Rlj}$ and $Igf2r^{tm1Wag}$ models have lung malformations, including retarded alveoli (226, 238), and the $Igf2r^{tm1Stw}$ model suffers from respiratory failure (114). Three of the models have organomegaly, which is due to cellular hyperplasia rather than hypertrophy (114, 128, 226). Serum IGF2 levels are increased in knockouts, and the phenotype is rescued in double *Igf2r/Igf2* mutants (61), confirming that the growth phenotype is due to the failure of *Igf2r* mutants to clear IGF2 and that the major role of the receptor in development is growth modulation. Disruption of the *Igf2r* gene also leads to impaired lysosomal enzymes because of the mannose 6-phosphate receptor function of the IGF2 receptor, which is thought to be its primary function in adults (226).

As with the *Kcnq1* locus, imprinting at the *Igf2r* cluster is regulated by an ncRNA transcript expressed antisense to the protein-coding transcripts. The ICR for the domain is the promoter for this noncoding transcript (*Airn*). It is methylated on the maternally inherited chromosome, which prevents maternal *Airn* expression. On the paternally inherited chromosome, the promoter is unmethylated and *Airn* is expressed (236); this expression is required for repression of the protein-coding genes, including *Igf2r*, on the paternally inherited chromosome. When a deletion of the ICR is inherited paternally, *Airn* cannot be expressed, and the expression of *Igf2r* and the neighboring imprinted genes becomes biallelic. This results in a 20% decrease in embryonic weight that continues into adulthood (237). Crossing the paternal ICR deletion (which deletes *Airn* and reactivates the normally silent paternal *Igf2r*) with maternal $Igf2r^{tm1Wag}$ (which deletes the normally active maternal allele) corrects the dosage of *Igf2r* and produces animals with a normal phenotype, demonstrating that *Airn* itself does not contribute to the growth phenotype (237). A premature polyadenylation signal inserted downstream of the promoter on the paternal chromosome truncates the *Airn* transcript and leads to the reactivation of *Igf2r*, *Slc22a2*, and *Slc22a3*, demonstrating that the function of *Airn* RNA is specifically to silence the other genes in the domain in *cis* (151, 189).

Slc22a2 and *Slc22a3* are imprinted only in the placenta, where they are expressed from the maternal allele. Knockout models are available for both genes, but a placental phenotype has been reported only for *Slc22a3*: Nulls have impaired uptake-2 system transport between the placenta and fetus (261). The role of *Slc22a3* in placental transport, along with the prenatal role of *Igf2r* in clearing IGF2 protein, indicates that the key functions of this imprinted cluster are, as in other clusters, in placental resource allocation and embryonic growth.

Maternal uniparental disomy (UPD): inheritance of both homologous chromosomes from the mother without any paternal copies

Paternal uniparental disomy (UPD): inheritance of both homologous chromosomes from the father without any maternal copies

Erratum

Snrpn/Ube3a: MOUSE CHROMOSOME 7 (HUMAN CHROMOSOME 15)

The *Snrpn/Ube3a* domain is associated with prenatal growth and multiple postnatal phenotypes and is located on mouse central chromosome 7, the orthologous human region of which is chromosome 15q11–q13. Microdeletions and UPD of this region in humans cause two distinct neurological conditions (242). A paternally inherited deletion or maternal UPD causes PWS, which is characterized by neonatal hypotonia and failure to thrive; children and adults with PWS have moderate mental retardation, hyperphagia, and severe obesity. A maternally inherited deletion or paternal UPD causes AS, which is characterized by mental retardation, ataxic movements, and behavioral anomalies such as inappropriate bouts of laughter. The imprinted region in mice consists of one maternally expressed gene (*Ube3a*); four paternally expressed protein-coding genes (*Frat3*, *Mkrn3*, *Magel2*, and *Ndn*); and a large, paternally expressed polycistronic transcript (*Snrpn*) that codes for the Snurf and SmN proteins as well as a large array of C/D small nucleolar RNAs (snoRNAs) and an antisense transcript (*Ube3a-as*) (154).

In terms of phenotypes associated with maternal duplication/paternal deficiency of the region, mouse models with large deletions and uniparental duplications recapitulate some of the phenotypes of PWS. Paternal inheritance of a 5-Mb deletion between *Mkrn3* and the nonimprinted *Herc2* (which deletes the entire imprinted domain as well as six nonimprinted neighbors) causes embryonic growth restriction from E17.5 onward. Neonatal pups have reduced movement, irregular breathing, and dehydration, and most die within the first week (69). At postnatal day 3 (P3), pups have altered levels of the appetite regulators AgrP and α-MSH, resulting in reduced appetite (73), hypoglycemia, and increased fat metabolism (192). These animals also have decreased α and β cells in the pancreas, leading to a reduction in insulin (193). A smaller paternal deletion between *Snrpn* and *Ube3a* also leads to growth retardation, hypotonia, and neonatal death, suggesting that *Ndn*, *Magel2*, *Mkrn3*, and *Frat3* are not major contributors to the neonatal failure-to-thrive phenotype (213). Deletions of the PWS ICR, which controls imprinting of the whole cluster, also cause reduced birth weight, failure to thrive, and neonatal death upon paternal inheritance (20, 50, 244). Teasing apart the contribution of each gene to these complex phenotypes will require studying individual gene models.

Paternal inheritance of a deletion of exon 1 or 2 of the *Snurf/Snrpn* gene results in a lack of the Snurf protein but phenotypically normal mice (20, 213). Also, paternal deletion of exon 7 of the *Snurf/Snrpn* transcript results in a lack of the SmN protein but, again, phenotypically normal mice (244). However, paternal deletion of the snoRNA *Snord116* cluster results in normal embryonic growth but postnatal growth restriction (48, 188). The animals are leaner, with increased oxygen consumption and some weak evidence of hyperphagia. They fail to gain weight despite sufficient food, and on a high-fat diet they gain less weight than normal. They also have delayed puberty and increased anxiety (48). This suggests a contribution of the *Snurf/Snrpn*/snoRNA transcripts to the PWS phenotype, although the individual models are not strongly similar to PWS.

Targeted mutations of *Magel2* (encoding melanoma antigen, family L, member 2) at the active paternally inherited allele also lead to a reduction in postnatal viability (110, 174). Magel2$^{Tm1.1Mus}$ mice fail to suckle, although this phenotype can be rescued by an oxytocin injection 3–5 h after birth (174). Both *Magel2* knockout models show reductions in orexins, which regulate appetite (110, 174). Magel2$^{Tm1.1Stw}$ mice have increased weight gain and adiposity after weaning but are hypophagic (15). These mice also have reduced fertility: Females have extended and irregular estrus cycles and delayed puberty, whereas males have reduced testosterone and do not respond appropriately to female odors (138). This also suggests a contribution to the phenotypes observed in PWS patients, although, again, there are intriguing differences.

The *Ndn* gene encodes necdin, which promotes neural differentiation and survival (112). Mouse models with a deletion of the active paternal allele are associated with numerous reported phenotypes, including partial neonatal lethality, breathing defects, increased tolerance to thermal pain, skin scraping, improved spatial learning, and defects in motor coordination and balance (3, 75, 112, 149, 168). In addition, Ndn^{Tm1Ky} paternal heterozygotes have decreased levels of thyroid-stimulating hormone and the thyroid hormones T3 and T4 (suggesting a role for necdin in the hypothalamic–pituitary–thyroid axis) and increased adiposity (68, 85). Thus, deficiencies of *Snord116*, *Magel2*, and *Ndn* all appear to contribute to the PWS phenotype. Mice with deletions of *Mkrn3* or *Frat3* are phenotypically normal (99, 217).

