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REVIEW

Genomic imprinting, growth and maternal–fetal interactions

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ABSTRACT

In the 1980s, mouse nuclear transplantation experiments revealed that both male and female parental genomes are required for successful development to term (McGrath and Solter, 1983; Surani and Barton, 1983). This non-equivalence of parental genomes is because imprinted genes are predominantly expressed from only one parental chromosome. Uniparental inheritance of these genomic regions causes paediatric growth disorders such as Beckwith–Wiedemann and Silver–Russell syndromes (reviewed in Peters, 2014). More than 100 imprinted genes have now been discovered and the functions of many of these genes have been assessed in murine models. The first such genes described were the fetal growth factor *insulin-like growth factor 2* (*Igf2*) and its inhibitor *Igf2 receptor* (*Igf2r*) (DeChiara et al., 1991; Lau et al., 1994; Wang et al., 1994). Since then, it has emerged that most imprinted genes modulate fetal growth and resource acquisition in a variety of ways. First, imprinted genes are required for the development of a functional placenta, the organ that mediates the exchange of nutrients between mother and fetus. Second, these genes act in an embryo-autonomous manner to affect the growth rate and organogenesis. Finally, imprinted genes can signal the nutritional status between mother and fetus, and can modulate levels of maternal care. Importantly, many imprinted genes have been shown to affect postnatal growth and energy homeostasis. Given that abnormal birthweight correlates with adverse adult metabolic health, including obesity and cardiovascular disease, it is crucial to understand how the modulation of this dosage-sensitive, epigenetically regulated class of genes can contribute to fetal and postnatal growth, with implications for lifelong health and disease.

KEY WORDS: Adipose tissue, Leptin, Pregnancy

Introduction: genomic imprinting

The process of genomic imprinting results in the monoallelic expression of genes based on their parental origin. This is an epigenetic process because copies of identical deoxyribonucleic acid (DNA) sequence may be either expressed or silenced. This haploid expression is usually present in all tissues where the gene is expressed, although there are examples of tissue-specific imprinting. Following the discovery of the first imprinted genes in the 1990s, the work of multiple groups has established that the differential expression of imprinted genes is mediated by *cis*-regulatory regions where DNA is differentially methylated on cytosine residues (reviewed in Ferguson-Smith, 2011). This differential methylation at crucial sites (known as imprinting control regions or ICRs) is acquired during germline development and maintained during the epigenetic reprogramming of the early embryo and in somatic cells. Imprinted genes are often clustered in the genome, where neighbouring maternally and paternally expressed genes are co-ordinately controlled by a single ICR. The ICRs regulate monoallelic transcription by modulating further local epigenetic states, such as promoter methylation, expression of long non-coding ribonucleic acids (RNAs) and histone modifications (Ferguson-Smith, 2011).

Our knowledge of imprinting in humans has been informed by a number of paediatric growth disorders with over-lapping phenotypes and diverse aetiology, but which all alter the dosage of imprinted genes. Mutations or epimutations in an ICR, or a duplication of a section of chromosome from one parent (uniparental disomy or UPD), results in imprinted genes being either over-expressed or not expressed. Because many imprinted regions contain both paternally and maternally expressed genes, it is difficult to determine which genes are causal for their respective syndromes; in some cases, imprinting disorders are likely to be caused by polygenic gene dosage disruption. A common feature of imprinting disorders is developmental abnormality, resulting in altered growth and nutrient acquisition in early life (Table 1) and, hence, they highlight the key role of imprinted genes in these physiological processes.

Imprinting has been described across Therian mammals (including marsupials but not monotremes) and conservation of many imprinted genes [e.g. insulin-like growth factor 2 (*IGF2*) and its inhibitor *IGF2* receptor (*IGF2R*), paternally expressed gene 1/mesodermally expressed transcript (*PEG1/MEST*) and insulin (*INS*)] within this group implies that imprinting was present in a common vertebrate ancestor that exhibited viviparity and had a placenta (Graves and Renfree, 2013). Coincident with their taxonomic correlation with the presence of a placenta, many imprinted genes are expressed in that organ and play a crucial role in the process of placentation. Moreover, genes involved in placentation are more likely to be imprinted (Graves and Renfree, 2013). In mammals, the level of dependence on the placenta correlates with both the known number of imprinted genes within each group (Renfree et al., 2009) and the complexity of their regulation (Graves and Renfree, 2013). The presence of parental-sex-dependent haploid expression has also been described in flowering plants, a subset of plants that utilise a nourishing external organ in their reproductive strategy. The endosperm in flowering plants provides nourishment from the parent's energy stores, much like the placenta; similar selection pressures are likely to have led to the convergent evolution of imprinted genes in these distant groups (Pires and Grossniklaus, 2014).

