DISEASE MECHANISMS

The role of genomic imprinting in biology and disease: an expanding view

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Abstract | Genomic imprinting is an epigenetic phenomenon that results in monoallelic gene expression according to parental origin. It has long been established that imprinted genes have major effects on development and placental biology before birth. More recently, it has become evident that imprinted genes also have important roles after birth. In this Review, I bring together studies of the effects of imprinted genes from the prenatal period onwards. Recent work on postnatal stages shows that imprinted genes influence an extraordinarily wide-ranging array of biological processes, the effects of which extend into adulthood, and play important parts in common diseases that range from obesity to psychiatric disorders.

Pronuclear
Pertaining to the pronucleus (that is, the haploid nucleus from a male or female gamete).

Genomic imprinting was first recognized in mammals nearly 30 years ago when pronuclear transplantation experiments showed that both maternal and paternal genomes are needed for the normal development of mouse embryos to term[1,2]. In parallel, mouse genetic experiments provided strong evidence that, in some regions of the genome, genes function differently when inherited maternally than when inherited paternally[3], which provided an explanation for earlier genetic findings[4,5]. Uniparental inheritance of these imprinted regions in mice was associated with abnormal phenotypes that affected development, viability, growth and behaviour, which suggested that defects in imprinting could be an important cause of human disease. These implications were subsequently shown to be well founded with the recognition of Prader–Willi syndrome as an imprinted disorder in humans in 1989 (REF. 6) and the identification of the first three imprinted genes in mice in 1991: *Igf2* (which encodes insulin-like growth factor 2), *Igf2r* (which encodes IGF2 receptor) and *H19* (which encodes an imprinted maternally expressed non-coding transcript)[7–9]. So far, various human imprinted syndromes with loss or gain of expression at imprinted genes have been described in addition to Prader–Willi syndrome, including Angelman syndrome, Beckwith–Wiedemann syndrome, pseudohypoparathyroidism types 1a and 1b, and Silver–Russell syndrome (TABLE 1). Furthermore, around 150 imprinted genes have been verified in the mouse (see the MouseBook Imprinting Catalog), and about half of these genes have been found in humans (see the Catalogue of Parent of Origin Effects). Now, high-throughput sequencing strategies are increasingly being used to identify imprinted genes; for example, this technique has recently been used to generate high-resolution maps of parental allele-specific DNA methylation, which may indicate the location of imprinted genes[10].

Genomic imprinting must have arisen with the development of the placenta in mammals possibly >125 million years ago, but the underlying reasons remain obscure. Given that imprinted genes are monoallelically expressed, there are probably strong selective advantages for the evolution and maintenance of this phenomenon. Two widely cited theories — the kinship theory and the coadaptation theory — have implications for both prenatal and postnatal stages (BOX 1). The kinship theory proposes that there is a conflict between the 'interests' of maternal and paternal genes in a fetus or an infant at stages when it is reliant on the mother's resources for nutrition[11]. By contrast, the coadaptation theory proposes that imprinted genes act coadaptively to optimize fetal development as well as maternal provisioning and nurturing[12]. Theories for the evolution of imprinting remain under active debate[13], and it seems probable that no one theory can account for the evolution of genomic imprinting at all imprinted loci.

For more than a decade, it has been established that many imprinted genes play a part in regulating fetal growth. However, it has become increasingly evident that imprinting also has an essential role after birth, and

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doi:10.1038/nrg3766
Published online 24 June 2014

recent studies show that imprinted genes are involved in a wide range of activities that are vital for the survival of neonates, such as feeding, maintenance of body temperature and regulation of metabolism, as well as in infant and maternal behaviours that optimize maternal care. Moreover, imprinting has been implicated in areas as diverse as sleep, and stem cell maintenance and renewal. An increasing amount of evidence indicates that altered expression of imprinted genes is a contributory factor in a wide range of common diseases, for example, intrauterine growth restriction (IUGR), obesity, diabetes mellitus, psychiatric disorders and cancer.

Table 1 | **Human imprinted syndromes and corresponding mouse models**

Human syndrome	Location	Major features	Causes	Mouse models
Pseudo-hypoparathyroidism type 1a (OMIM 103580)	20q13.3	Dysmorphism, obesity, cognitive impairment, end-organ resistance to PTH (which results in hypocalcemia and hyperphosphatemia) and resistance to other hormones[131]	Maternal inactivating mutations of *GNAS* result in 50% expression of non-imprinted *GNAS*, which causes the dysmorphic phenotype AHO; loss of imprinted *GNAS* expression causes obesity and hormone resistance	Maternal knockout models of *Gnas* exon 1 (REFS 54,94) and point mutation in exon 6 (REFS 55,132), which show neonatal lethality, dysmorphism, adult obesity and multiple hormone resistance
Pseudo-hypoparathyroidism type 1b (OMIM 603233)	20q13.3	End-organ resistance to PTH (which results in hypocalcemia and hyperphosphatemia) and occasional resistance to TSH[131]	Lack of maternal *GNAS* methylation imprinting results in loss of expression of imprinted *GNAS*	Knockout model with loss of maternal *Gnas* methylation imprints, which shows neonatal lethality, end-organ resistance to PTH, hypocalcemia and hyperphosphatemia[52,53]
Prader–Willi syndrome (OMIM 176270)	15q11–13	Developmental delay, poor suckling, hyperphagia, obesity, hypogonadism, cognitive impairment and characteristic behavioural profile (which includes temper tantrums and obsessive–compulsive features)[133,134]	Loss of paternal expression of up to 11 genes in 15q11–13 mainly as a result of paternal deletion or MatUPD15; rare imprinting defects	Thirty mouse models[76] that recapitulate some features of Prader–Willi syndrome; defects include neonatal lethality, poor suckling, postnatal growth retardation, adult obesity, subfertility and respiratory defects
Angelman syndrome (OMIM 105830)	15q11–13	Developmental delay, microcephaly, severe intellectual disability, absent or limited speech, gait ataxia, sleep disturbance, characteristic EEG and behavioural profile with happy demeanour[133]	Loss of maternal expression of *UBE3A* mainly due to maternal deletion, *UBE3A* mutation or PatUPD15; rare imprinting defects	Four mouse models comprising maternal knockouts and PatDp(prox7), which show cognitive impairment, motor abnormalities, gait abnormalities and abnormal EEGs[104,135]
Beckwith–Wiedemann syndrome (OMIM 130650)	11p15.5	Prenatal and/or postnatal overgrowth, enlarged tongue (macroglossia), abdominal wall defects (omphalocele), placental overgrowth and predisposition to embryonal tumours (for example, Wilms tumour)	Complex: mostly epigenetic errors that lead to silencing of *CDKN1C* or biallelic expression of *IGF2* and silencing of *H19*; inactivating mutations in *CDKN1C*; PatUPD11	*Igf2* transgenic[50] and *Cdkn1c*-knockout[136] mouse models, which show fetal and neonatal lethality; collectively, they have most features of Beckwith–Wiedemann syndrome
Silver–Russell syndrome (OMIM 180860)	Up to 65% of cases map to 11p15.5, and 10% of cases show MatUPD7	Dysmorphism, IUGR and postnatal growth retardation	Complex: in cases of 11p15.5, hypomethylation of *H19* DMR results in silencing of *IGF2* and biallelic expression of *H19*; *MEST* and *GRB10* are candidates for MatUPD7 cases	No specific mouse model
Transient neonatal diabetes mellitus type 1 (OMIM 601410)	6q24	Neonatal hyperglycaemia and IUGR[137]	Overexpression of *PLAG1* and *HYMAI*	A transgenic model that shows neonatal hyperglycaemia but no growth retardation[138]
MatUPD14 syndrome	14q32	Prenatal and postnatal growth retardation, premature puberty and obesity	Loss of paternal expression of *DLK1* and *RTL1* (REF. 139)	A MatDp(dist12) model, which shows perinatal lethality and prenatal growth retardation[139]
PatUPD14 syndrome	14q32	Dysmorphism, placentomegaly and excessive amniotic fluid (polyhydramnios)	Increased expression of *RTL1* (REF. 140)	A PatDp(dist12) model, which shows prenatal lethality and placentomegaly[139]

AHO, Albright's hereditary osteodystrophy; *CDKN1C*, cyclin-dependent kinase inhibitor 1C; *DLK1*, delta-like 1 homologue; DMR, differentially methylated region; EEG, electroencephalography; *GNAS* encodes the G protein α-subunit $G_s\alpha$; *GRB10*, growth factor receptor-bound protein 10; *H19* encodes an imprinted maternally expressed non-coding transcript; *HYMAI*, hydatidiform mole-associated and imprinted; *IGF2*, insulin-like growth factor 2; IUGR, intrauterine growth restriction; MatDp(dist12), maternal duplication of distal chromosome 12; MatUPD15, maternal uniparental disomy for chromosome 15; *MEST*, mesoderm-specific transcript; OMIM, Online Mendelian Inheritance in Man; Pat, paternal; PatDp(prox7), paternal duplication of proximal chromosome 7; *PLAG1*, pleiomorphic adenoma gene 1; PTH, parathyroid hormone; *RTL1*, retrotransposon-like 1; TSH, thyroid-stimulating hormone; *UBE3A*, ubiquitin protein ligase E3A.