Erratum

The reciprocal imprinting effects to those associated with PWS (maternal deletion/paternal duplication at this imprinted domain) lead to AS. The cluster includes two maternally expressed genes, *Atp10a* and *Ube3a*, the former of which is not imprinted in humans. *Ube3a* codes for ubiquitin-protein ligase E3A, and disruption of this gene leads to AS in humans. In mice, deletions of *Ube3a* from the maternally inherited chromosome but not the paternally inherited chromosome cause motor dysfunction, reduced coordination, and impaired learning (96, 139). Tonic-clonic seizures

occur in ~25% of Ube3a^{Tm1Alb} maternal heterozygotes (96), whereas fast cerebellar oscillations, which are associated with ataxia in humans, occur in Ube3a^{Tm1Jwf} animals (33). These findings are consistent with the human phenotypes associated with AS being caused by the absence of expression of this single gene.

Unlike the genes in the clusters discussed above, those in this cluster predominantly function in postnatal life, with gene deletion models affecting sugar and fat metabolism, appetite, and behavior. This indicates roles for imprinted genes not only prenatally in resource allocation but also postnatally in resource acquisition and energy homeostasis.

Dlk1/Dio3 DOMAIN: MOUSE CHROMOSOME 12 (HUMAN CHROMOSOME 14)

Apart from mouse chromosome 7, the distal portion of chromosome 12 is the only imprinted domain in which both maternal and paternal UPDs result in prenatal lethality. This indicates the presence of important developmentally regulated imprinted genes. The *Dlk1/Dio3* domain is located on mouse distal chromosome 12 and consists of four paternally expressed protein-coding genes (*Dlk1*, *Rtl1*, *Dio3*, and one transcript of *Begain*) and several maternally expressed noncoding genes [including *Gtl2*, *Rtl1-as*, microRNAs (miRNAs), and C/D snoRNAs] (44) (**Figure 2**). The domain ICR is the intergenic DMR (IG-DMR), which is hypermethylated during spermatogenesis but remains hypomethylated during oogenesis (123, 205).

The importance of this domain to development was demonstrated using UPDs and ICR deletion. PatDp(dist12) embryos die by E17.5 and have placental, muscular, and skeletal defects, whereas MatDp(dist12) embryos die by P2 and have skin defects (74, 211). Deletion of the paternal IG-DMR does not alter the imprinting of the domain, whereas deletion of the maternal IG-DMR paternalizes the maternal chromosome, causing embryonic or perinatal death, ossification defects, and muscular hypertrophy (123). Some of the defects observed in UPD12 animals and IG-DMR mutants are attributable to individual genes in the cluster.

Loss of paternal expression of *Dlk1*, which encodes the delta-like 1 homolog (DLK1) protein, results in partial neonatal lethality, postnatal growth retardation, and altered B-lymphocyte populations (5, 146, 166, 172). Additionally, paternal inheritance of the Dlk1^{tm1Sul} knockout results in skeletal abnormalities, genetic-background-dependent eyelid abnormalities, and increased adult adiposity on a high-fat diet (146). Expression of a single extra dose of *Dlk1* results in embryonic overgrowth, skeletal abnormalities, ~33% neonatal lethality, and neonatal growth retardation (43). Postnatally, this increase in dosage results in perturbed mechanisms of lipid storage, suggesting a role for DLK1 in the modulation of adult nutrient metabolism (M. Charalambous, S.T. Da Rocha, E.J. Radford, G. Medina-Gomez, S. Curran, et al., manuscript in review). Expression of two extra doses of *Dlk1* results in a more severe phenotype: severe skeletal abnormalities and embryonic growth enhancement until E15.5, followed by edema and 100% lethality by P1 (43). Thus, a reduction or increase in *Dlk1* expression causes significantly reduced viability, illustrating the dosage sensitivity of this imprinted gene.

Rtl1 is a paternally expressed gene that encodes the retrotransposon-like 1 (RTL1) protein (250) and has a maternally expressed noncoding antisense transcript, *Rtl1as* (180). *Rtl1as* is a host for multiple miRNAs, at least some of which function to repress *Rtl1* posttranscriptionally via RNA interference in a *trans*-homolog interaction (45, 180). Thus, paternal transmission of an *Rtl1* deletion results in loss of RTL1 protein, whereas maternal transmission results in loss of *Rtl1as* and therefore increased RTL1 protein levels owing to the absence of the negatively regulating miRNAs. RTL1 loss causes placental growth retardation, other placental defects, up to 100% perinatal lethality, and strain-specific delayed parturition, which results in the death of the mother

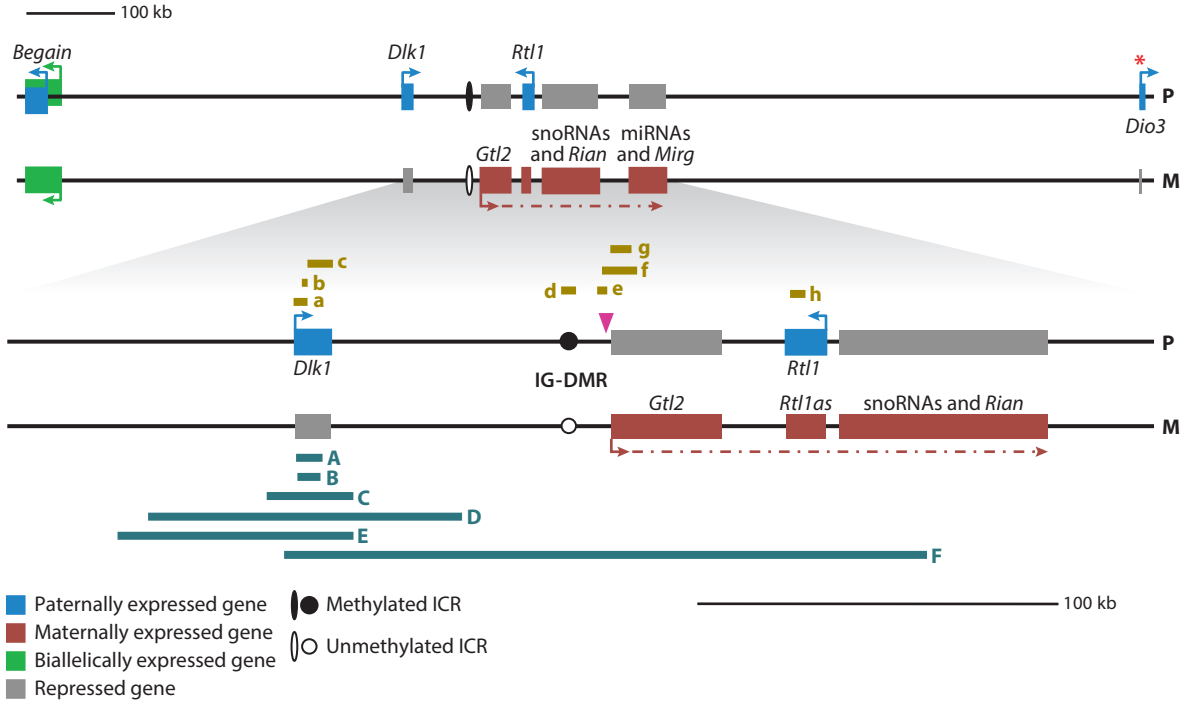

Figure 2

Collated mouse models in the *Dlk1/Dio3* domain. The mouse *Dlk1/Dio3* cluster is located on chromosome 12. Imprinted expression in the region is controlled by the intergenic differentially methylated region (IG-DMR) (*black* and *white ovals*). Thick dark yellow lines with lowercase letters represent targeted deletions in the cluster: a, $Dlk1^{tm1Hsul}$ (146); b, $Dlk1^{tm1Srba}$ (166); c, $Dlk1^{tm1.1Jvs}$ (221); d, $Igdmr^{tm1Ace}$ (123); e, $Meg3^{tm1Jvs}$ (194); f, $Meg3^{tm1Kono}$ (209); g, $Meg3^{tm1Igl}$ (258); h, $Rtl1^{tm1Fuis}$ (181). Thick aqua lines with capital letters represent transgenes overexpressing the genes in the cluster: A, TG Dlk1-Fc (115); B, TG DLK1-Hs (1); C, TG Dlk1-31 (43); D, TG Dlk1-84 (171); E, TG Dlk1-70 (43); F, TG Dlk1/Gtl2-178 (245). The magenta arrowhead represents the Gtl2LacZ [$Meg3^{Gt(pGTi)216Gos}$] insertion site (177), and the red asterisk represents the $Dio3^{tm1Stg}$ mutation site (88, 89). Abbreviations: ICR, imprinting control region; M, maternal; miRNA, microRNA; P, paternal; snoRNA, small nucleolar RNA.

and the entire litter (181). Loss of *Rtl1as* results in placentomegaly, placental defects, postweaning growth retardation, and strain-specific neonatal lethality (181).