Imprinted genes control maternal resource allocation during pregnancy and lactation

This selective pressure for the imprinting of a gene has acted almost exclusively at the maternal–offspring interface, giving imprinted genes a unique role in influencing maternal resource allocation. Maternal resource allocation is dependent on both the conceptus and the mother, and the role of imprinted genes reflects this, with imprinted gene action affecting both and involved in communication between the two. Imprinted genes that act in the conceptus to modulate maternal energy

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Table 1. Summary of human imprinting disorders

Disorder	Phenotype	Reference
Silver–Russell syndrome (SRS, OMIM 180860)	Small for gestational age at birth, distinctive features, including a triangular-shaped face and macrocephaly, body asymmetry	Netchine et al., 2012
Beckwith–Wiedemann syndrome (BWS, OMIM 130650)	Large for gestational age with neonatal hypoglycaemia, overgrowth; macroglossia, often have asymmetrical growth and embryonic tumours	Netchine et al., 2012
Angelman syndrome (AS, OMIM 105830)	Microcephaly, a number of neurological disorders, including intellectual and speech deficiencies, behavioural and sleep abnormalities and epilepsy	Buiting et al., 2016
Prader–Willi syndrome (PWS, OMIM 176270)	Hypotonia and feeding difficulties at birth followed by hyperphagia and obesity in childhood and adulthood	Holm et al., 1993
Temple syndrome (TS, OMIM 616222)	Small for gestational age at birth, with postnatal failure to thrive and hypotonia, followed by reduced growth, early puberty and childhood truncal obesity	Ioannides et al., 2014
Transient neonatal diabetes type 1 (TNDM, OMIM 601410)	Small for gestational age at birth, failure to thrive in the first few postnatal weeks, hyperglycaemia and dehydration	Mackay and Temple, 2010

supply can be further subdivided into those acting in the placenta and those acting in the fetus.

Fetal resource acquisition via the placenta

The rate at which nutrients can be supplied to the developing fetus is dependent upon the placenta. The primary substrate for fetal growth is glucose. Maternal–fetal glucose transfer is mediated by multiple factors, including the maternal–fetal blood glucose concentration gradient, the rate of blood flow and the placental glucose transporter density (Hay, 1991). Placental amino acid transport to the fetus from the maternal circulation operates against a concentration gradient and is an energy-dependent process requiring specialised transporter proteins (Hay, 1991).

Imprinting has been most extensively studied in humans and mice. These organisms share similar placental physiology: both use a hemochorial placenta where the maternal blood travels through sinusoidal spaces lined by trophoblast cells, rather than an endothelium, allowing for more efficient nutrient transfer (reviewed in Rai and Cross, 2014). The distinct populations of cells in the placenta, the trophoblast and the extraembryonic mesoderm, differentiate early in embryogenesis with the trophoblast cells invading the maternal uterine decidua following fertilisation. In mice, at mid-gestation the definitive placenta is formed by intercalation of the extraembryonic mesoderm (which will form the embryonic vasculature) with trophoblast cells that channel maternal blood flow through the placenta. The portion of the placenta where maternal and fetal circulation is closely opposed is known as the labyrinth in mice. The trophoblast further differentiates into multiple cell types that have both endocrine and energy-storage properties (Rai and Cross, 2014).

The action of imprinted genes on placental development and nutrient transport capacity is well established and has been reviewed extensively (Fowden et al., 2011; Monk, 2015). Most imprinted genes are monoallelically expressed in the placenta, and have been shown to control early developmental processes that establish the organ [e.g. *Peg10* (Ono et al., 2006)]. In addition, imprinted gene products can modulate labyrinth size and the surface area for exchange [*Igf2* (Sibley et al., 2004) and *Growth factor bound protein 10* (*Grb10*; Charalambous et al., 2010)] as well as vascular branching density [*Aquaporin* (Guo et al., 2016)]. Placental imprinted genes can also directly influence maternal physiology by controlling the differentiation of the trophoblast-derived endocrine cells (reviewed in John, 2017).

Imprinted genes involved in fetal resource acquisition

Nutrient uptake from maternal tissues and fetal growth rates are interconnected processes (Hay, 1991). Imprinted genes can act directly on fetal growth-promoting pathways and, thus, increase the demand for maternal resources. The IGF pathway is a major fetal growth-promoting pathway and includes two key components encoded by imprinted genes (*Igf2* and *Igf2r*; DeChiara et al., 1991; Lau et al., 1994; Wang et al., 1994). Fetal growth is also restrained by imprinted genes, including the *Cyclin-dependent kinase inhibitor 1C* [Cdkn1c (Tunster et al., 2011)] and *Grb10* (Charalambous et al., 2003). *Grb10* encodes an adapter protein that acts downstream of tyrosine-kinase receptors to inhibit signalling, and is a crucial auto-regulatory component of the nutrient-sensing mechanistic target of rapamycin (mTOR) pathway (Hsu et al., 2011; Yu et al., 2011). *Grb10* may act in the same signalling pathway as the fetal growth promoter *Delta-like homologue 1* (*Dlk1*; Madon-Simon et al., 2014). *Dlk1* is a paternally expressed gene encoding a single-pass transmembrane protein that can be cleaved to produce a soluble form that circulates in the blood (Smas et al., 1997). *Dlk1* expression levels in the embryo are positively correlated to embryonic mass in the second half of gestation (Cleaton et al., 2016). *Dlk1* deletion causes growth retardation (Cleaton et al., 2016; Moon et al., 2002) and overexpression causes overgrowth independently of the placenta (da Rocha et al., 2009). The signalling pathway by which DLK1 acts has yet to be elucidated. However, DLK1 from the fetus is secreted into the maternal circulation and acts to modify the pregnancy-specific response to nutrient restriction (Cleaton et al., 2016).