Box 1 | **The origin of imprinting**

Kinship theory

The kinship theory (also known as the parental conflict hypothesis) proposes that there is a conflict between the 'interests' of maternal and paternal genes in a fetus or an infant at stages when it is reliant on the mother's resources for nutrition[11]. The idea behind the kinship theory is that mothers can bear and raise offspring from multiple fathers; whereas all the offspring from one female are equally related to their mother, each father is only related to a subset of these offspring. It is postulated that this difference in relatedness gives rise to different interests of paternal and maternal genomes in the offspring. Hence, for optimal fitness for the father, it is advantageous for paternal genes in the fetus or infant to maximize acquisition of maternal resources, regardless of any detrimental effect to the mother or to other siblings. This is to ensure larger sized offspring, which will have a better chance of surviving to reproduce. By contrast, for optimal fitness for the mother, it is advantageous for maternal genes in the fetus or infant to be sparing in demands for maternal resources, so that the mother has a better chance of continuing to bear further offspring. This theory accords with the finding that many paternally expressed genes enhance growth, whereas many maternally expressed genes repress growth, and this may apply to adult phenotypes such as maternal care and social behaviour[130].

Coadaptation theory

The coadaptation theory proposes that imprinted genes act coadaptively to optimize fetal development as well as maternal provisioning and nurturing[12]. The coadaptation theory is relevant to a subset of mainly paternally expressed genes that are expressed in both the placenta and the hypothalamus region of the brain. During mammalian development, a complex set of interactions occurs between the fetus, the placenta and the mother's hypothalamus that influences fetal growth and brain development, the provision of maternal resources at both prenatal and postnatal stages, and postnatal maternal care. Regulation of these functions by genomic imprinting is likely to be due to parent–infant coadaptation through selection for co-expression of genes in the placenta and the mother's hypothalamus. Paternally expressed 3 (*Peg3*) is a key example of an imprinted gene in which this hypothesis may apply[12].

This Review brings together studies that reveal common detrimental effects of abnormal imprinted gene dosage on phenotype in mice and humans. I start with a brief overview of the organization and control of expression of imprinted genes. Next, I focus on studies that delineate the role of imprinted genes in growth, metabolism, and neurological and behavioural processes, which indicates the expanding part played by genomic imprinting in biology. The role of imprinting in common diseases such as obesity and cancer is also discussed.

Gene organization and expression

Since the discovery of the first imprinted genes, there has been intense interest in uncovering the mechanisms by which their monoallelic expression according to parental origin is initiated and maintained.

Imprinted gene clusters. Mouse studies have shown that >80% of the known imprinted genes are clustered together (see the MouseBook Imprinting Catalog). At least 13 clusters have been identified on 8 chromosomes; these clusters contain 2–15 genes and vary in size from <100 kb to several megabases[14] (FIG. 1). For the most part, orthologous clusters are arranged similarly in mice and humans. All clusters contain both maternally and paternally expressed genes, as well as genes that encode proteins and those that encode non-coding RNAs.

With one exception, all imprinted genes discovered so far show exclusive or predominant expression from either the maternal or the paternal allele. The exception is the growth factor receptor-bound protein 10 (*Grb10*) gene, which encodes an adaptor protein. It is predominantly maternally expressed and acts as a growth inhibitor during embryogenesis[15], but is paternally expressed in the brain and regulates adult social behaviour[16]. This reciprocal pattern of imprinted gene expression is achieved through the use of tissue-specific promoters and the loss of a repressive chromatin mark on the paternal allele in the brain[17]. Thus, through tissue-specific expression, alternative functions of a single gene can be regulated by different parental alleles, which shows the adaptability of imprinting.

Imprinting control regions. Parent-specific expression of multiple genes within a cluster is under the overall control of a *cis*-acting imprinting control region (ICR)[14]. This region shows parental allele-specific DNA methylation and chromatin modifications. DNA methylation of the ICR is acquired in either maternal or paternal germ cells by a mechanism that involves transcription[18,19]. This germline methylation is robust and resistant to the extensive reprogramming of the genome that occurs in the embryo after fertilization, but is erased and reset during germ-cell development. Most ICRs acquire methylation in the female germ line during oogenesis, and these ICRs typically contain the promoters of long non-coding RNA (lncRNA) genes that run antisense to at least one of the protein-coding genes within the cluster[14] (FIG. 1a,b). ICRs that acquire methylation in the male germ line seem to be located in intergenic regions[14] (FIG. 1c,d). Reasons for the difference in position of maternally and paternally methylated ICRs are unknown. An ICR is active when unmethylated and inactive when methylated. The mechanisms by which unmethylated ICRs control imprinted gene expression are only partially understood, and two different models — the lncRNA model and the insulator model — have been described. From studies of four clusters, lncRNAs that arise from a promoter within the ICR have a key role in silencing imprinted genes in *cis*[20–23] (FIG. 1a,b). How lncRNAs silence imprinted genes is an active field of research, and there is evidence for both involvement of the lncRNA product in silencing some imprinted

Gene dosage
The number of expressed copies of a gene in a cell.