The *Dio3* gene codes for type 3 iodothyronine deiodinase (D3) and is preferentially expressed from the paternal allele (212). D3 is a negative regulator of thyroid hormone metabolism. Homozygous loss of *Dio3* expression results in partial embryonic lethality and defects in hearing and sight owing to thyrotoxicity before P11. The animals then transition into a hypothyroid state by P15, resulting in low fertility, impaired ability to clear infections, and impaired insulin secretion (17, 88, 89, 136, 152, 153). Loss of paternal *Dio3* expression results in mild auditory impairments, suggestive of haploinsufficiency (153). Embryos with paternal UPD for chromosome 12 have increased *Dio3* levels and are hypothyroid; those with maternal UPD for chromosome 12, in contrast, have increased T3 levels owing to biallelic repression of *Dio3*.

The chromosome 12 imprinted genes expressed solely from the maternally inherited chromosome are all ncRNAs. *Gtl2* produces a maternally expressed long ncRNA (176, 206). There are several mouse models that knock out *Gtl2* expression (177, 194, 209, 258). Interpreting these models is difficult: The knockout also alters the expression of other noncoding transcripts in the region because most of the maternally expressed transcripts are regulated by the *Gtl2* promoter

region. This makes it difficult to ascertain the contributions of individual ncRNAs to the phenotypes associated with altered imprinting on chromosome 12 (29). The functions of the other protein-coding gene in the domain (*Begain*) and the miRNAs and C/D snoRNAs are currently unknown because there are no published mouse models that alter the expression of these transcripts independently of any other.

The *Dlk1/Dio3* domain is one of the few imprinted regions where key genes (*Dlk1* and *Rtl1*) have been experimentally demonstrated to be dosage sensitive, with lethal consequences upon both under- and overexpression. Insertion of a *LacZ* transgene upstream of the *Gtl2* promoter causes partial loss of imprinting and, when maternally inherited, an increase in the expression of the protein-coding genes. This increase in DLK1 and D3 levels causes delayed brown adipose tissue (BAT) development, perturbed β-adrenergic receptor signaling, and impaired growth (**Figure 1**). The dysfunction in BAT results in lethality upon weaning because the mice cannot carry out nonshivering thermogenesis, and after leaving the nest, they are unable to thermoregulate. This failure in the transition to independent life is rescued if additional warmth is supplied at weaning (29).

Like the IC1 and IC2 clusters, genes of the *Dlk1/Dio3* domain have roles in embryonic and placental growth. However, the domain also has other roles postnatally, particularly in nutrient metabolism, energy homeostasis, and the adaptations required for independent life.

Gnas DOMAIN: MOUSE CHROMOSOME 2 (HUMAN CHROMOSOME 20)

The *Gnas* imprinted domain is located on mouse distal chromosome 2 and contains a paternally expressed noncoding antisense transcript (*Nespas*) and a single gene comprising multiple alternatively spliced protein-coding exons that are maternally, paternally, or biallelically expressed, depending on the tissue (159, 233). The alternative splicing generates different isoforms of the stimulatory G-protein subunit $G_s\alpha$. Differential methylation at the *Nespas* promoter (*Nespas* DMR) acts as the ICR; this region is hypermethylated during oogenesis but remains unmethylated during spermatogenesis (40, 234).

Both maternal and paternal UPDs for distal chromosome 2 are lethal postnatally, with reciprocal phenotypes. MatDp(dist2) neonates are dehydrated; have long, flat-sided bodies with arched backs; do not suckle; are inactive; and almost always die within 24 h of birth (25, 231). PatDp(dist2) neonates survive for several days but have occasional subcutaneous edema; have short, square bodies with broad, flat backs; are hyperkinetic; and suffer from progressive tremor and balance defects before neonatal death (25, 231). Deletion of the maternal *Nespas* DMR has no phenotypic effect. However, deletion of the paternal *Nespas* DMR results in neonates that suckle poorly, become thin and lethargic, and die by P2 (234), reflecting the regulatory role of the antisense transcript in the control of the protein-coding transcripts in the cluster. The majority of mouse models of the *Gnas* locus were designed to answer mechanistic questions about its complex transcriptional and imprinting profile. However, a few models result in alterations of single genes or specific transcripts.

Gnas, *Gnasxl*, and *Nesp* are the transcript variants created via alternative splicing at the locus (159). *Nesp* encodes a maternally expressed protein, neuroendocrine-specific Golgi protein P55 (NESP55) (93, 159), and *Gnas* encodes the heterotrimeric G-protein subunit $G_s\alpha$, which is preferentially expressed from the maternal allele in a tissue-specific manner (159, 227, 252). *Gnasxl* encodes two paternally expressed proteins from overlapping open reading frames: an extra-large variant of the $G_s\alpha$ subunit, XLαs (104), and a eutherian-specific protein, ALEX (108, 222). Loss of maternal *Nesp* expression, through a deletion of 26 base pairs at its start codon that does not affect the other transcripts, results in a subtle behavioral phenotype of abnormal reactions to novel environments.

In humans, *Gnas* mutations are associated with pseudohypoparathyroidism types 1a and 1b, pseudopseudohypoparathyroidism, and progressive osseous heteroplasia (12, 185). In mice, deletion of *Gnas* exon 1, which results in loss of *Gnas* expression but normal expression of the other transcripts, causes phenotypes that vary between models, including partial neonatal lethality, altered energy homeostasis, parathyroid hormone resistance, and skin defects (32, 76, 92, 125, 239). Many of these phenotypes are apparent in both maternal and paternal heterozygotes and thus cannot be attributed to imprinted *Gnas* expression. However, some phenotypes are specific to one heterozygote: For example, deletion of the maternal *Gnas* exon 1 allele results in subcutaneous edema and partial neonatal lethality [partially recapitulating the phenotype of PatDp(dist2) mice] but also glucose intolerance and obesity in adulthood. In contrast, deletion of the paternal *Gnas* exon 1 causes elevated energy expenditure and activity levels in adulthood (32, 124, 239).

Deletion of *Gnas* exon 2 (common to all transcripts in the region except *Nespas*) from the maternal chromosome causes mice to become obese, whereas deletion from the paternal chromosome results in lean mice with elevated activity levels and energy expenditure (251). In contrast, specific deletion of paternal *Gnasxl* results in impaired suckling, hypoactivity, and 90–100% neonatal lethality, depending on the genetic background. Surviving adults display growth retardation; a lean, hypermetabolic phenotype with hyperphagia; increased sympathetic nervous system activity; and improved insulin sensitivity (161, 240). This recapitulates the majority of the paternal *Gnas* exon 2 deletion phenotype and demonstrates that *Gnasxl* is the principal affected gene in this complex model.

The other transcripts at the locus, *Exon 1A* and *Nespas*, lack specific mouse models. The *Exon 1A*–specific exon overlies a DMR whose deletion abolishes tissue-specific imprinting of *Gnas* (52, 124, 230), whereas *Nespas* knockout or hypomorphic mutation results in altered *Nesp* expression (229, 234).

The *Gnas* locus is unique within imprinted clusters because of the range and complexity of alternative splicing that occurs within it. Although the functions of several of these transcripts have yet to be elucidated, it is clear that the locus acts mainly postnatally, with effects on behavior via *Nesp* and reciprocal effects on metabolism via *Gnas* and *Gnasxl*.

