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REVIEW

Placental glycogen stores and fetal growth: insights from genetic mouse models

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Abstract

The placenta performs a range of crucial functions that support fetal growth during pregnancy, including facilitating the supply of oxygen and nutrients to the fetus, removal of waste products from the fetus and the endocrine modulation of maternal physiology. The placenta also stores glucose in the form of glycogen, the function of which remains unknown. Aberrant placental glycogen storage in humans is associated with maternal diabetes during pregnancy and pre-eclampsia, thus linking placental glycogen storage and metabolism to pathological pregnancies. To understand the role of placental glycogen in normal and complicated pregnancies, we must turn to animal models. Over 40 targeted mutations in mice demonstrate the defects in placental cells that store glycogen and suggest that placental glycogen represents a source of readily mobilized glucose required during periods of high fetal demand. However, direct functional evidence is currently lacking. Here, we evaluate these genetic mouse models with placental phenotypes that implicate glycogen trophoblast cell differentiation and function to illuminate the common molecular pathways that emerge and to better understand the relationship between placental glycogen and fetal growth. We highlight the current limitations in exploring the key questions regarding placental glycogen storage and metabolism and define how to experimentally overcome these constraints.
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Introduction

The placenta is a unique organ that exists only during pregnancy to perform a diverse range of functions that collectively support optimal fetal growth while maintaining maternal well-being. Arguably, the principal role of the placenta is to mediate the supply of oxygen and nutrients from the mother to fetus (Burton & Fowden 2015). The placenta also removes waste products from the fetal circulation and forms a physical barrier that affords some protection against certain pathogens and toxins (Gude *et al.* 2004). Furthermore, the placenta also synthesizes and secretes hormones into the mother's blood to adapt maternal physiology and behavior, ultimately to ensure that the pregnancy is sustained and fetal growth is supported (Napso *et al.* 2018). In addition to facilitating glucose transport, the placenta metabolizes glucose for its own use and stores it in the form of the multi-branched polysaccharide glycogen. The purpose of placental glycogen stores in normal pregnancy remains unclear. The most widely accepted theory is that placental glycogen stores ensure that fetal glucose supply is maintained at times of maximal demand, such as during late gestation (Barash & Shafrir 1990, Coan *et al.* 2006). However, direct experimental evidence to support such a function is currently lacking.

Placental glycogen in humans

Despite the earliest reference to glycogen storage in the human placenta dating to the early twentieth century (Driessen 1907), understanding its role during normal or complicated pregnancies has remained an elusive goal. In the human placenta, glycogen is predominantly found in the cytotrophoblast cells (Wislocki & Bennett 1943), with the highest levels in the distal cytotrophoblast closest to the decidua (Georgiades *et al.* 2002). In uncomplicated pregnancies, placental glycogen content peaks toward the end of the first trimester before declining towards term (Villee 1953, Boyd 1959, Robb & Hytten 1976). Aberrant glycogen storage was reported in gestational diabetes (Heijkenskjold & Gemzell 1957, Gabbe *et al.* 1972, Diamant *et al.* 1982, Desoye *et al.* 1992, Gheorman *et al.* 2013) and preeclampsia (PE) (Arkwright *et al.* 1993, Tsoi *et al.* 2003), suggesting a possible role in the pathogenesis of these two common pregnancy complications. The relevance of aberrant glycogen storage in the context of human pregnancy complications has been reviewed elsewhere (Akison *et al.* 2017). However, one hypothesis suggests that, in diabetic pregnancies, the placenta stores excess glucose as glycogen to protect the fetus from hyperglycaemia, thus limiting fetal overgrowth (Desoye *et al.* 2002).

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Considerable constraints limit our ability to explore placental glycogen function in humans, such as the lack of available tissue at the appropriate developmental stage. Accordingly, it is necessary to turn to the mouse model, which benefits from large litter sizes, a short gestation period that allows for the investigation of early pregnancy and a similar genome to humans that is easily manipulated. There are 47 known mouse models with targeted mutations that display phenotypes associated with the trophoblast cell subtype that stores glycogen. A common feature among these placental mutants is that fetal growth is co-ordinately affected. Therefore, the use of these mutant mouse lines will help to elucidate the normal physiological role of placental glycogen and may provide further insight to the relevance of placental glycogen in the human placenta during normal and complicated pregnancies.