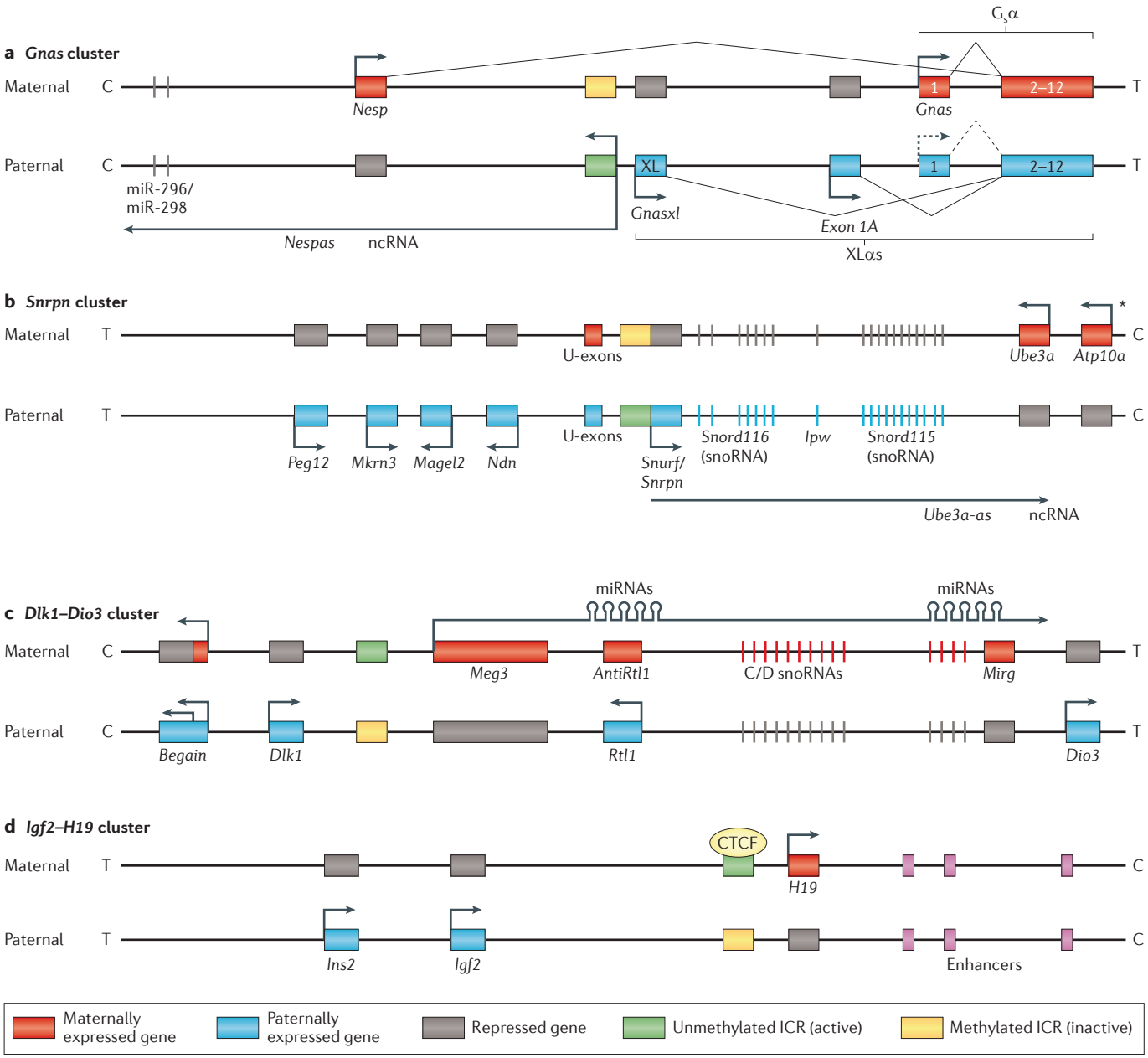

Figure 1 | **Representative mouse imprinted gene clusters. a** | The *Gnas* cluster gives rise to the maternally expressed *Nesp* (which encodes a neuroendocrine secretory protein) and *Gnas* (which encodes the G protein α-subunit G_sα), and the paternally expressed *Gnasxl* (which encodes a variant G_sα subunit known as XLαs), *Nespas* and *Exon 1A* (which are long non-coding RNAs). *Nespas* arises from the active paternal imprinting control region (ICR) and silences *Nesp*. *Gnas* is preferentially maternally expressed in subsets of cells in some tissues but is mainly biallelically expressed. **b** | The small nuclear ribonucleoprotein N (*Snrpn*) cluster gives rise to the maternally expressed ubiquitin protein ligase E3A (*Ube3a*) gene, five paternally expressed protein-coding genes and several paternally expressed non-coding RNAs, including small nucleolar RNAs (snoRNAs) and the long non-coding RNA *Ube3a-as*, which silences *Ube3a*. *Ube3a* is exclusively maternally expressed within the brain and biallelically expressed in other tissues. U-exons are expressed in the oocyte, where they may regulate methylation of the maternal ICR, but are exclusively paternally expressed in neurons. In humans, the *SNRPN* cluster is associated with Prader–Willi syndrome and Angelman syndrome. Imprinting of *Atp10a* (ATPase, class V, type 10A; indicated by an asterisk) is controversial in humans and mice. **c** | The delta-like 1 homologue (*Dlk1*)–*Dio3* (which encodes deiodinase, iodothyronine type III) cluster gives rise to four paternally expressed protein-coding genes and multiple maternally expressed non-coding RNAs, which include microRNAs (miRNAs) and snoRNAs. The ICR is intergenic and active on the maternal allele. **d** | The insulin-like growth factor 2 (*Igf2*)–*H19* (which encodes an imprinted maternally expressed non-coding transcript) cluster comprises the paternally expressed *Igf2* and *Ins2* (which encodes insulin II), as well as the maternally expressed *H19* gene. The ICR is intergenic, and the active maternal ICR binds to CCCTC-binding factor (CTCF) to form an insulator that blocks access of enhancers to *Igf2*, thereby silencing *Igf2*. The clusters and genes are not drawn to scale. *Begain*, brain-enriched guanylate kinase-associated; C, centromere; *Ipw*, imprinted gene in the Prader–Willi syndrome region; *Magel2*, melanoma antigen, family L, 2; *Meg3*, maternally expressed 3 (also known as *Gtl2*); miR, miRNA; *Mirg*, miRNA-containing gene; *Mkrn3*, makorin, ring finger protein, 3; *Ndn*, necdin; *Peg12*, paternally expressed 12 (also known as *Frat3*); *Rtl1*, retrotransposon-like 1; *Snurf*, SNRPN upstream reading frame; T, telomere.

genes[24,25] and transcription of the lncRNA in silencing others[26,27]. The insulator model has been described for the well-studied *Igf2–H19* cluster, where the active ICR forms an insulator by binding to the zinc-finger protein CCCTC-binding factor (CTCF), thereby blocking access of downstream enhancers to *Igf2* promoters and resulting in silencing of *Igf2* (REFS 28,29) (FIG. 1d).

The mechanisms for regulating imprinted gene expression are evidently complex, which is unsurprising given that deviation from monoallelic expression of imprinted genes can result in a range of abnormal phenotypes and disease from prenatal stages to adulthood.

Disrupted imprinting that leads to disease. Both genetic and epigenetic mechanisms can result in perturbed expression of imprinted genes and lead to disease (TABLE 1). One of the genetic mechanisms is mutations that result in either loss of function or deletion of an imprinted gene (or genes). Another mechanism is the occurrence of uniparental disomy (UPD) or uniparental partial disomy, in which both copies of a chromosome or part of a chromosome come from only one parent and none from the other parent. Diseases resulting from UPD can be due to loss or gain of imprinted gene expression. For example, Angelman syndrome can result from paternal UPD for chromosome 15 (PatUPD15), which is due to loss of maternal expression of the ubiquitin protein ligase E3A (*UBE3A*) gene (TABLE 1), whereas transient neonatal diabetes mellitus type 1 can arise from UPD for chromosome 6 (PatUPD6) owing to overexpression of paternally expressed pleiomorphic adenoma gene 1 (*PLAG1*) and the hydatidiform mole associated and imprinted (*HYMAI*) gene (TABLE 1). Epigenetic mechanisms include alteration in DNA methylation marks within an imprinted cluster, which results in altered expressed dosage of one or more genes; this mechanism is, for example, a major cause of Beckwith–Wiedemann syndrome and pseudohypoparathyroidism type 1b (TABLE 1).

Survival and growth

Investigations of mouse mutants have been important for unravelling the roles of imprinted genes and for elucidating some of the pathophysiological mechanisms involved in various human imprinted syndromes. These studies have shown that imprinted genes have major effects on prenatal and postnatal development, survival and growth (TABLE 2).

Prenatal viability and growth. In general, paternally expressed imprinted genes enhance fetal growth, whereas those that are maternally expressed restrict it[30], which accords well with the predictions of the kinship theory (BOX 1). Some genes show imprinted expression only in the placenta, although a recent re-analysis of placenta-specific imprinted gene expression by RNA sequencing indicates that the number of such genes may have been overestimated owing to contamination of samples with maternal cells[31]. However, placenta-specific achaete–scute complex homologue 2 (*Ascl2*; also known as *Mash2*) and paternally expressed 10 (*Peg10*) are essential for the formation of a viable placenta and, in their absence, embryonic lethality ensues[32–34]. Other imprinted genes have the potential for regulating fetal growth by controlling the nutrient supply. For example, disrupted expression of the placenta-specific *Igf2 P0* transcript results in a smaller placenta, which leads to fetal growth retardation[35]. Others such as solute carrier family 22 member 2 (*Slc22a2*) and *Slc22a3* encode transporters involved in transplacental solute exchange[36,37].

Imprinted genes that are expressed in both the fetus and the placenta can potentially affect fetal growth through effects on fetal demand for or placental supply of nutrients. Disrupted imprinting of these genes can result in fetal and placental growth enhancement or retardation[38]. Many of these genes are expressed in developing fetal tissues that are important in postnatal metabolic regulation and are downregulated after birth[38]. Furthermore, various imprinted genes that are expressed in both the placenta and the embryo seem to belong to a network that co-regulates embryonic and fetal growth and differentiation[39].