Grb10 DOMAIN: MOUSE CHROMOSOME 11 (HUMAN CHROMOSOME 7)

The *Grb10* domain is under the control of the maternally methylated *Grb10* DMR (184). It has an interesting imprinting profile, being expressed from the maternal chromosome in all tissues except the central nervous system, where it is paternally expressed under the control of a brain-specific promoter. The imprinting of the *Grb10* domain and the presence of the brain-specific *Grb10* promoter are unique to eutherians (195). Maternal and paternal UPDs for chromosome 11 result in reciprocal phenotypes, but the mice are viable and fertile. As with many of the other loci described above, the *Grb10* domain controls growth in addition to other postnatal behavioral and metabolic phenotypes. MatDp(prox11) animals are smaller at birth, whereas PatDp(prox11) animals are larger at birth; both retain these size differences throughout life (22). Deletion of the ICR, which causes maternalization of the paternally inherited region, results in embryonic, placental, and postnatal growth retardation upon paternal transmission, but the reciprocal deletion on the maternal chromosome has no effect (184).

Grb10 encodes a protein with isoform-dependent imprinting: Type I transcripts are maternally expressed in almost all tissues, whereas type II transcripts are brain specific and paternally expressed (7, 90, 141). In humans, *GRB10* has been implicated in Silver–Russell syndrome (145). There are

several mouse knockouts of *Grb10* with similar phenotypes upon maternal transmission—namely, placental and embryonic overgrowth, reduced placental efficiency, brain-sparing postnatal growth enhancement with increased lean mass, and resistance to adiposity induced by a high-fat diet (28, 30, 71, 91, 190, 223). One model, $Grb10^{Gt(Betageo)1Ward}$, also showed 12% lethality soon after birth, which was attributed to abnormal lung development (30). In contrast, 1.2–1.4-fold-greater expression of *Grb10δ* cDNA resulted in growth retardation from 4 weeks postnatum onward, early- and late-onset type 2 diabetes, severe pancreatic abnormalities, and reduced body mass index (183). Observations of behavioral phenotypes also revealed that paternal transmission of a *Grb10* deletion results in increased social dominance and allogrooming (71).

In addition to *Grb10*, this domain contains the imprinted genes *Ddc*, which is paternally expressed in the heart (137), and *Cobl*, which is maternally expressed in the yolk sac (184). The phenotypic consequences of alterations of these genes are not clear because there are no models that alter *Ddc* expression in the heart (254) and constitutive *Cobl* deletion does not result in a mutant phenotype (72).

The *Grb10* domain is one of many imprinted domains that affect metabolism and energy homeostasis. Like the IC1, IC2, *Igf2r*, and *Dlk1/Dio3* domains, it affects embryonic growth. However, similarly to the *Gnas* and *Dlk1/Dio3* domains, it has postnatal roles in behavior and/or metabolism and energy homeostasis

Peg10: MOUSE CHROMOSOME 6 (HUMAN CHROMOSOME 7)

The *Peg10* imprinted domain spans 1.8 Mb and consists of two paternally expressed genes, *Peg10* and *Sgce*, and one maternally expressed gene, *Asb4* (142, 158). The ICR for this region is believed to be the CpG island covering the bidirectional *Peg10/Sgce* promoter, which becomes methylated in oocytes and remains unmethylated in sperm (158). However, a targeted deletion of this element has not been performed to confirm its ICR status. MatDp(prox6) causes early embryonic lethality (13), indicating either that the paternally inherited complement of this domain is required for development or that a double dose of maternally expressed imprinted genes is incompatible with development. Five additional genes (*Tfpi2*, *Neurabin/Ppp1r9a*, *Pon2*, *Pon3*, and *Calcr*) may be imprinted but show only tissue-specific allelic biases in expression (142, 144, 156, 158, 160). Targeted deletions have been created for many of the genes in the cluster, and the absence of obvious parent-of-origin effects in the phenotypes associated with these deletions is consistent with either the absence of imprinting or a lack of functional relevance for any parent-of-origin-specific expression bias that might exist.

Asb4 (encoding ankyrin repeat and SOCS box–containing 4) is a maternally expressed imprinted gene in all tissues tested at this locus. Its expression in the rat brain is restricted to regions of the amygdala and hypothalamus involved in energy homeostasis, including the proopiomelanocortin neurons of the arcuate nucleus (120). Moreover, *Asb4* expression is downregulated in fasted rats (120). No knockout is available, but there is a transgene driven by a mouse proopiomelanocortin enhancer. These transgenic mice exhibit increased energy expenditure and decreased serum leptin levels; they eat more food than control animals yet weigh less (119).

Mouse *Sgce* is expressed from the paternally inherited chromosome in all tissues tested (144, 160, 247). In humans, functional mutations in the *SGCE* gene lead to myoclonus-dystonia syndrome, which is characterized by bilateral, myoclonic jerks and dystonia (259). In mice, paternal inheritance of a targeted deletion of exon 4 that creates a premature stop codon results in the complete absence of *Sgce* in the brain (247). These mice are viable and fertile but exhibit myoclonic twitches, motor deficits, hyperactivity, depression, and anxiety-like behaviors, recapitulating symptoms of myoclonus-dystonia syndrome (246).

Peg10 is a Ty3/gypsy retrotransposon-derived gene that is expressed exclusively from the paternally inherited chromosome and is highly expressed in the placenta (156). Targeted mutations of the *Peg10* gene in mice cause early embryonic lethality: Embryos develop normally until E9.5 but by E10.5 are growth restricted and have no evident heartbeat. Growth restriction in the placenta is evident from E9.5 onward. The knockout placentas lack spongiotrophoblast cells, and tetraploid rescue experiments indicate that the embryonic lethality is due to placental failure (157). Together, these models indicate that the embryonic lethality in MatDp(prox6) mice (13) is caused by the lack of *Peg10*. No mutant phenotypes are associated with PatDp(dist6), indicating that *Asb4* is not necessary for development and that any effects of double doses of *Peg10* and *Sgce* are subtle.

These findings indicate that the key imprinted genes in this cluster are important for regulating placental development, morphogenesis, and function, and that loss of function has a greater impact than increased dosage. This suggests that dosage sensitivity is not a key feature of imprinted genes at this locus, and thus that dosage modulation is not the sole impetus for the evolution of imprinting. Suzuki et al. (200) have proposed that imprinting at this locus is associated more with the retrotransposon origins of *Peg10* than with the need for dosage control.

Rasgrf1: MOUSE CHROMOSOME 9 (HUMAN CHROMOSOME 15)

The *Rasgrf1/A19* domain is one of three imprinted clusters with a paternally methylated ICR. The cluster is located on distal chromosome 9, and maternal duplications of this region lead to postnatal growth retardation (24). The ICR is located in the intergenic region between *Rasgrf1* and the paternally expressed ncRNA *A19* (AK015891). The differentially methylated ICR becomes methylated in the male germline through the action of Piwi-interacting RNAs on 40 copies of a 41-mer repeat adjacent to the ICR. This is the only germline-derived ICR that is known to be regulated via small-RNA-mediated mechanisms. *Rasgrf1* is most highly expressed in the brain. Paternally inherited expression is present in neonatal whole brain, and paternally biased expression occurs in the adult cerebellum and cerebrum (163). Paternal allelic expression also occurs in the heart and stomach, but expression in the testes, ovaries, lungs, and thymus is biallelic (163). More recent data indicate that imprinting relaxes around weaning (49).

There are no mouse models that alter *A19* expression, but *Rasgrf1* has been studied extensively. Like many other imprinted genes, *Rasgrf1* is implicated in growth control. Studies of three knockout and two induced point mutation models reported 10–13% postnatal growth retardation compared with wild types, which continues into adulthood (35, 66, 78, 95). Paternal transmission of the tandem repeat knockout (+/Rasgrf1^{Tm1Pds}) produces transient nulls that are also significantly growth retarded from P16 onward (49, 249). Conversely, functionally biallelic mice that ectopically express *Rasgrf1* from the maternal allele (Rasgrf1^{Tm2Pds}/+) show significant growth enhancement from P16 onward (49, 248). Serum IGF1 and growth hormone (GH) are decreased in the *Rasgrf1*-null growth-restricted animals (49, 95). However, because the growth defect in some of the models manifests before GH is produced, this defect is believed to be independent of GH, at least in early neonatal stages (35, 49). In a more detailed hormonal analysis, Font de Mora et al. (66) also showed that *Rasgrf1* is expressed in pancreatic islets and that nulls have 30% fewer β cells owing to a reduction in neogenesis and proliferation. These mice also showed reduced serum insulin and reduced glucose tolerance at 6 weeks, indicating a metabolic phenotype associated with this gene.