In summary, imprinted genes in the conceptus produce products that alter maternal resource allocation by: (i) altering the transport capacity of the placenta; (ii) increasing fetal demand for resources by their action on the intrinsic growth rate; and (iii) signalling to the mother by the production of fetal/placental hormones that modify maternal metabolism.

Energy homeostasis and resource allocation during pregnancy

The energetic cost of pregnancy is distributed amongst three compartments, i.e. the energy invested in the products of conception, the energy deposited as adipose tissue in the mother and the energy required to maintain these new tissues (Thomson and Hytten, 1961). Well-nourished humans and rodents accumulate adipose tissue reserves during pregnancy in the first half of gestation (Herrera and Ortega-Senovilla, 2014; King, 2000). The total cost of pregnancy is correlated with pre-pregnancy fatness – individuals with a higher body mass index (BMI) gain more weight during pregnancy, both the adipose tissue reserve and conceptus mass (Prentice and Goldberg, 2000). This suggests that adipose reserves in the mother can produce a signal to the body that directs the level of nutritional allocation after conception (Prentice and Goldberg, 2000). Leptin and other adipokines are ideal candidates for such signalling molecules.

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Leptin, a cytokine secreted exclusively by adipose tissue in proportion to adipose mass, is a major determinant involved in signalling the status of the energy reserve to central pathways controlling appetite, energy expenditure and reproductive behaviour (van Swieten et al., 2014). The action of leptin on the hypothalamic control of energy homeostasis is complex, and a detailed description is beyond the scope of this Review. Briefly, leptin signals to its receptors expressed on the surface of first-order neurons in the arcuate nucleus of the hypothalamus (ARH). Two populations of neurons in the ARH have been well defined – the orexigenic neuropeptide Y (NPY)/Agouti-related peptide (AGRP) neurons, which are inhibited by leptin, and the anorexigenic pro-opiomelanocortin (POMC) neurons, which are activated by leptin (reviewed in van Swieten et al., 2014). These neurons project to secondary hypothalamic sites, such as the paraventricular nucleus (PVN), which controls feeding behaviour, and the dorsomedial hypothalamus (DMH), which regulates the level of sympathetic neuronal activity. A crucial intermediate signalling molecule is the neuropeptide α-melanocyte-stimulating hormone (αMSH), which is secreted by POMC neurons and acts on melanocortin receptors to mediate downstream actions of leptin (van Swieten et al., 2014). AGRP antagonises αMSH. Simply, a high leptin level from adequate adipose stores suppresses appetite by deactivating orexigenic neuronal pathways, and increases energy expenditure by increasing activity and sympathetic neuronal activity. Increased sympathetic neuronal activity in turn increases body temperature by activating the expression of thermogenic genes in brown and beige adipose tissue (van Swieten et al., 2014). Low stores of adipose result in an opposite series of responses. Taken together, these mechanisms maintain a homeostatic 'set point' that maintains body weight within a narrow range.

Genetic and environmental modulations of leptin signalling pathway components can cause alterations to the value of these set points, or cause a failure to achieve homeostasis. For example, the pups of mice exposed to a high-fat maternal diet during the perinatal period fail to establish neuronal connectivity between the ARH and PVN, resulting in altered body composition and a predisposition to metabolic disease as adults (Vogt et al., 2014). Leptin-deficient (*ob/ob*) mice and people with mutations in components of the central melanocortin pathway are hyperphagic and obese – maintaining a higher body weight set point owing to leptin deficiency or resistance (reviewed by Farooqi and O'Rahilly, 2014).

Elegant studies utilising timed leptin-replacement therapy in *ob/ob* mice have demonstrated that this adipokine has a broad role in reproduction (Malik et al., 2001). Leptin signalling is required for fertility [by controlling gonadotrophin release (Padilla et al., 2017)], conception and implantation. Moreover, leptin is required during the perinatal period for lactation and for appropriate maternal behaviours following parturition (Malik et al., 2001).

Pregnancy is associated with leptin resistance. Circulating levels of leptin rise in the maternal blood in the second half of pregnancy; however, the expression of orexigenic peptides NPY and AGRP in the ARH remain stable. Moreover, injecting pregnant rats with leptin does not suppress food intake or activate downstream αMSH pathways (Ladyman et al., 2010). The leptin resistance of late pregnancy is thought to be mediated by placental secretion of hormones, specifically the prolactin and placental lactogen family of molecules. Intracerebroventricular injection of prolactin into female rats mimics the leptin resistance of pregnancy, and prolactin receptor expression is widespread in the hypothalamic areas associated with energy homeostasis (Ladyman et al., 2010). Consistently, global deletion of the prolactin receptor in mice causes changes to glucose homeostasis during pregnancy; however, the contribution of leptin signalling to this phenotype has yet to be explicitly tested (Rawn et al., 2015). Therefore, interactions between placental hormone secretion and central leptin sensitivity are crucial for an appropriate maternal response to pregnancy.