Although expression levels of imprinted genes can influence birth weights within the normal range[40], their disrupted expression can have severe consequences for human fetal growth. IUGR is a serious but not uncommon condition with increased risk of perinatal mortality and morbidity, as well as of developing cardiovascular and metabolic diseases in later life[41]. IUGR is a defining feature of the rare imprinted disorder Silver–Russell syndrome (TABLE 1) and, in a subset of cases, can be associated with reduced expression of the growth enhancer *IGF2* (REF. 42). IUGR is also associated with loss of expression of *GNASXL*[43] — a paternally expressed transcript at the *GNAS* cluster (FIG. 1a). However, most cases of IUGR are not associated with known human imprinted syndromes; for many of these, the cause is unclear, although some are due to fetal chromosomal abnormalities or to maternally transmitted infection. Imprinted genes are also implicated in these non-syndromic cases, as alterations in the expression of some imprinted genes (*PEG10, PEG3, PHLDA2* (pleckstrin homology-like domain, family A, member 2) and *PLAG1*) have been consistently found in gene expression studies in placentas in non-syndromic IUGR[42].

Postnatal viability and growth. The newborn mammal must overcome various challenges that are associated with an independent life, including the maintenance of body temperature, acquisition of food and regulation of its own metabolism. Impairment in one or more of these activities occurs with loss of expression of various imprinted genes (TABLE 2) and is probably a major contributor to neonatal death[44–47]. For most cases of disrupted imprinted gene expression, the cause of death cannot be established, but breathing difficulties, lung abnormalities and heart defects may account for some neonatal lethalities[15,48–50]. In mice, the neonatal lethality that occurs with loss of *Gnasxl* is probably due to a combination of poor or absent suckling (see below) and flawed metabolism that results in defective glucose counter-regulation[46]. Defective glucose counter-regulation is also found with overexpression of *Gnasxl*, and may also contribute to the failure to thrive and to perinatal lethality that ensues in these animals[51–53].

CCCTC-binding factor
(CTCF). A highly conserved zinc-finger protein that influences chromatin organization and architecture; it is implicated in diverse regulatory functions, including transcriptional activation, repression and insulation.

Epigenetic
Pertaining to heritable but potentially reversible changes in gene expression that are caused by mechanisms other than changes in the underlying DNA sequence.

Uniparental disomy
(UPD). A cellular or organismal phenomenon in which both chromosome homologues are derived from one parent and none from the other parent. It can be the result of fertilization that involves a disomic gamete and a gamete that is nullisomic for the homologue.

Table 2 | **Imprinted genes associated with adult obesity or leanness**

Gene and imprinted cluster	Expressed allele	Gene expression in mutant mice	Prenatal to weaning viability and birthweight	Suckling ability	Birth to weaning growth and metabolic phenotype	Adult feeding	Adult metabolic phenotypes	Refs
Gnas distal chromosome 2	Mat	Loss	• Viable to birth but most die neonatally • Increased birth weight	NR	• Growth retardation followed by catch-up growth • Euglycaemia	Hypophagia	Insulin resistance, hyperglycaemia, obesity, small body size, hypometabolism, glucose intolerance and hyperinsulinemia	48,54, 55,63, 69,70, 141,142
	Mat	Increase	• Fully viable • Decreased birth weight	Unimpaired	• Growth retardation followed by catch-up growth • Euglycaemia	Unaffected	Slightly smaller body size, proportional decrease in lean and fat mass, and euglycaemia	51,62, 143
Gnasxl distal chromosome 2	Pat	Loss	• Viable to birth but most die neonatally • Decreased birth weight	Grossly impaired	• Growth retardation followed by catch-up growth • Glucose counter-regulation defect	Hyperphagia	Leanness, small body size, hypermetabolism, hypolipidemia, increased glucose tolerance and increased insulin sensitivity	46,55, 82,83, 143
	Pat	Increase	• Viable to birth but all die by ten days • Decreased birth weight	Impaired	• Growth retardation • Possibly glucose counter-regulation defect	NA	NA	51–53
Mest proximal chromosome 6	Pat	Loss	• Viable to birth with much preweaning lethality • Decreased birth weight	NR	Growth retardation followed by catch-up growth	NR	Small body size	45
	Pat	Increase	NR	NR	NR	NR	Obesity	75
Peg3 proximal chromosome 7	Pat	Loss	• Some neonatal lethality • Decreased birth weight	Impaired	Growth retardation	Hypophagia	Obesity, hypometabolism and euglycaemia	44,59
Ndn central chromosome 7	Pat	Loss	Viable to birth with some neonatal lethality	NR	NR	Unaffected	Obesity	49,73, 144
Magel2 central chromosome 7	Pat	Loss	Midgestation loss and much neonatal lethality	Impaired	Growth retardation followed by catch-up growth	Hypophagia	Increased susceptibility to obesity and increased insulin sensitivity	47,77, 78,99, 145
Snord116 central chromosome 7	Pat	Loss	• Viable to birth; weaning viability not recorded • Normal birth weight	Unimpaired	Growth retardation with slight catch-up post-weaning	Late-onset hyperphagia	Leanness, small body size, increased glucose tolerance and increased insulin sensitivity	79,146
Igf2 distal chromosome 7	Pat	Brain-specific loss	NR	NR	NR	Hypophagia	Obesity	147
	Pat	Increase	NR	NR	NR	NR	Leanness	121

Table 2 (cont.) | **Imprinted genes associated with adult obesity or leanness**

Gene and imprinted cluster	Expressed allele	Gene expression in mutant mice	Prenatal to weaning viability and birthweight	Suckling ability	Birth to weaning growth and metabolic phenotype	Adult feeding	Adult metabolic phenotypes	Refs
Rasgrf1 chromosome 9	Pat	Loss	• Viable to birth; weaning viability not recorded • Normal birth weight	NR	Growth retardation followed by catch-up growth	Unaffected	Leanness, increased lipid catabolism, glucose intolerance and hypoinsulinemia	80,148
Grb10 proximal chromosome 11	Mat	Loss	• Some perinatal lethality • Increased birth weight	NR	Larger at birth and weaning	Unaffected	Leanness, increased glucose tolerance and increased insulin sensitivity	15,149
	Mat	Increase	• Viable • Decreased birth weight	NR	Smaller at birth and weaning	NR	Small body size, insulin resistance and impaired glucose tolerance	150–152
Dlk1 chromosome 12	Pat	Loss	• Viable to birth with much perinatal lethality • Decreased birth weight	NR	Small at birth and weaning	NR	Obesity	74
	Pat	Increase	• Viable to birth with some neonatal lethality • Increased birth weight	Impaired	• Growth retardation followed by catch-up growth by adulthood • Euglycaemia	NR	Leanness, insulin resistance and impaired glucose tolerance	81,92
Dio3 chromosome 12	Pat	Loss	Some lethality around or before birth	NR	Growth retardation at weaning	NR	Leanness and glucose intolerance	88,153

Dio3, deiodinase, iodothyronine type III; *Dlk1*, delta-like 1 homologue; *Gnas* encodes the G protein α-subunit $G_s\alpha$; *Gnasxl* encodes XLαs; *Grb10*, growth factor receptor-bound protein 10; *Igf2*, insulin-like growth factor 2; *Magel2*, melanoma antigen, family L, 2; Mat, maternal; *Mest*, mesoderm-specific transcript; NA, not applicable; *Ndn*, necdin; NR, not recorded; Pat, paternal; *Peg3*, paternally expressed 3; *Rasgrf1*, RAS protein-specific guanine nucleotide-releasing factor 1; *Snord116*, small nucleolar RNA, C/D box 116 cluster.