Because the major site of *Rasgrf1* expression is the brain, extensive behavioral assessments have been performed in these models. However, the data regarding the effects of *Rasgrf1* deletion on behavior are conflicting. Analysis of Rasgrf1^{tm1Sva} nulls implies impaired hippocampal-dependent spatial learning and memory but normal amygdala function (78). Conversely, behavioral tests

on Rasgrf1^{tm1Kln} and Rasgrf1^{enu1H} nulls suggest limited spatial memory impairments but impaired contextual fear conditioning, indicating abnormal amygdala function (19). As with the PWS region, the *Rasgrf1* cluster seems to be required mainly postnatally for appropriate growth, metabolism, and behavior.

Peg1/Mest DOMAIN: MOUSE CHROMOSOME 6 (HUMAN CHROMOSOME 7)

In humans, the *PEG1/MEST* domain is associated with the intrauterine growth retardation disorder known as Silver–Russell syndrome (100). The region contains the paternally expressed gene *Peg1/Mest*, encoding the mesoderm-expressed transcript protein; the reciprocally imprinted maternally expressed genes *Copg2* and *Klf14*; and two paternally expressed ncRNAs, *Copg2as* and *Mestit1*. In mice, imprinting in the cluster is thought to be controlled by the *Peg1* germline DMR, which becomes hypermethylated during oogenesis (127), but this remains unconfirmed owing to the lack of mouse knockout models of this DMR. MatDp(prox6) animals, which lack *Peg1*, die early in development, but PatDp(prox6) animals are viable and healthy (23). The *Peg1/Mest* locus is understudied, with only two models generated to date. Only one of these models, Mest^{tm1Masu}, deletes a single gene. Interestingly, upon paternal transmission of Mest^{tm1Masu}, female mice have compromised maternal behavior that affects nest building and caring for pups. This results in low pup survival irrespective of genotype (117). Additionally, *Peg1/Mest* paternal heterozygous mutants have embryonic, placental, and postnatal growth retardation and a subtle cardiomyopathy but normal longevity and male fecundity (107, 117). The other transcripts of the *Peg1/Mest* domain have not been studied in mouse models.

This locus not only controls pre- and postnatal growth and placental development but also impacts offspring phenotype by regulating maternal behavior. This adds an interesting dimension to the functions of imprinted genes.

Peg3 DOMAIN: MOUSE CHROMOSOME 7 (HUMAN CHROMOSOME 19)

The *Peg3* domain contains four paternally expressed imprinted genes (*Peg3/Pw1*, *APeg3*, *Usp29*, and *Zfp264*) and three maternally expressed zinc-finger proteins (*Zim1*, *Zim2*, and *Zim3*). The cluster is located on mouse proximal chromosome 7 (101, 111). Imprinting in the region is controlled by the *Peg3* DMR; this region is located at its promoter, which is hypermethylated during oogenesis but remains hypomethylated during spermatogenesis (106). Study of the *Peg3* domain using UPDs is complicated by the presence of other imprinted regions on mouse chromosome 7. However, there is a mouse model that deletes the *Peg3* ICR: Maternal transmission of this deletion results in animals that are transiently heavier than controls at weaning (106), whereas paternal transmission results in partial embryonic lethality and reduced body weight (106).

Like the *Peg1/Mest* domain, the *Peg3* domain has very few mouse models. Similarly to the *Peg1/Mest* mutant, *Peg3* mutants show a maternal behavioral phenotype in pup nurturing. Loss of paternal *Peg3* expression results in reduced food intake during pregnancy, impaired milk letdown, and compromised maternal care, leading to low pup survival (41, 121). Embryos are growth retarded, and after birth the mice have impaired suckling and defective nonshivering thermogenesis that extends into adulthood (41). Adult animals are underweight and hypophagic but have elevated adiposity, a secondary effect of dysregulated hypothalamic control of their metabolism (42). *Peg3* mutations lead to delayed puberty in females, an anxiety-like phenotype, and a lack of the typical improvement in male sexual behavioral responses after sexual experience (27, 41, 42, 202, 203).

Intriguingly, transgenic overexpression of *Peg3* did not result in any phenotypic change (204). However, the transgene was minimally overexpressed in embryos and failed to rescue the majority of *Peg3*$^{+/-}$ phenotypes in intercrosses. Thus, it likely lacked the full complement of *Peg3* enhancers. The other transcripts of the *Peg3* locus have not been studied using specific mouse models.

P1-derived artificial chromosome (PAC): a bacteriophage-derived DNA construct containing a genomic insert of up to 300 kb that can be used to make transgenes

Like the *Peg1/Mest* locus, the *Peg3* locus demonstrates that imprinted genes may act on offspring both directly and via the mother. Also like *Peg1/Mest*, *Peg3* affects pre- and postnatal growth, but it also affects postnatal metabolism, thermogenesis, and behavior. Additionally, the effects of *Peg3* on milk letdown hint at undiscovered roles for imprinted genes in another mammal-specific process: lactation.

SINGLETON GENES

In addition to the imprinted gene clusters, several unclustered imprinted genes exist as singletons. The majority of these genes do not have mouse mutant or overexpression models; the exceptions are *Plagl1/Zac1*, *Commd1*, *U2af1-rs1*, and *Htr2a*, which are all expressed from the paternally inherited chromosome and have maternally methylated germline DMRs that are likely responsible for their monoallelic expression.

Plagl1/Zac1, located on mouse proximal chromosome 10 and human chromosome 6, encodes a transcription factor and shows imprinting in embryonic tissues. After birth, it is biallelically expressed in the liver (160). In humans, paternal UPD6 and duplications of the region containing *Plagl1/Zac1* are associated with intrauterine growth retardation and transient neonatal diabetes mellitus (70). *Plagl1* therefore represents another growth-regulating imprinted gene with a postnatal impact on metabolism. In mice, overexpression of *PLAGL1* from a human P1-derived artificial chromosome (PAC) transgene causes neonatal hyperglycemia, recapitulating the human transient neonatal diabetes mellitus phenotype (129). However, loss of paternal murine *Plagl1* expression results in variable phenotypes. Both knockout models exhibit partial neonatal lethality, curly tails, and wrinkled skin (219, 253). However, +/Plagl1$^{GT(OST181461)Lex}$ animals also display partial embryonic lethality, neonatal weight loss, and, often, heart defects or neural tube defects (253). In contrast, +/Plagl1^{tm1Jour} animals display only intrauterine growth retardation and abnormal ossification of some bones (219).

Commd1/Murr1, located on mouse chromosome 11 and human chromosome 2, encodes a preferentially maternally expressed protein and contains the paternally expressed antisense transcript *U2af1-rs1* within its first intron (150, 225). Its ICR is the *U2af1-rs1* DMR, which acquires methylation in oocytes (150). Human *COMMD1/MURR1* lacks *U2AF1-RS1* and thus is not imprinted (256). Homozygous deletion of *Commd1* in mice is embryonic lethal between E9.5 and E10.5, likely because of placental failure, with marked embryonic and placental developmental delay present at E9.5 (218). However, heterozygous mice lack a mutant phenotype (218), suggesting lack of functional relevance for any imprinted *Commd1* transcription. A mouse model with 200–300% *U2af1-rs1* expression did not exhibit any gross abnormality (196).