A second adipokine, adiponectin, has important functions during pregnancy. Adiponectin is secreted from adipocytes according to their size and acts on multiple tissues, predominantly to increase insulin sensitivity (Stern et al., 2016). Adiponectin levels drop in both rodent and human pregnancy and remain low during lactation (Combs et al., 2003; Howell and Powell, 2017). Low adiponectin levels during the second half of gestation are thought to increase maternal insulin resistance, thus reducing maternal glucose uptake and increasing the maternal–fetal glucose concentration gradient in favour of fetal uptake. Consistent with this, obese pregnant women and rodents who are insulin resistant give birth to large babies and have further reduced adiponectin levels (Howell and Powell, 2017). Furthermore, supplementing obese mice with adiponectin during pregnancy can reverse both maternal insulin resistance and fetal overgrowth (Aye et al., 2015).

Experiments utilising deletion and overexpression models in mice have demonstrated that imprinted genes have important roles in energy homeostasis – resulting in altered steady-state levels of adiposity, adipokine production and sensitivity. However, many of these phenotypic alterations have not yet been tested directly for their contributions to the outcome of pregnancy, and adipokine levels during pregnancy have been measured for only very few imprinted gene manipulations (available data for murine models published to date are summarised in Table 2).

The actions of imprinted genes in energy homeostasis can be partitioned into those that modulate the central pathways, and those that act in peripheral tissues to alter adipose mass and type (Fig. 1).

Imprinted genes mediating the central control of energy homeostasis

Gnas locus

The *Gnas* locus encodes maternally and paternally expressed products (Gsα and XLαs), as well as several regulatory RNAs (reviewed in Peters, 2014). Although Gsα is widely and predominantly biallelically expressed, it is imprinted in only a small number of tissues, including the kidney and neuroendocrine tissues, where it is maternally expressed. Gsα encodes the stimulatory G-protein alpha subunit that couples receptor-mediated signalling to intracellular cyclic adenosine monophosphate – causing the activation of protein kinase A (PKA). A second isoform from the *Gnas* locus encodes XLαs, containing a distinct amino-terminal of the protein from Gsα. This isoform is exclusively expressed from the paternally inherited chromosome, predominantly in the brain and endocrine tissues (reviewed by Peters, 2014).

Maternally inherited Gsα mutations cause pseudohypoparathyroidism type A, a syndrome of multiple hormone resistance, reduced sympathetic tone, early severe obesity and insulin resistance (reviewed in Weinstein, 2014). This is phenocopied in mice that inherit a deletion in the first exon of *Gnas* from their mother. Central melanocortin signalling limits food intake and stimulates sympathetic tone and energy expenditure, and the melanocortin receptor 4 (MC4R) is known to couple to Gsα (Weinstein, 2014). Maternally expressed Gsα is required for melanocortin signalling in the DMH. Deletion of Gsα in this tissue causes obesity and insulin resistance by reducing energy expenditure, both by reducing physical activity and the expression of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT). Food intake is not affected. The mice were resistant to the effect of melanocortin agonists, and these phenotypes

Table 2. Murine models of imprinted gene regulation with details of energy homeostasis and maternal reproductive phenotypes and adipokine levels