Animals with aberrant imprinted gene expression that survive past the first few days of birth show a broadly similar postnatal growth trajectory (TABLE 2). This group includes mutants of maternally and paternally expressed genes, as well as loss and gain of expression mutants[54–56]. Growth retardation commences prenatally or within a few days of birth and becomes more severe over the first 2–3 weeks of life. This is followed by a period of catch-up growth to a greater or lesser degree and normal viability after weaning. Thus, mouse studies show that aberrant dosage of both maternally and paternally expressed imprinted genes almost invariably results in failure to thrive in the early weeks of life. Failure to thrive in the early months of life can also be seen in human imprinted disorders, such as in Prader–Willi syndrome and Silver–Russell syndrome[57,58].

Metabolism

Imprinted genes are emerging as key regulators of mammalian metabolic processes from infancy to adulthood (FIG. 2a; TABLE 2).

Imprinting and thermogenesis. Maintenance of body temperature in the cold is vital for the survival of newborn mammals and is particularly challenging for mammals with young that are born naked. Mice cannot reliably regulate their own body temperature until one week after birth[59]; until then, young mice depend on the mother to provide body heat and to keep the litter together in the nest as a way of conserving heat. Temperature regulation in neonates relies on the process of non-shivering thermogenesis (NST) in brown adipose tissue (BAT), which has evolved in mammals to prevent hypothermia[60]. In BAT, chemical energy is dissipated in the form of heat through the actions of mitochondrial brown fat uncoupling protein 1 (UCP1)[60]. BAT is present in the fetus and undergoes major recruitment in the first few days after birth[60].

Several imprinted genes affect BAT and potentially NST. Two of these — *Gnas* (which encodes the G protein α-subunit $G_s\alpha$) and *Gnasxl* (which gives rise to a variant $G_s\alpha$ subunit known as XLαs) — lie within the *Gnas* cluster (FIG. 1a). *Gnas* is mainly biallelically expressed but shows preferential maternal expression in a few tissues,

whereas *Gnasxl* is exclusively paternally expressed in a tissue-specific manner[46,61]. Maternally expressed $G_s\alpha$ and paternally expressed XLαs act antagonistically and have opposite effects on BAT metabolism from birth through sympathetic nervous system (SNS) signalling and/or directly through β-adrenergic receptor activity of BAT, with $G_s\alpha$ promoting and XLαs repressing heat production[46,62,63] (FIG. 2). Further effects on thermoregulation are that $G_s\alpha$ increases and XLαs reduces adult core body temperature[64,65].

The paternally expressed genes necdin (*Ndn*) and delta-like 1 homologue (*Dlk1*) (FIG. 1b,c) inhibit differentiation of brown adipocytes[66], although a role for *Dlk1* in BAT differentiation before weaning has not been established[67]. However, *Dlk1* and the paternally expressed imprinted gene *Dio3* (which encodes deiodinase, iodothyronine type III) (FIG. 1c) are required for a recently recognized second phase of BAT recruitment two weeks after birth; this stage is necessary for thermoregulation when mice start to leave the nest and become independent of the mother[67]. Overexpression of *Dlk1* impairs BAT differentiation and β-adrenergic signalling, and overexpression of both *Dlk1* and *Dio3* results in diminished UCP1 expression. The result is that thermogenesis is impaired and body temperature cannot be maintained. Thus, the combined overexpression of *Dlk1* and *Dio3* results in BAT that is defective in its response to cold.

Overall, the actions of *Gnas, Gnasxl, Ndn, Dlk1* and *Dio3* on thermogenesis are broadly consistent with the prediction of the kinship theory of imprinting that paternally expressed genes act to reduce thermogenic output (as they favour investment in growth) and that maternally expressed genes act to increase thermogenic output[68] (BOX 1).

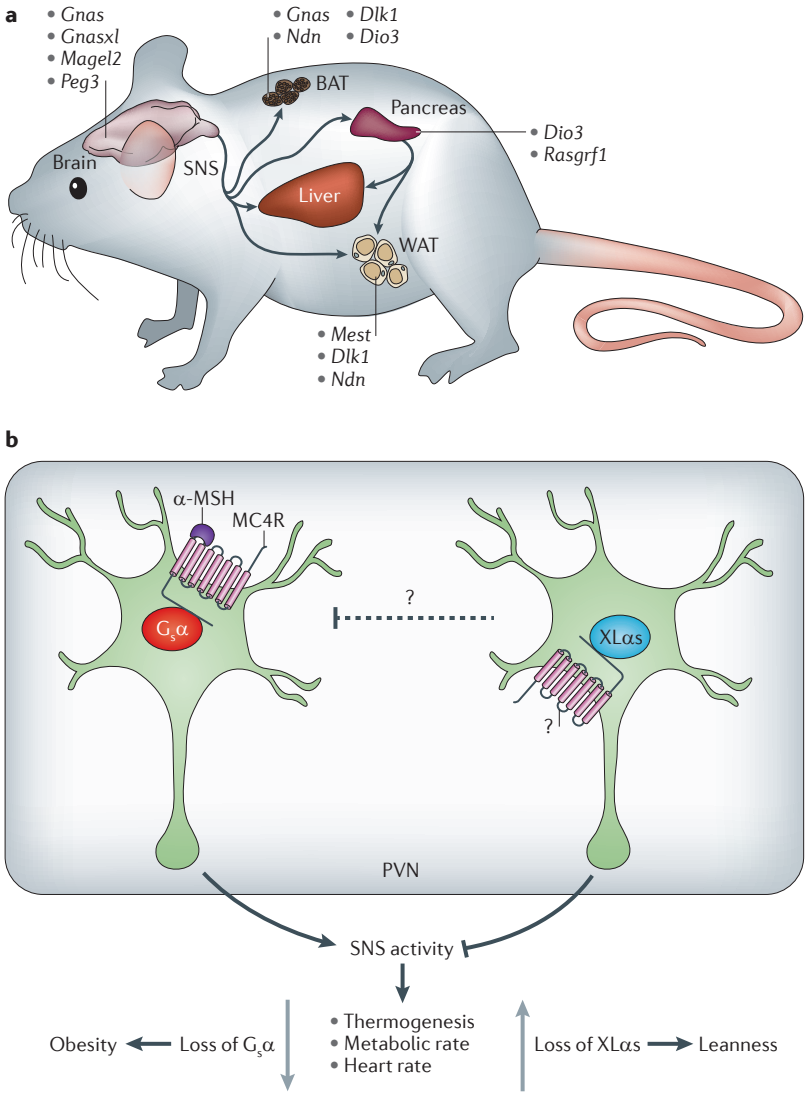

Figure 2 | **Imprinted genes affect metabolism.** **a** | Imprinted genes can act in a range of tissues and by various processes to affect metabolism. Selected examples described in the text are illustrated here. **b** | Maternally expressed $G_s\alpha$ (encoded by *Gnas*) and paternally expressed XLαs (encoded by *Gnasxl*) act antagonistically. Melanocortins signal through maternally expressed $G_s\alpha$-coupled melanocortin receptor 4 (MC4R) at multiple sites in the central nervous system (CNS), including neurons of the paraventricular nucleus (PVN) of the hypothalamus to increase sympathetic nervous system (SNS) outflow, energy expenditure and thermogenesis. Paternally expressed XLαs is also expressed in neurons that regulate SNS outflow but suppresses SNS activity, thereby antagonizing the action of $G_s\alpha$. XLαs may also signal through MC4R receptors, but this has not yet been established. It is not known whether the antagonization of $G_s\alpha$ occurs by acting at CNS sites that are distinct from $G_s\alpha$ or whether XLαs directly inhibits $G_s\alpha$ signalling. α-MSH, α-melanocyte-stimulating hormone; BAT, brown adipose tissue; *Dio3*, deiodinase, iodothyronine type III; *Dlk1*, delta-like 1 homologue; *Magel2*, melanoma antigen, family L, 2; *Mest*, mesoderm-specific transcript; *Ndn*, necdin; *Peg3*, paternally expressed 3; *Rasgrf1*, RAS protein-specific guanine nucleotide-releasing factor 1; WAT, white adipose tissue.