Htr2a, located on mouse chromosome 14 and human chromosome 13, encodes serotonin receptor type 2A and is expressed from the maternally inherited chromosome (102). The two mouse models of homozygous *Htr2a* deletion seem to have distinct phenotypes: Htr2a^{tm1Grch} results in normal depression-related and fear-conditioned behaviors but reduced anxiety-related behaviors (228), and Htr2a^{tm1Rhn} results in intestinal abnormalities, reduced non-REM sleep, hyposensitivity to chemical-induced pain, and alterations in responses to hallucinogens and amphetamines. Heterozygotes have not been investigated in either model (62, 81). Whether these models actually exhibit different specific phenotypes is not entirely clear, however, as they were not submitted to the same behavioral tests.

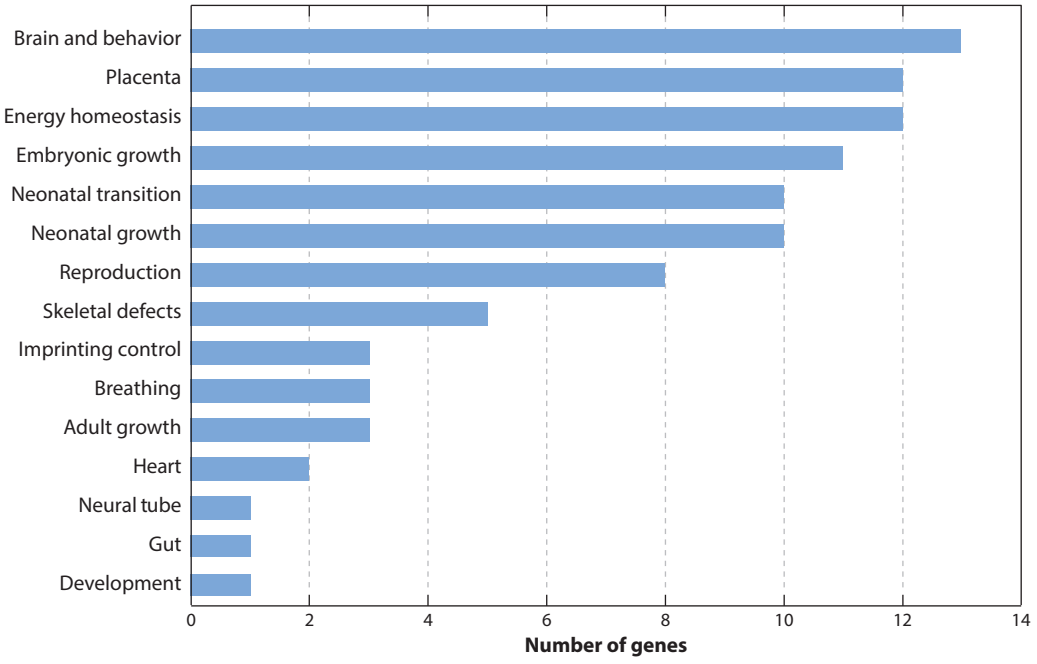

Figure 3

Functions of imprinted genes identified by mouse models.

GENERAL CONCLUSIONS

Imprinted Gene Function in Utero

The systematic cataloging of phenotypes associated with the altered dosages of imprinted genes highlights their common functions. Two of the most common functions involve the regulation of placentation and embryonic growth (**Figure 3**), indicating that appropriate imprinting is vital for normal in utero development. Indeed, androgenote and gynogenote/parthenogenote embryos fail early in development (11, 135, 197). However, these conceptuses successfully complete fertilization and gastrulation, indicating that the first developmental time point at which a full complement of maternally and paternally inherited genomes is required is considerably after implantation, after germ-layer specification, and most likely during tissue development and growth.

Targeted deletions of only two imprinted genes cause early embryonic lethality: paternally expressed *Peg10* and maternally expressed *Ascl2*. Animals without functional copies of either of these genes have normal-looking placentas early in development but die by E10.5 because of placental failure (83, 157). Both models lack spongiotrophoblast cells and have a poorly developed labyrinth zone (83, 157). Paternal UPD of the *Peg10* region does not lead to any visible phenotype (232), suggesting that a double dose of *Peg10* is not detrimental. Moreover, overexpression of an *Ascl2* transgene also causes no visible phenotype (169), suggesting that functional copies of both genes are required for placentation but that dosage is not important.

Deletion of the active copies of nine other genes results in a noticeable placental phenotype, with most deletions affecting the size of the placenta. Deletion of paternally expressed genes (*Igf2*, *Rtl1*, *Peg1*, and *Plagl1*) causes growth restriction, and deletion of maternally expressed genes

(*Igf2r*, *Cdkn1c*, *Phlda2*, and *Grb10*) causes growth enhancement. *Igf2* and *Slc22a3* deletion models have defects in placental transport (36, 37, 186, 261), and *Igf2* knockout, *Igf2* overexpression, and *Phlda2* models show reciprocal changes in glycogen cell number (36, 57, 67, 126). However, this is clearly not the full story, as there are genes that are imprinted only in the placenta for which either no mouse models are available or placental phenotypes have not been reported or studied, suggesting that there may be more roles for imprinted genes in placental development and physiology.

Embryonic growth defects are seen in deletion models of *Igf2*, *Dlk1*, *Rtl1*, *Peg1*, *Peg3*, *Peg10*, *Plagl1*, *Igf2r*, *Cdkn1c*, *Phlda2*, *Ascl2*, *Gnas*, and *Grb10*. Generally, deletion of paternally expressed genes leads to growth retardation, and deletion of maternally expressed genes leads to embryonic growth enhancement. The exceptions are *Phlda2*, where maternal transmission of a deletion causes mild growth restriction (67), and *Ascl2*, where maternal transmission results in placental failure. Most of these genes are also expressed in the placenta; thus, placental defects may drive embryonic growth effects. However, in some cases, placental and embryonic functions can be separated. For example, deletion of the placenta-specific *Igf2* transcript results in intrauterine growth retardation, but these mice catch up after birth, unlike full *Igf2* nulls, which remain growth retarded throughout life (38, 39); this indicates that IGF2 plays a growth regulatory role that is both dependent on and independent of the placenta. Moreover, overexpression of *Rtl1* leads to placentomegaly but has no effect on embryonic growth (181), and deletion of *Phlda2* causes placentomegaly but also embryonic growth restriction. Thus, a larger placenta does not necessarily lead to larger offspring, but reduced placental growth or efficiency does generally lead to embryonic growth restriction.

Mouse models that are embryonic lethal usually cause death late in gestation, consistent with postgastrulation functions. However, PatDp(dist7) animals die at midgestation, around E8.5–10.5, and MatDp(dist7) animals usually die after E15.5. This is likely the result of increased *Cdkn1c* and *Phlda2* expression combined with a lack of *Igf2*. UPD of the *Dlk1/Dio3* region on chromosome 12 also causes embryonic lethality: PatDp(12) animals die by E16.5, and MatDp(12) animals die between E18.5 and birth (211). These phenotypes, which are caused by the altered dosage of whole clusters, are, unsurprisingly, more severe than those of single-gene models. Paternal deletion of *Rtl1* leads to a delay in parturition that, depending on the mouse strain, can cause the whole litter (including wild-type littermates) to die, and even when delivered by Caesarean section, the *Rtl1*-deficient neonates die within 24 h (181).

Additionally, cataloging the phenotypes of mouse imprinted gene models has revealed a clear role for imprinted genes in the development of mesodermally derived tissues, including the skeleton. Ossification defects are reported to be associated with *Igf2*, *Cdkn1c*, *Plagl1*, *Dlk1*, and *Igf2r* models. Paternal disruption of the *Igf2*-P2 promoter causes delayed ossification and shorter, thinner bones (84), as does maternal deletion of *Cdkn1c* (208, 243, 255). Overexpression of *Dlk1* also causes delayed ossification and thinner bones (43). *Igf2r* nulls have advanced ossification in digits and vertebrae in addition to fused ossification centers in the sternum and extra digits (226). Finally, deletion of the transcription factor gene *Plagl1* also causes skeletal defects, but because these animals also show altered expression levels of *Igf2*, *Cdkn1c*, and *Dlk1*, it is difficult to determine whether this is a direct effect (219).