Gene	Model	Energy homeostasis phenotype	Phenotype of mutant mothers	Adipokine levels	Expressed allele	References
Gnas	Global maternal deletion of Gnas exon 1 – specific to Gsα	Increased adipose deposition, reduced glucose tolerance and insulin sensitivity. Metabolic rate reduced owing to reduced SNS activity	NR	Leptin high Adiponectin unchanged	Maternal	Chen, 2005
	DMH deletion of Gnas exon 1	Increased adipose deposition, reduced glucose tolerance and insulin sensitivity. Reduced energy expenditure owing to reduced activity. Food intake unchanged	NR	Leptin high		Chen et al., 2017
GnasXL	Global deletion of Gnasxl exon 1 – specific to XLαs	Reduced adiposity, increased metabolic rate, increased glucose tolerance and insulin sensitivity. Increased SNS activity	NR	Leptin low Adiponectin low	Paternal	Xie et al., 2006
Peg1/ Mest	Deletion	Reduced adipose stores	Poor maternal care	NR	Paternal	Nikonova et al., 2008
	Transgenic over expression in mesenchymal cells (aP2 promoter)	Increased adipocyte size	NR	Leptin messenger RNA expression increased		Takahashi et al., 2005
Peg3	Deletion	Increased body fat, reduced lean mass. Reduced food intake and body temperature. Central leptin resistance	Poor maternal care Reduced maternal investment in pregnancy	Leptin high	Paternal	Curley et al., 2005
Asb4	Overexpression in hypothalamic POMC neurons	Reduced body fat, increased food intake, increased energy expenditure by thermogenesis and activity	NR	Leptin low	Maternal	Li et al. 2010
Snord116	Global deletion	In mature animals, reduced body fat, increased food intake, increased energy expenditure by activity	NR	NR	Paternal	Ding et al., 2008
	Conditional deletion in NPY neurons	In mature animals, reduced body fat, increased food intake, increased energy expenditure by activity	NR	NR	Paternal	Qi et al., 2016
Magel 2	Global deletion	Maturity-onset insensitivity to leptin causes increased adipose mass and reduced energy expenditure	NR	Leptin high	Paternal	Bischof et al., 2007; Mercer et al., 2013; Pravdivyi et al., 2015
Necdin	Global deletion	Increased adipose mass on HFD Food intake unchanged	NR	NR	Paternal	Fujiwara, 2012
Cdkn1c	Tissue-appropriate overexpression (BAC transgenic)	Reduced white adipose mass, increased BAT activity Food intake reduced	NR	NR	Maternal	Van De Pette, 2016
Grb10	Global deletion of somatic expression (maternal transmission of deletion)	Reduced white adipose mass, increased lean mass, improved glucose tolerance and insulin sensitivity Food intake unchanged	Reduced resource allocation to litter	Leptin low	Maternal	Smith et al., 2007; Cowley et al., 2014
	Deletion in adipocytes (adiponectin promoter-Cre)	Increased WAT mass, impaired glucose and insulin sensitivity. Thermogenic gene expression is reduced in BAT, and animals do not 'brown' their WAT on cold challenge and have impaired ability to defend BT	NR	NR		Liu et al., 2014
Dlk1	Global deletion	Increased WAT mass, impaired glucose and insulin sensitivity Food intake unchanged	Increased maternal allocation to litter	Leptin high	Paternal	Moon et al., 2002; Cleaton et al., 2016
	Tissue-appropriate overexpression (BAC transgenic)	Reduced WAT mass, improved glucose tolerance and insulin sensitivity Food intake unchanged	NR	Leptin low Adiponectin unchanged		Charalambous, 2012

Abbreviations: BAC, bacterial artificial chromosome; BAT, brown adipose tissue; BT, body temperature; DMH, dorsomedial hypothalamus; HFD, high-fat diet; mRNA, messenger ribonucleic acid; NPY, neuropeptide Y; NR, not reported; POMC, pro-opiomelanocortin; SNS, sympathetic nervous system; WAT, white adipose tissue.

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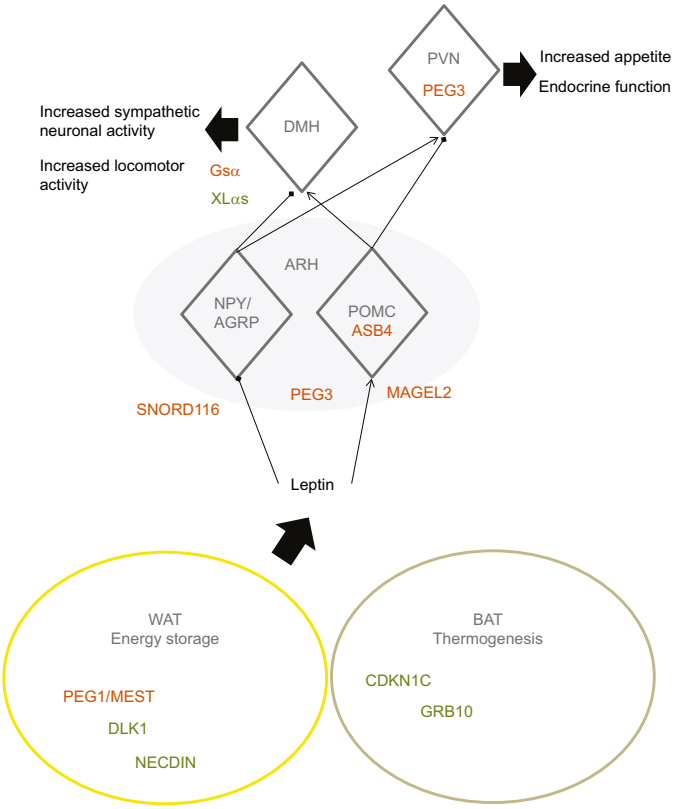

Fig. 1. Summary of the action of imprinted gene products on energy homeostasis pathways. Imprinted genes act on the secretion of adipokines by altering the balance of thermogenic brown adipose tissue (BAT) to white adipose tissue (WAT). Imprinted genes may act on additional pathways to modify body composition – for brevity these mechanisms are omitted from this Review. In the hypothalamus, imprinted gene products are expressed in the first order orexigenic (neuropeptide Y/Agouti-related peptide, NPY/AGRP) and anorexigenic (pro-opiomelanocortin, POMC) arcuate nucleus (arcuate nucleus of the hypothalamus, ARH) neurons and modulate their sensitivity to leptin. These neurons interact with other hypothalamic structures such as the dorsomedial hypothalamus (DMH) and paraventricular nucleus (PVN) to mediate the physiological responses to leptin, such as suppression of appetite and increased energy expenditure. Gene products that promote leptin production or the activity of leptin signalling are shown in orange, those that suppress leptin production or its actions are shown in green. References are listed in Table 2. Abbreviations: ASB4, Ankyrin repeat, SOCS box-containing 4; CDKN1C, cyclin-dependent kinase inhibitor 1C; DLK1, Delta-like homologue 1; GRB10, growth factor bound protein 10; PEG1/MEST, paternally expressed gene 1/mesodermally expressed transcript.