Imprinting and adult adiposity. Aberrant expression of either maternally or paternally expressed imprinted genes can affect body weight and metabolism in adults. The obesity that may result is generally not associated with hyperphagia (TABLE 2). For example, maternal inheritance of inactivating mutations in the human *GNAS* gene — the underlying cause of pseudohypoparathyroidism type 1a (TABLE 1) — or its mouse orthologue *Gnas* results in severe obesity and symptoms of type 2 diabetes mellitus, such as hyperglycaemia, glucose intolerance, hyperinsulinaemia and insulin resistance[54,55,69,70]. Mutant mouse studies have shown that the molecular abnormality that underlies the obesity associated with disruption in imprinted *Gnas* expression seems to be a defect in $G_s\alpha$-dependent melanocortin receptor 4 (MC4R) signalling — which is known to regulate SNS activity, glucose metabolism and insulin sensitivity[69,71,72] — in as yet unidentified regions of the central nervous system and in the paraventricular nucleus of the hypothalamus[69] (FIG. 2b). There is no evidence of increased food intake in mice or humans with loss of imprinted *Gnas* or *GNAS* expression, which implies that the obesity resulting from loss of imprinted expression of this gene is due to reduction in energy expenditure as a result of decreased SNS activity.

Reduced energy expenditure can also account for the obesity in mice that occurs with loss of expression of the paternally expressed gene *Peg3* (REFS 56,59), whereas either loss of *Dlk1* and *Ndn* or overexpression of mesoderm-specific transcript (*Mest*; also known as *Peg1*) results in obesity owing to defects in adipogenesis[73–75].

The effects on adipogenesis vary depending on the affected gene and its protein function; for example, in healthy states, *Dlk1* inhibits the differentiation of preadipocytes into mature adipocytes[56], whereas *Mest* and *Ndn* regulate adipocyte size[75] and number, respectively[73]. Specifically, *Ndn* strongly suppresses cell proliferation, and loss of *Ndn* expression increases adiposity owing to hyperplasia of white adipose tissue cells[73]. *NDN* is implicated in Prader–Willi syndrome which, similar to pseudohypoparathyroidism type 1a, is characterized by severe obesity (TABLE 1). Although infants with Prader–Willi syndrome feed poorly, by about two years of age they develop hyperphagia and become severely obese; however, the pathophysiology of the hyperphagia and obesity is unclear. Prader–Willi syndrome is due to loss of expression of up to 11 contiguous genes in chromosome 15q11–13 (REF. 76). Two of the genes within the orthologous region in the mouse — *Magel2* (melanoma antigen, family L, 2) and *Ndn* — have a role in obesity (FIG. 1b). *Magel2*-null mice show increased susceptibility to obesity in adulthood[77,78] and are defective in their ability to sense leptin, which is a hormone secreted from adipocytes that regulates feeding behaviour and energy expenditure. Thus, in *Magel2*-null mice, proopiomelanocortin neurons in the hypothalamus are unresponsive to leptin, which leads to defective MC4R signalling[78]. In patients with Prader–Willi syndrome, loss of *MAGEL2* might result in defective leptin sensing, leading to increased appetite and weight gain. Nevertheless, other genes in the Prader–Willi domain, including *NDN,* are likely to have major roles in the development of obesity in patients with this disorder.

Disrupted expression of various imprinted genes in the mouse is associated with a lean adult phenotype in the absence of decreased food intake[55,79–82] (TABLE 2). Indeed, food intake in adults can be increased[79,82], and there may be resistance to weight gain on a high-fat diet[55,79]. Increased energy expenditure can account for the lean phenotype found with loss of XLαs[82,83]. Although $G_s\alpha$ and XLαs share biochemical properties, they exert opposite physiological effects after birth (FIG. 2b). As mentioned above, loss of $G_s\alpha$ or XLαs results in decreased or increased SNS activity, respectively[84]. The normal role of XLαs is to downregulate sympathetic output from the central nervous system, but it remains to be determined whether this occurs by acting at central sites that are distinct from $G_s\alpha$ or whether XLαs directly inhibits $G_s\alpha$ signalling. Glucose metabolism is also disrupted with loss of XLαs[82].

Further work has shown reduced activity in the nutrient-sensing mammalian target of rapamycin 1 (mTOR1)–ribosomal S6 kinase (S6K) signalling pathway in the hypothalamus of *Gnasxl*-deficient mice, which accords with their metabolic status[83]. The mTOR1–S6K pathway has a key role in coordinating nutrient sensing and metabolism in peripheral tissues and the hypothalamus[85]. Furthermore, dysfunction of the mTOR1–SK6 signalling pathway is also implicated in the lean phenotype that results from loss of maternally expressed *Grb10* (REFS 86,87). Pancreatic defects can account for the lean phenotype that is found with the loss of either *Dio3* or the RAS protein-specific guanine nucleotide-releasing factor 1 (*Rasgrf1*) gene[80,88]. Loss of *Dio3* expression increases thyroid hormone signalling in the developing pancreas, which results in impaired islet function[88]. When *Rasgrf1* is not expressed, cell proliferation in the maturing postnatal pancreas is impaired[80]. Insulin secretion is reduced as a result of the pancreatic defects mediated by both *Dio3* and *Rasgrf1*, which leads to impaired glucose tolerance and reduced adiposity due to increased lipid catabolism[80,88].

Thus, imprinted genes control body weight and metabolism by acting on multiple tissues and pathways. The findings that loss of both maternally and paternally expressed genes can result in adult obesity or leanness are contradictory to the kinship theory (BOX 1). However, obesity or leanness in adulthood may be a consequence of events that begin during fetal or infant development. In humans, growth impairment during fetal and early postnatal stages gives rise to an increased risk of developing the metabolic syndrome in later life, which is attributable to the flawed setting of metabolic responses in early life — a phenomenon known as metabolic programming[89]. For example, the adult obesity that occurs with loss of expression of imprinted genes may be secondary to growth retardation that occurs *in utero* and in infancy, and major selective pressures act at the early stages of life. Nevertheless, definitive evidence that imprinted genes are targets of metabolic programming is lacking.

Neurological and behavioural effects

Many imprinted genes are expressed in the brain[90] and affect not only metabolism but also behaviour after birth (FIG. 3).

Imprinting and neonatal feeding. Before birth, mammals acquire nutrients from the mother via the placenta but, after birth, the neonate must quickly adapt to oral feeding in order to survive. Disrupted expression of several paternally expressed genes is associated with impaired suckling in the mouse[3,46,51–53,83,91,92] (TABLE 2); however, with the exception of *Magel2*, detailed investigations of the nature of the feeding defects have not been undertaken.

Suckling is a complex process that involves searching for and latching onto the mother's nipples, having a rhythmic suckling reflex and being able to swallow. Feeding problems from birth are characteristic of infants with Prader–Willi syndrome, in whom muscle tone is poor and suckling activity is weak or absent. These individuals also show failure to thrive, slow weight gain and growth retardation. Work in the mouse implicates loss of expression of *Magel2* in the infant feeding deficit[47]. *Magel2* has a key role in the initiation of infant feeding in the mouse. *Magel2*-deficient newborns either fail to attach to the nipple and suckle or show delayed attachment with weak suckling, which results in considerable neonatal lethality. They have low levels of mature oxytocin in the hypothalamus, which indicates an as yet undefined role for *Magel2* in oxytocin maturation. Interestingly, this phenotype can be rescued by a single injection of oxytocin shortly after birth[47], which suggests that administration of oxytocin could

Metabolic syndrome
A group of metabolic conditions that occur together and that increase the risk of developing cardiovascular disease, stroke and diabetes.

Metabolic programming
The response to adverse conditions during early development that results in resetting of metabolic responses and predisposition to metabolic syndrome in adulthood.