Clearly, roles in growth, placentation, and skeletogenesis are not the only functions of imprinted genes in utero, and embryonic defects likely program at least some of the observed postnatal phenotypes. This may contribute to the range of defects described in the brain, muscle, fat, and lungs, where phenotypes are evident mostly postnatally. Interestingly, although only a few imprinted genes appear to be involved in in utero development, at least one gene from each major cluster has an impact prenatally, even within the PWS cluster, where failure to correctly imprint the region causes lower birth weight (50, 244).

In Utero to Ex Utero Transition

The next major step where failure to correctly imprint genes has dramatic consequences is the neonatal period. Loss of paternal *GnasXL*, *Dlk1*, or *Rtl1* and maternal *Cdkn1c* or *Igf2r* is associated with almost complete perinatal/neonatal lethality. Deletion of *Gnas* exon 2, which is common to all transcripts, causes neonatal death when inherited from either parent (251). *Gtl2* and *Rtl1as* maternal knockouts also cause strongly penetrant neonatal lethality, but they both cause changes in the expression of other genes in the domain that are likely to contribute to the phenotype. Disruptions of paternally expressed *Gnas*, *Sno116*, *Magel2*, *Ndn*, *Igf2*, and *Plagl1* and maternal *Gnas*, *Nesp*, and *Grb10* all result in partial neonatal lethality. Changes in neonatal growth phenotypes are also seen in many of the models. Deletions of the expressed copies of *Gnas Exon 1A*, *Gnasxl*, *Nespas*, *Peg1*, *Peg3*, *Snord116*, *Magel2*, *Ube3a*, *Igf2*, *Cdkn1c*, *Rasgrf1*, *Plagl1*, *Dlk1*, and *Gtl2* all cause growth restriction, whereas deletions of maternal *Grb10*, *Igf2r*, *H19*, and *Gnas* exon 2 (which affects all transcripts) cause increased birth weight. The overgrowth phenotypes seen in *Igf2r* and *H19* deletions are due to increased levels of IGF pathway activation in these animals.

Life outside the womb brings many challenges for newborn mammals, and failure to overcome these can be fatal. First, they need to be able to breathe efficiently from the moment they exit the birth canal. Failure to initiate breathing is implicated in neonatal morbidity in *Igf2*, *Igf2r*, *Cdkn1c*, *Ndn*, *Grb10*, and *Plagl1* mutants. In *Cdkn1c* mutants, this has been attributed to skeletal defects of the rib cage (208). Immature alveoli are associated with some deletions of *Igf2r* (226, 238), whereas *Igf2* knockouts have disorganized alveoli and increased levels of surfactant B (187). Neonatal death of maternal *Grb10* knockouts is associated with blood in the lungs (30).

Second, neonates must adapt to feeding by suckling. Lactation is one of the defining characteristics of mammals, and suckling defects have been observed in models that lack *Peg3*, *GnasXL*, *Magel2*, *Cdkn1c*, and *Igf2r* (41, 114, 161, 174, 243). The cause of the impairment is different in each model. In *Cdkn1c* mutants, the reduced feeding is explained primarily by a cleft palate (243), whereas the *GnasXL* phenotype has been attributed to lack of *GnasXL* expression in key facial motor nuclei in the brain (161). In paternal *Magel2* nulls, the failure to initiate suckling is likely due to a lack of oxytocin in the hypothalamus, because the phenotype is rescued by a single oxytocin injection after birth (174). In *Peg3* mutants, the reasons for reduced feeding activity are twofold: The offspring fail to initiate feeding, but the mothers also fail to let down milk owing to hypothalamic dysfunction (41).

Finally, neonates need to regulate energy homeostasis, a complex process involving sugar metabolism, fat metabolism, appetite regulation, activity levels, and nonshivering thermogenesis. There are at least 13 imprinted genes whose deletion results in an energy homeostasis phenotype: *Gnas*, *Gnasxl*, *Asb4*, *Peg3*, *Magel2*, *Snord116*, *Ins2*, *Kcnq1*, *Rasgrf1*, *Plagl1*, *Grb10*, *Dlk1*, and *Dio3*. *Ins2*, *Rasgrf1*, *Dio3*, and *Plagl1* have roles in sugar metabolism. *Ins2* has a key role in sugar metabolism but is imprinted only in the yolk sac, and thus imprinting does not regulate its major postnatal pancreatic function (51, 58). *Rasgrf1*$^{+/-}$ mice are lean, lighter, and hypoinsulinemic owing to a reduced β cell number and are thus glucose intolerant (66). Similarly, *Dio3*$^{-/-}$ mice are lighter, have a reduced pancreatic islet area, and are glucose intolerant owing to impaired glucose-stimulated insulin secretion (136). Overexpression of *Plagl1* in mice using a human PAC transgene leads to hyperglycemia in neonates, recapitulating transient neonatal diabetes mellitus, which occurs in humans with loss of imprinting at this locus (129).

The *Gnas* complex is involved in many mammalian processes and provides the only clear example of proteins transcribed from the same imprinted locus having reciprocal effects on energy homeostasis. *Gnas* encodes $G_s\alpha$, an intermediate in nonshivering thermogenesis in BAT, whereas *GnasXL* encodes XLαs, an extra-long isoform of $G_s\alpha$. Deletion of the common exon 2 from

the maternal chromosome causes mice to become obese, with increased levels of pale BAT and a decreased metabolic rate. Conversely, when the deletion is inherited paternally, the mice are lean, with small amounts of dark BAT and an increased metabolic rate (251). *Gnasxl*-deficient mice have a phenotype similar to that of paternal transmission of the exon 2 deletion (161), and because maternal deletion of *Nesp* has only a subtle neurological phenotype, we can deduce that the phenotypes of maternal exon 2 deletion are attributable to loss of maternal *Gnas* expression. Thus, *Gnas* and *Gnasxl* have opposite effects on energy homeostasis.

Several other paternally expressed genes are associated with increased adiposity upon deletion. *Peg3*$^{+/-}$ mice have increased fat mass, likely because of their reduced metabolic rate and core body temperature, despite being leptin resistant and hypophagic (42). *Dlk1*$^{+/-}$ mice have increased fat mass and are more susceptible to obesity induced by a high-fat diet (146). Finally, *Magel2*^{tm1Stw} (but not *Magel2*$^{tm1.1Mus}$) paternal heterozygotes are hypophagic, hypoactive, and hyperinsulinemic and develop obesity after weaning (15, 174). Intriguingly, despite these phenotypes, *Magel2*^{tm1Stw} paternal heterozygotes do not gain additional weight on a high-fat diet (15).

Interestingly, deletions of both paternally and maternally expressed genes are associated with reduced adiposity. *Snord116*$^{+/-}$ mice are smaller and leaner (despite their hyperphagia), have increased insulin sensitivity, and are resistant to obesity induced by a high-fat diet (48). *Grb10*$^{-/+}$ mice are larger and heavier, with increased lean mass and reduced fat mass, owing partly to increased muscle mass (190, 223). They have increased glucose tolerance and are less susceptible to the increased insulin resistance induced by a high-fat diet (190). In contrast, mice that overexpress *Grb10* cDNA from a chicken β-actin promoter become growth retarded after weaning and have a reduced body mass index, pancreatic abnormalities, and either early- or late-onset type 2 diabetes (183). These phenotypes are more severe on a high-fat diet (241).

Finally, two imprinted genes appear to alter energy homeostasis through their effect on activity levels. *Asb4* transgenic mice, which specifically overexpress *Asb4* in the proopiomelanocortin neurons of the arcuate nucleus, are hyperactive and have an increased metabolic rate, which likely causes their hyperphagia, reduced weight, and reduced serum leptin levels (119). *Kcnq1*$^{-/-}$ mice are also hyperactive owing to their shaker/waltzer phenotype, which likely also causes their hyperphagia, improved insulin sensitivity, and, in *Kcnq1*^{tm2Kpfe} homozygotes, reduced weight (18, 21).