were recapitulated when the MC4R was ablated in the DMH, suggesting that they are a direct result of aberrant signalling through this pathway. Surprisingly, cold tolerance was not affected in either the Gsα or MC4R mutant mice, and both were able to mount a transcriptional response to cold challenge in both BAT and by promoting browning of white adipose tissue (WAT) (Chen et al., 2017).

Loss of XLαs causes a metabolic phenotype that is opposite to that caused by maternal Gsα mutations – a reduced adiposity and an increased metabolic rate owing to increased sympathetic nervous system activity (Xie et al., 2006).

Peg3

Paternally expressed gene 3 (*Peg3*), which encodes a zinc-finger protein is expressed in the placenta and ovaries as well as in some neuroendocrine tissues (Hiby et al., 2001; Kuroiwa et al., 1996; Relaix et al., 1996). Mice that inherit a *Peg3* deletion from their father are growth retarded at birth and remain small throughout life. After weaning, males show delayed adipose accumulation and females enter puberty late. In adulthood, the animals have increased white adipose deposition and very high levels of circulating leptin. The animals show reduced energy expenditure, reduced resting body temperature and are less able to defend body temperature following cold challenge (Curley et al., 2005). PEG3 is expressed in the developing hypothalamus (Li et al., 1999). In young mice with deleted *Peg3*, the transcriptional regulation of neuropeptides in the feeding centres of the hypothalamus is disrupted. Although this is resolved in adulthood, the animals remain less sensitive to leptin challenge, suggesting a permanent change in the set points of central energy homeostasis (Curley et al., 2005).

Females with an ablated *Peg3* gene are poor mothers (Li et al., 1999). During pregnancy, *Peg3* mutant mothers fail to increase food intake in early gestation and, consequently, have a reduced post-partum adipose reserve. In addition, wild-type pups born to *Peg3* mutant mothers do not gain weight appropriately and enter puberty late. Mutant mothers fail to express maternal-specific behaviours such as pup-retrieval and nest building, and litters from such mothers are much less likely to survive to weaning (Curley et al., 2004; Li et al., 1999).

Interestingly, the consequences of either the mother or offspring having a *Peg3* mutation are strikingly similar, with pups having reduced protection from the cold and reduced growth pre- and postnatally. Despite the functional similarity, *Peg3* is expressed primarily in the paraventricular nucleus of the hypothalamus in the adult, an area known to regulate maternal behaviours and milk let-down, whereas the dysfunction due to mutation in the pups is most likely to be because of the actions of *Peg3* in the placenta. As noted earlier, the placenta regulates maternal metabolic adaptation and maternal behaviours through endocrine signalling, and the loss of *Peg3* in the offspring is associated with the altered expression of prolactin-like genes (Broad and Keverne, 2011; John, 2013). The placenta and hypothalamus may interact through endocrine signalling, leading to a cumulative effect seen when both mother and offspring lack *Peg3* (Curley et al., 2004).

Asb4

Ankyrin repeat, SOCS box-containing 4 (Asb4) is a ubiquitin ligase that is maternally expressed in mice but not imprinted in humans. It is expressed in the first-order POMC and NPY/AGRP neurons of the hypothalamus and its transcription is regulated by fasting, leptin and insulin injection. Overexpression of *Asb4* in POMC neurons causes a hypermetabolic phenotype with increased energy expenditure, reduced adipose mass and resistance to weight gain on a high-fat diet. Therefore, ASB4 dosage mediates the sensitivity of POMC neurons to leptin given that increased gene dosage promotes leptin signalling (Li et al., 2010). In mice, *Asb4* also plays a role in the early vascular differentiation of the placenta (Townley-Tilson et al., 2014).

Prader–Willi syndrome cluster

Snord116 maps to the critical region for Prader–Willi syndrome (PWS) (characterised by early hypertonia and a failure to thrive followed by hyperphagia at 2–3 years and obesity). It encodes a C/D box small nucleolar RNA (SnoRNA). This family of catalytic RNAs regulate RNA processing; however, the targets of *Snord116* are unknown. In the mouse, *Snord116* is exclusively expressed in the brain, and localised to the appetite centres (Cavaille et al., 2000). Deletion of *Snord116* in mice causes postnatal growth retardation

with an age-dependent effect on appetite. In early life, appetite is reduced; however, later food intake is increased. The mice do not become obese, rather they increase energy expenditure and maintain a steady state of reduced adipose stores with respect to wild-type littermates (Ding et al., 2008). Deletion of *Snord116* in NPY/AGRP neurons recapitulates all the phenotypes of the global deletion, suggesting that *Snord116* acts to alter the sensitivity of orexigenic neurons to stimulus (Qi et al., 2016).