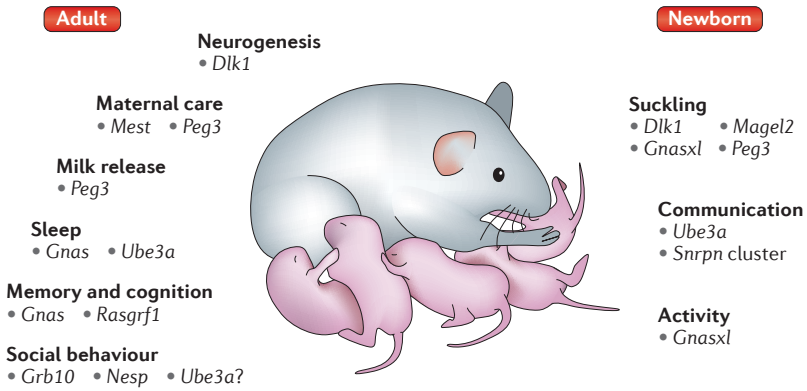

Figure 3 | **Imprinted genes regulate behaviours.** In adults, imprinted genes affect milk release, maternal care of offspring, sleep and other behaviours. In infants, imprinted genes act on feeding behaviour by regulating nipple attachment, suckling ability, locomotor activity and communication with the mother. *Dlk1*, delta-like 1 homologue; *Gnas* encodes the G protein α-subunit $G_s\alpha$; *Gnasxl* encodes a variant $G_s\alpha$ subunit known as XLαs; *Grb10*, growth factor receptor-bound protein 10; *Magel2*, melanoma antigen, family L, 2; *Mest*, mesoderm-specific transcript; *Nesp* encodes a neuroendocrine secretory protein; *Peg3*, paternally expressed 3; *Rasgrf1*, RAS protein-specific guanine nucleotide-releasing factor 1; *Snrpn*, small nuclear ribonucleoprotein N; *Ube3a*, ubiquitin protein ligase E3A.

be a therapeutic option for neonates with Prader–Willi syndrome or other early-onset feeding disorders.

Both *Dlk1* and *Gnasxl* are highly expressed in tissues that are relevant for suckling; *Dlk1* is expressed in the tongue and lips[92], and *Gnasxl* is expressed in the tongue and the facial nucleus in the brain that innervates jaw muscles[46,83]. *Gnasxl* is transiently expressed in neonatal muscle[83]. Thus, the severe feeding deficit and inertia that occur with loss of *Gnasxl* expression may be due to muscle dysfunction, which results in the inability of the infant mouse to seek out the mother's nipple and to suckle. Conversely, overexpression of *Gnasxl* also results in a feeding deficit[52], albeit of much reduced severity compared with that seen with loss of *Gnasxl*. Overexpression of *Gnasxl* results in hyperactivity within a day of birth[3,51,53], which could conceivably impair the ability of the infant mouse to latch onto and stay attached to the nipple, thereby limiting the acquisition of milk. Loss of expression of the orthologous *GNASXL* gene in humans may also account for some cases of intractable feeding difficulties in infancy[93].

Maternal behaviour also has a role in infant feeding[44,45,94]. Loss of expression of the imprinted genes *Mest* or *Peg3* (REFS 44,45) results in mothers having scant care for their offspring. This is compounded in *Peg3*-null mothers by impairment of milk release, which leads to poor or even absent nutrient supply to the infant. Furthermore, infant mice can emit ultrasonic vocalizations (USVs), which are thought to be distress calls to attract the mother and elicit maternal care. Both loss of expression of maternally expressed *Ube3a* and overexpression of paternally expressed genes in the small nuclear ribonucleoprotein N (*Snrpn*) cluster (FIG. 1b) result in increased USVs and increased demand for maternal resources[95,96].

Taken together, the findings on the roles of imprinted genes (such as *Peg3*) on maternal care and infant behaviours have been interpreted to fit with expectations of both the kinship theory and the coadaptation theory[12,97] (BOX 1).

Imprinting and sleep. Sleep has a role in infant feeding and growth; many mammals that are helpless at birth suckle while asleep, and mothers sleep while nursing their young. Rapid eye movement (REM) sleep is the predominant form of sleep in pre-weaning mice and promotes suckling, whereas non-REM (NREM) sleep is the predominant form of sleep in the mother and promotes milk ejection[98]. Growing evidence indicates that deficits in imprinted gene expression can result in abnormal circadian rhythms, and that abnormalities in REM and NREM sleep give rise to sleep disorders[91,99–101]. Sleep problems are features of both Prader–Willi syndrome and Angelman syndrome. *Ube3a*-deficient mice are characterized by reduced NREM sleep and poor REM sleep[91], and these animals model the sleep reduction seen in patients with Angelman syndrome.

The *Gnas* locus also affects sleep, as loss of imprinting of *Gnas* and the consequent increase in expression of $G_s\alpha$ lead to inhibition of REM sleep and enhancement of NREM sleep[64]. Body temperature is known to affect sleep[102]; hence, the sleep abnormalities can be attributed to the increased body temperature that occurs with loss of imprinting of maternally expressed *Gnas*[64]. Sleep-dependent adult behaviours are also affected by loss of imprinting of *Gnas*; REM-linked memory consolidation of fear conditioning is impaired, whereas NREM-linked cognition is enhanced in these animals. Interestingly, the same behavioural defect — failure to consolidate context-dependent fear conditioning — has also been found with loss of expression of paternally expressed *Rasgrf1* (REF. 103). Nevertheless, it remains unclear how these behaviours fit with either the kinship or the coadaptation hypotheses for the evolution of genomic imprinting.

It would be of interest to test other imprinted genes that are known to affect thermogenesis and/or suckling (such as *Dlk1*, *Dio3*, *Ndn*, *Gnasxl* and *Peg3*) for effects on sleep to ascertain whether there is a common link between sleep, thermogenesis and suckling. *Gnasxl* is a good candidate given that it is highly expressed in brain areas that are important in regulating sleep and wakefulness, such as the locus coeruleus[46].

Adult social behaviour and psychiatric disorders. In adults, imprinted genes in the brain influence behaviours such as maternal care, sex, feeding, emotionality and cognition. Deficits in social cognition are well-recognized features of Angelman and Prader–Willi syndromes: patients with Angelman syndrome have a happy disposition and can show autistic behaviours[104], whereas patients with Prader–Willi syndrome can show mood instability, have temper tantrums and be susceptible to psychotic episodes[105]. Patients with Prader–Willi syndrome as a result of either maternal UPD for chromosome 15 (MatUPD15) (TABLE 1) or ICR mutations are far more prone to psychotic episodes than those

Rapid eye movement
(REM). A phase of sleep that is characterized by rapid and random movement of the eyes, low muscle tone and a rapid low-voltage electro-encephalogram. It is associated with dreaming, and many brain areas are active during REM sleep.

Non-REM
(NREM). A phase of sleep that is characterized by slow or no eye movement. Non-REM sleep is divided into three stages, which have distinct brain wave patterns, and deep or slow wave sleep occurs in stage three. There is relatively little dreaming in non-REM sleep.

Fear conditioning
A behavioural paradigm in which organisms learn to predict adverse events.

with paternal deletions of 15q11–13 (REF. 105). Unlike the latter, patients with MatUPD15 or ICR mutations are predicted to have increased expressed gene dosage in the 15q11–13 region. It has been suggested that this increased gene dosage leads to psychosis. Furthermore, increased dosage of genes in the 15q11–13 region is associated with non-syndromic cases of psychosis in carriers of a maternally derived copy-number variant that spans the locus[105]. Of these genes, the major candidate *UBE3A*, which shows imprinted expression only in neurons, is known to influence behaviour and may affect two neurotransmitter systems in the brain: the inhibitory γ-aminobutyric acid (GABA) system and the excitatory glutamatergic system[90]. How overexpression of *UBE3A* could lead to psychosis remains to be established[90,105,106]. In addition, several psychiatric disorders with social impairments, including autism spectrum disorders, have shown linkage to imprinted regions or cytogenetic abnormalities that are predicted to disrupt imprinted gene expression[90,107,108].