Leaving the Nest

Within the postweaning stages of life, mammals must adapt to intermittent and varied feeding, which is dependent on their ability to explore and remember their surroundings. They also must regulate body temperature outside the huddle of the nest and must be able to successfully mate and reproduce. Much of this adaptation requires appropriate levels of anxiety and risk taking. Imprinted genes are involved in these three major areas of postweaning life—nonshivering thermogenesis, reproduction, and behavior—in addition to playing a continued role in energy homeostasis.

Dlk1 and *Dio3* together regulate nonshivering thermogenesis upon weaning through precisely regulated and coordinated control of dosage within the same cluster. The physiological function is achieved via DLK1's role in the temporal regulation of BAT development combined with D3's control of thyroid hormone action on β-adrenergic signaling (29).

Nine imprinted gene mutants have nonreproductive phenotypes that affect a variety of behaviors, including anxiety, learning, memory, sleep, and reaction to novelty. Deletion of paternal *Peg3*, *Snord116*, or *Sgce* results in increased anxiety, whereas deletion of maternal *Htr2a* results in reduced anxiety (27, 48, 228, 246). *Peg3*$^{+/-}$ and *Nesp55*$^{-/+}$ mice exhibit reduced exploration of novel environments (27, 162). *Snord116*$^{+/-}$, *Rasgrf1*$^{+/-}$, *Ube3a*$^{-/+}$, and some *Sgce*$^{+/-}$ mice exhibit

deficits in learning and memory (19, 48, 78, 96, 139). In contrast, *Ndn*$^{+/-}$ mice exhibit deficits in motor coordination and balance but improved spatial learning, reminiscent of human PWS (149). Sleep is altered in two mouse models: *Htr2a*$^{-/-}$ mice have reduced levels of non-REM sleep, whereas mice with loss of *Gnas* imprinting have reduced total levels of REM sleep (113, 228). Finally, seizures occur in *Sgce*$^{+/-}$ and *Ube3a*$^{-/+}$ mice plus some *Kcnq1* mutants, although the latter may be attributable to postnatal nonimprinted *Kcnq1* functions (80, 96, 246).

Although it is clear that the knockout of imprinted genes can result in behavioral phenotypes, it is far from clear whether these phenotypes follow any particular patterns that support or contradict any theories of imprinting evolution. A key problem is that few mouse models of imprinted genes have been subjected to behavioral tests, and those that have been have not been subjected to the same tests. Additionally, sex and genetic background often vary between behavioral studies, further complicating interpretations because both can influence mouse behavior independently of any mutation (220).

Five imprinted genes affect reproduction: *Peg1*, *Peg3*, *Snord116*, *Dio3*, and *Magel2*. Interestingly, all of these genes are paternally expressed and expressed in the hypothalamus. The reproductive effects of imprinted genes can be divided into behavioral and physical effects. The behavioral reproductive defects associated with imprinted genes are most evident in *Peg1* and *Peg3* mutants. *Peg1*$^{+/-}$ and *Peg3*$^{+/-}$ mice display severe deficits in maternal behaviors that result in a preweaning lethality of 88.7% and 92% of offspring, respectively, independent of pup genotype (41, 117, 121). *Peg1*$^{+/-}$ mothers fail to respond appropriately to newborns and show reduced or absent placentophagia, pup retrieval, and nest-building activities (117). *Peg3*$^{+/-}$ females show similar maternal behavioral deficits and increased maternal aggression (27, 41, 121). Behavioral reproductive defects are not confined to females: *Peg3*$^{+/-}$ and *Magel2*$^{+/-}$ males show an impaired response to female odors, resulting in reduced reproductive fitness (138, 202, 203).

Physical reproductive defects are associated with four imprinted genes. *Peg3*$^{+/-}$, *Snord116*$^{+/-}$, and *Magel2*$^{+/-}$ mice exhibit delayed female puberty (41, 48, 138), which may be a consequence of their underweight phenotype, and *Peg3*$^{+/-}$, *Magel2*$^{+/-}$, and *Dio3*$^{+/-}$ mice exhibit additional physical reproductive defects. *Magel2*$^{+/-}$ males and females both show a premature reproductive decline and early infertility, with females also having abnormal and extended estrus cycles that worsen with age (138). *Peg3*$^{+/-}$ females fail to increase food intake during pregnancy and exhibit a lactation defect likely caused by a reduced number of oxytocin-expressing hypothalamic neurons. Finally, *Dio3*$^{+/-}$ males and females have very low fertility rates owing to central hypothyroidism (89).

The Evolution of Imprinting in Mammals

The severe consequences of loss of imprinting have led to great speculation on the evolutionary impetus behind genomic imprinting. Among vertebrates, imprinting has been demonstrated only in the eutherians and at a handful of loci in marsupials. A key adaptation that is shared by these two mammalian infraclasses is viviparity via a placenta. The restriction of imprinting to the therian mammals indicates that placentation and imprinting arose at similar time points in evolution and may be inextricably linked. Imprinting may therefore have evolved alongside placentation to regulate the supply and demand of nutrients in utero; this theory suggests that genes imprinted in the placenta control the supply of nutrients to the fetus, whereas genes imprinted in the fetus control the demand for those nutrients (167). The marsupial genes known to be regulated via imprinting fit with this theory. *IGF2* controls in utero growth, as demonstrated by the mouse models, whereas the major function of *IGF2R* is to modulate growth in development via IGF2. The retrotransposon-like gene *PEG10* is essential for successful placentation in eutherians. This gene is present and imprinted in the wallaby, but the other genes in the region are not imprinted (200).

Only 5 genes are known to be imprinted in marsupials, compared with more than 80 that have been identified in mice and humans. This suggests that the evolutionary impetus to imprint genes is greater in eutherians than in marsupials. The major difference between marsupial and eutherian reproduction is that marsupials give birth to altricial young that are nursed in a pouch with a complex lactational profile, whereas eutherians allocate proportionally more time to in utero development. This implies that imprinting evolved in parallel with longer-lived, invasive placentas and an increased gestation length. This fits with the large number of imprinted genes that function in placentation or are imprinted solely in the placenta. For example, the retrotransposition event that created the *Rtl1* gene is present in marsupials, but the region is nonfunctional, whereas in eutherians, an imprinted neogene has evolved that is crucial for placentation and parturition in mice (53, 181).

An extension of the placental coevolution theory posits that imprinting arose as a result of coadaptation between mothers and offspring to achieve optimal fitness in the offspring through the coordination of resource allocation prenatally via the placenta and postnatally via lactation, maternal nurturing behavior, and adaptation to independent life (105). Although this model really works only in the context of the *Peg3* gene, the number of imprinted genes with roles in adapting to early postnatal life (e.g., *Gnas* and *Dlk1* clusters in thermogenesis and the PWS cluster in behavior and metabolism) suggests a further evolutionary impetus to imprint genes involved in the highly dependent relationship between mammalian neonates and their mothers. This suggests that genes involved in lactation would also tend to be subjected to imprinted control. Indeed, a lack of *Peg3* in mothers causes failure of milk letdown, and *INS* and *IGF2* were recently shown to be monoallelically expressed in the tammar wallaby mammary gland (195). Unfortunately, imprinted gene expression has not been extensively studied in the mouse mammary gland, so whether any other genes are imprinted in this mammal-specific organ is not known.

CONCLUDING REMARKS

Here, by cataloging the currently available mouse models, we show that imprinted genes are involved in many of the characteristic processes of mammals, including placentation, in utero development, and thermogenesis. Importantly, we also find critical roles for imprinted genes in the successful passage through major life transitions involving the relationship between mother and offspring, such as birth, feeding, and postweaning independence, thus reinforcing their importance in successful reproduction in mammals.

In addition, we find that the majority of models generated to date are targeted gene deletions that do not address the question of how a subtly increased or decreased dosage of imprinted genes affects phenotype. The dosage of some imprinted genes (e.g., *Dlk1* and *Igf2*) is critical for correct development (43, 235), whereas for others (e.g., *Ascl2*), appropriate development relies solely on the presence of the gene (169). The evaluation of further transgene and overexpressing models will help to address the role of genomic imprinting in the modulation of gene dosage.

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