Magel2 encodes an E3 ubiquitin ligase expressed in the brain. It is localised to the PWS locus in humans and the syntenic region in mice, and is paternally expressed (Boccaccio et al., 1999). Deletion of *Magel2* from the paternally inherited allele in mice phenocopies some aspects of PWS: the animals fail to thrive in the early postnatal period, but later experience catch-up growth and as adults have increased adiposity (Bischof et al., 2007). However, this energy imbalance is not caused by hyperphagia in the mutant mice because although they eat less, they have reduced energy expenditure. *Magel2* deletion causes multiple defects in hypothalamic function, including the insensitivity of first order POMC neurons to leptin (Mercer et al., 2013). Leptin insensitivity in *Magel2* knock-out animals is associated with an impairment in the developmental accumulation of neuronal connections between arcuate nucleus neurons and their targets, which occurs in the early postnatal period (Maillard et al., 2016; Pravdivyi et al., 2015).

Imprinted gene actions in peripheral tissues

Peg1/Mest

Peg1/Mest is paternally expressed and encodes a member of the α/β-hydrolase fold family (Kaneko-Ishino et al., 1995). When inbred C57BL6 mice are fed a high-fat diet they show a variable adipose tissue gain response. *Peg1/Mest* messenger RNA levels are induced in WAT following only 2 days of high-fat feeding, and expression positively correlates with weight gain (Koza et al., 2006). *Peg1/Mest* levels have been shown to correlate with adipocyte size in a variety of genetic and dietary models of obesity (Nikonova et al., 2008; Takahashi et al., 2005), and overexpression of *Peg1/Mest* in adipocytes causes increased cell size. Mice with deleted *Peg1/Mest* have reduced adipose stores and reduced adipokine production. It has been proposed that *Peg1/Mest* induction during positive energy balance controls the initial phase of adipose tissue expansion by adipocyte hypertrophy (Nikonova et al., 2008).

Peg1/Mest mutant mothers exhibit abnormal maternal behaviour – they fail to clean new-born pups and ingest extraembryonic tissues, they do not retrieve pups to the nest, and perform poorly at nest building. Consequently, pup survival is severely compromised (Lefebvre et al., 1998).

Necdin

Necdin function in multiple contexts has been associated with the restriction of cell proliferation, at least in part through interactions with p53 (Hasegawa and Yoshikawa, 2008). *Necdin* is paternally expressed in mice and humans, where it was mapped to the PWS cluster (Chibuk et al., 2001). Deletion of *Necdin* in mice causes hyperproliferation of the WAT compartment without affecting central energy balance (Fujiwara, 2012). In mutant mice, the WAT depot size increased without a concurrent increase in fat cell size, indicating that expansion occurred by a mechanism of preadipocyte hyperplasia (Fujiwara, 2012).

Cdkn1c

Increasing *Cdkn1c* expression in tissues where it is normally expressed results in a phenotype similar to Silver–Russell syndrome, pre- and postnatal growth retardation with failure to gain adipose mass. *Cdkn1c* is expressed in multiple adipose tissue depots in early perinatal life, and the level of expression reflects the propensity of that depot to 'brown' – i.e. to express thermogenic genes following stimulus. Mice with an increased *Cdkn1c* dosage showed increased thermogenic gene activity in WAT and BAT and increased body temperature. Deletion of *Cdkn1c* results in perinatal lethality and, therefore, the postnatal phenotype could not be assessed. However, ablation of *Cdkn1c* causes impaired BAT development during embryogenesis (Van De Pette, 2016).

Grb10

Unusually for an imprinted gene, *Grb10* is both maternally and paternally expressed, but in different tissues. Maternal expression is seen in the peripheral tissues whereas expression in the central nervous system is solely paternal, with maternally expressed *Grb10* controlling growth and energy homeostasis, and paternal *Grb10* regulating social dominance behaviours (Garfield et al., 2011). Postnatally, maternally inherited deletion of *Grb10* results in increased lean mass, reduced adipose mass (with low leptin) and improved glucose homeostasis (Smith et al., 2007). Food intake is not grossly affected. *Grb10* expression is low in mature fat, but can be induced in brown adipocytes by cold or adrenergic stimulation (Liu et al., 2014). The deletion of *Grb10* in mature fat cells causes the failure of BAT to respond to stimuli and, consequently, thermogenesis is impaired (Liu et al., 2014).