In contrast to the findings in human imprinted disorders, there was incomplete evidence in the mouse that imprinted genes affect social behaviour until a study in 2011 indicated that paternally expressed *Grb10* has a function in mouse social behaviour[16]. Specifically, observations of facial barbering and results from the tube test showed that adult mice deficient in *Grb10* expression in the brain are socially dominant over wild-type animals, which implies that the normal role of paternally expressed *Grb10* is to suppress social dominance. This study is of considerable interest, as *Grb10* is the first mouse gene that has been identified to influence a specific adult behaviour outside the realm of parental care; there is incentive to investigate the phenotype in more detail and to test other imprinted genes for specific roles in adult social behaviours. Most animal societies are organized according to a dominance hierarchy, which seems to be essential for well-being[109]. Social dominance has been correlated with predisposition to take risks[110], and it has been speculated that paternally expressed *Grb10* may be involved in risk-aversive behaviour[111]. Interestingly, lack of expression of the maternally expressed gene *Nesp* (which is expressed from the *Gnas* locus and encodes a neuroendocrine secretory protein) increases reluctance to explore novel environments[112], and *Nesp* may therefore increase risk tolerance. More work is required to ascertain whether *Grb10* and *Nesp* genuinely influence risk taking; however, as this characteristic is prominent in several psychiatric disorders, it is possible that imprinted genes have a role in these diseases. Although the kinship theory can be applied to adult social interactions in groups in which the two parental alleles are unequally represented[113,114], it is unclear how the findings with *Grb10* and *Nesp* on adult social behaviour fit with current hypotheses for the evolution of imprinting[16].

Imprinting and adult neurogenesis. Some imprinted genes have essential roles in adult stem cell maintenance and renewal[115–119]. Adult stem cells renew somatic tissue, are few in number and occupy specific niches within tissues. In the adult brain, neurogenesis occurs in two discrete regions — the subventricular zone and the subgranular zone — and neural stem cells continually give rise to adult neurons[116]. A key player in this process is the paternally expressed gene *Dlk1*. Of note, normal neurogenesis requires loss of imprinting of *Dlk1* but not its imprinted expression in neural stem cells in the neurogenic niche from early postnatal stages[116]. Imprinted expression of *Dlk1* is maintained in mature neurons in non-neurogenic regions[116]. The loss of imprinting of *Dlk1* is brought about by the postnatal acquisition of DNA methylation at the maternal ICR (FIG. 1c). The requirement for increased *Dlk1* dosage in neurogenesis is not yet understood. The results are of considerable interest, as they reveal new roles for imprinting and indicate that local loss of imprinting can be a way of regulating development. Furthermore, these findings imply that the imprinted status of a gene can be adapted to local conditions and that its alteration can be a dynamic method of changing dosage and expression levels within specific environments, such as the neurogenic niche. By contrast, recent work has shown that imprinting at the *Igf2* cluster (FIG. 1d), but not the loss of this cluster, is required for the maintenance and functioning of adult haematopoietic stem cells[118]. However, both studies imply that imprinted gene dosage can be crucial for the maintenance of adult stem cell populations.

Imprinting and cancer

Given the important role of imprinted genes in growth and development, it is unsurprising that aberrant expression of imprinted genes is associated with cancer (reviewed in REFS 120,121). Global loss of imprinting is known to be associated with increased tumorigenesis in mice[122]. In humans, dysregulated imprinting that results from somatic events (or from germline events in known imprinted syndromes) and the global imprinting disorder complete hydatidiform mole are associated with increased cancer risk[120,121]. The risk of developing tumours, especially embryonal tumours such as Wilms tumour and rhabdomyosarcomas, is increased in patients with Beckwith–Wiedemann syndrome[120,121]. The causes of this syndrome are complex (TABLE 1) and comprise mutations, epimutations or uniparental inheritance of imprinted genes in the 11p15.5 imprinted region. These can result in loss of imprinting and overexpression of the potent growth factor gene *IGF2*, as well as loss of expression of the tumour suppressor genes *H19* and cyclin-dependent kinase inhibitor 1C (*CDKN1C*), which accounts for the increased cancer risk[120,121].

Aberrant expression, which is often due to loss of imprinting of imprinted genes, has been found in various cancers from individuals without human imprinting disorders. Although loss of imprinting of *IGF2* is the most frequently reported, abnormal expression of >30 imprinted genes has been found[121]. A recent addition to the list is retrotransposon-like 1 (*RTL1*), which is a retrotransposon gene within the *DIO3–DLK1* imprinted domain. Overexpression of *RTL1* has been found in a subset of human hepatocellular carcinoma samples[123], and overexpression of the orthologous gene *Rtl1* within the mouse *Dio3–Dlk1* domain promotes

Facial barbering
The trimming and plucking of the whiskers and fur of one mouse by another.

Tube test
A test of social dominance in which two unfamiliar mice are placed head first at opposite ends of a tube. The socially dominant mouse remains in the tube, whereas the more submissive mouse retreats from the tube.

Complete hydatidiform mole
A conceptus that lacks a set of normal maternal chromosomes and that forms a tumour-like mass. Known causes include a failure to set imprints in the female germ line and the occurrence of a conceptus that has both sets of chromosomes of paternal origin.

Epimutations
Mutations that result in heritable changes in gene expression that are caused by mechanisms other than changes in the underlying DNA sequence.

Retrotransposon
A genetic element that can be transposed to a new site in the genome by forming an RNA transcript that can be copied to DNA using reverse transcriptase, which can then be integrated into the genome.

Reciprocal hybrids
F_1 hybrid mice produced from reciprocal crosses between two mouse strains or between *Mus musculus* subspecies.

hepatocarcinogenesis[123]. These findings are important, as they suggest that *RTL1* is a relevant therapeutic target for human hepatocellular carcinoma, which is the third leading cause of cancer deaths worldwide[123].

Conclusions and future perspectives

Clearly, the roles of imprinted genes are more wide-ranging than those indicated by early studies. In addition to well-defined effects before birth, imprinted genes have profound metabolic, neurological and behavioural effects throughout life. These include effects that are essential to mammalian survival, such as regulation of metabolism and body temperature, as well as mother–infant interactions. Furthermore, imprinted genes regulate growth, metabolism and behaviour in various ways, and our understanding of the underlying pathways — from imprinted gene to phenotype — is ever increasing.

It has become clear that disrupted expression of imprinted genes has implications for disease in both childhood and adulthood, and that imprinted genes are not only implicated in the known imprinted syndromes but may also be involved in the development of common diseases such as obesity, diabetes mellitus and cancer. Indeed, our knowledge of the role of imprinted genes in disease has reached a stage at which the development of therapies can be considered for treating aspects of some human imprinted syndromes and cancer[47,123,124].

Not only has a new role for imprinting been recently identified in stem cell maintenance, but the work on *Dlk1* in neurogenesis[116] has also shown that imprinting can be unexpectedly malleable and used by the organism as a way of modulating gene dosage when required in specific developmental and environmental contexts. Whether *Dlk1* is unique in this regard may emerge in the future.

A remaining question is whether effects on adult metabolism and behaviour are the consequence of earlier developmental events. This question should be resolvable in the future using mouse conditional knockout models. Imprinted genes affect a range of adult behaviours in humans, including social behaviour. The recent identification of a social cognition phenotype in the mouse gives increased motivation for finding further models, possibly through phenotypic screens of knockouts of imprinted genes known to be monoallelically expressed in brain regions that are crucial for social behaviour.

To fully comprehend the role of imprinted genes in biology and disease, a complete list of imprinted genes is needed. UPD models, mouse mutants (particularly those arising from targeted mutagenesis) and reciprocal hybrids have been successfully used in the past for identifying imprinted genes. High-throughput sequencing methods using reciprocal hybrids are currently being used. One approach is to generate high-resolution maps of parental allele-specific DNA methylation that may facilitate the localization of previously unidentified differentially methylated regions that might signal the presence of imprinted genes[10]. Another approach is to use high-throughput transcriptome sequencing[125]. This strategy holds considerable promise for the identification of imprinted transcripts but can suffer from a high number of false positives, and new candidate imprinted genes need to be thoroughly validated using independent methods[126]. Nevertheless, in the future, the use of high-throughput sequencing should provide the opportunity to identify all imprinted genes and their spatiotemporal specificity not only in humans and mice but also in any species with a sequenced genome. Together with ever-growing mouse genetic resources that enable detailed testing of gene function[127–129], a more complete understanding of the function, adaptability and evolution of imprinted genes should ensue.

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Acknowledgements

The author thanks B. Cattanach, G. Moore, A. Plagge and V. Tucci for discussions and comments. Given the broad scope of this Review, the author apologizes to colleagues whose work was not cited owing to space limitations.

Competing interests statement

The author declares no competing interests.

FURTHER INFORMATION

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