Cross-fostering studies have been used to disentangle the effects of *Grb10* loss in either the mother or the offspring on offspring growth. These studies showed that *Grb10* plays complementary roles in the offspring and the mother. Wild-type pups cross-fostered onto *Grb10*-deleted mothers (inheriting a deleted allele from their mother) show reduced growth in early life compared with those reared by wild-type mothers, suggesting that the normal role for *Grb10* in the mother is to increase resource allocation to the offspring (Cowley et al., 2014). Interestingly, *Grb10* mutant pups fostered onto wild-type mothers gain more weight than wild-type pups; however, this effect is negated if they are raised by mutant mothers – indicating that the balance of *Grb10* dosage in the mother and pup is a crucial determinant of nutrient flow from the mother to her offspring (Cowley et al., 2014). Maternal care provision and milk let-down were unaltered by *Grb10* deletion, but in response to increased demand and corresponding increased prolactin levels, *Grb10* mutant mothers could not increase milk production, suggesting resistance to elevated prolactin. *Grb10* is expressed in the mammary epithelium during pregnancy; however, its role here is unclear because no defect was found in the tissue morphology or the constituents of the milk produced by *Grb10* mutant females (Cowley et al., 2014).

Dlk1

Dlk1 has been widely reported as an inhibitor of preadipocyte differentiation because the soluble form of the protein can inhibit *in vitro* differentiation of 3T3-L1 cells or mesenchymal cells into adipocytes (Smas and Sul, 1997). Moreover, ectopic expression of *Dlk1* in adult WAT causes lipodystrophy (Lee et al., 2003). However, lineage-appropriate overexpression of *Dlk1* from a bacterial artificial chromosome transgene (*Dlk1-TG*) does not cause a failure of adipose expansion in adults, even when animals are challenged with the hyperphagic leptin-deficient background (Charalambous et al., 2014). Instead, *Dlk1-TG* mice have a larger proportion of small adipocytes with increased insulin sensitivity compared with wild-type mice. Conversely, *Dlk1* null mice have

larger adipocytes than wild-type mice (Moon et al., 2002). Manipulation of *Dlk1* dosage has the expected effect on circulating leptin levels: *Dlk1* null mice have elevated leptin and *Dlk1-TG* have reduced levels. However, despite this, food intake is not altered by either genetic manipulation (Charalambous et al., 2014; Cleaton et al., 2016), suggesting impaired leptin sensitivity.

Dlk1 is likely to be acting during early life to increase adipocyte number given that a lineage tracing study of cells expressing GFP from a minimal *Dlk1* promoter labelled adipose tissue in all depots of the adult mouse. Moreover, ablation of this embryonic DLK1-positive cell population caused a reduction in adipose depot size (Hudak et al., 2014).

Dlk1 null mothers give birth to larger litters than wild-type mothers (approximately one extra pup per litter), and overall litter mass is increased. Moreover, the conceptus mass of like-genotype offspring in litters from *Dlk1* null mothers is not different from that in litters from mothers with an intact *Dlk1* gene. Therefore, *Dlk1* null mothers increase their investment in each litter, rather than reducing offspring size to offset offspring number. In addition, although *Dlk1* null mothers enter pregnancy with increased adipose stores, they gain less adipose mass during pregnancy than wild-type mothers. Altogether, females without a functional copy of *Dlk1* invest more resources in pregnancy, suggesting that the normal role for *Dlk1* in female reproduction is to decrease nutrient allocation (Cleaton et al., 2016).

Summary

Imprinted gene products act at multiple levels in the adipose–hypothalamic axis to modulate set points of energy homeostasis. Experiments from murine models with ablation or overexpression of imprinted genes demonstrate that they are required in the hypothalamus to modulate the sensitivity of neuroendocrine pathways to leptin. Moreover, the dosage of imprinted genes in developing and mature adipose tissue can modulate leptin secretion (summarised in Fig. 1).

The degree of maternal investment in pregnancy is positively correlated with the pre-pregnancy adipose reserve. Mothers with high BMI invest more in their own adipose reserves, and in the products of conception (Prentice and Goldberg, 2000). Leptin has been proposed as a potential mediator of this effect. Given that imprinted gene products can modulate both leptin production and central sensitivity to leptin, we predict that an impaired imprinted gene dosage in females may influence their resource allocation as mothers. To date, few imprinted genes have been tested explicitly for their role in maternal physiology. However, where data exist, the prediction that low leptin/leptin-resistant mothers should invest less in a reproductive cycle appears consistent. *Peg3*, *Peg1/Mest* and *Grb10* deletions in the mother all reduce leptin signalling, and maternal investment is reduced; conversely *Dlk1* deletion increases leptin production and maternal investment in pregnancy. Further work is required to establish whether other models of imprinted gene misregulation cause defects in the maternal phenotype, and if other adipokines (such as adiponectin) are involved.

Conclusions

Since their discovery nearly 30 years ago, imprinted genes have been a paradigm for exploring the epigenetic control of gene expression. Moreover, their roles in early life growth and placentation are undisputed. However, it is becoming increasingly clear that imprinted gene function has a wider role in maternal physiology during reproduction – both by modulating fetal and placental endocrine products that signal to alter maternal energy homeostasis, and by altering maternal energetic set points, thus producing downstream actions on nutrient provisioning. Uncovering the molecular nature of these pathways has a broad application in terms of understanding natural reproductive strategies and provides a basis for preventing complications to pregnancy in human populations.

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Competing interests

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