Placental development in the mouse

The mouse is a particularly useful model for the human placenta, since they are both hemochorial, architecturally similar and express many of the same genes that regulate placental development and function (Rossant & Cross 2001). In particular, imprinted genes, which are characterized by expression derived wholly, or predominantly, from one or other parental allele, exert significant influence on placental development in both humans (Frost & Moore 2010) and mice (Tunster *et al.* 2013). Furthermore, the mouse placenta also accumulates glycogen stores in a specialized trophoblast cell subtype called the glycogen trophoblast (GlyT), some of which interact intimately with the maternal decidua (Rampon *et al.* 2005, Coan *et al.* 2006, Bouillot *et al.* 2006). For this reason, GlyT cells share similarities with human cytotrophoblast cells and establish the mouse as an ideal model in which to elucidate the function(s) of placental glycogen stores.

In the mouse, GlyT cells appear as clusters in the junctional zone (Jz) of the mature placenta, before a proportion of these cells invade the maternal decidua to congregate around maternal spiral arteries (Adamson *et al.* 2002). When considering how defects in placental glycogen storage and metabolism emerge, it is important to consider when GlyT first arise during placental development and whether defective GlyT differentiation is the primary cause of altered placental glycogen content. Development of the mouse placenta under normal circumstances has been extensively reviewed elsewhere (Rossant & Cross 2001, Watson & Cross 2005). Briefly, by implantation at embryonic day (E) 4.5, the mouse blastocyst comprises a layer of trophectoderm that surrounds the inner cell mass (ICM). The trophectoderm cells immediately adjacent to the ICM proliferate to give rise to two main trophoblast progenitor populations: the ectoplacental cone (EPC), which will differentiate to yield cells of the centrally located Jz that is predominantly endocrine in function and the extraembryonic ectoderm (ExE), which will eventually give rise to the labyrinth zone (Lz) where, when vascularized by fetal capillaries, nutrient, gas and waste exchange between maternal and fetal blood circulations occurs (Fig. 1).

To date, nine trophoblast cell sub-types are known to differentiate from these progenitor populations. These include the spongiotrophoblast (SpT) and GlyT cells, which share a common EPC progenitor and together with parietal trophoblast giant cells (P-TGCs) comprise the mature Jz. SpT cells function predominantly in the production of hormones, including members of the *Prl* (prolactin/placental lactogen-related) and *Psg* (pregnancy specific glycoprotein) gene families. The GlyT lineage is named for the abundant stores of glycogen that they accumulate and metabolize during gestation. In total, five TGC sub-types have been identified. Based upon lineage tracing, gene-expression profiles and spatial localization within the placenta (Fig. 1), it is hypothesized that most of the TGCs also derive from the EPC (Simmons *et al.* 2007, Gasperowicz *et al.* 2013). TGCs associate with the maternal blood sinuses throughout the placenta and secrete hormones into the maternal circulation to modulate maternal physiology and placenta function. Sinusoidal-TGCs (S-TGCs) are located within the Lz, and together with a bilayer of syncytiotrophoblast (SynT-I and SynT-II) cells derived from the ExE, they create a trilaminar cellular arrangement that separates the maternal circulation from the extensively branched fetal vasculature (Adamson *et al.* 2002, Watson & Cross 2005, Simmons *et al.* 2008*a*).

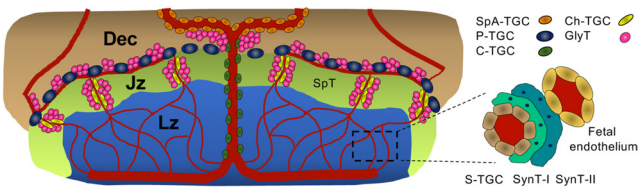

Figure 1 Structure and trophoblast lineages of the mature mouse placenta: The mature mouse placenta comprises three structurally and functionally distinct layers: the maternal decidua (Dec), junctional zone (Jz) and labyrinth zone (Lz). The junctional zone predominantly comprises the spongiotrophoblast (SpT) and non-migratory glycogen trophoblast (GlyT) cells. A proportion of GlyT migrate to the maternal decidua where they associate with maternal spiral arteries bringing maternal blood to the placenta. Five trophoblast giant cell sub-types have been described based on the localization on gene expression patterns: the parietal TGCs (P-TGCs) form a discontinuous cell layer at the boundary between the Jz and maternal decidua; spiral artery-associated TGCs (SpA-TGCs) line maternal spiral arteries through the decidua; canal-TGCs (C-TGCs) line maternal blood canals through the Jz and Lz; sinusoidal-TGCs (S-TGCs) replace the endothelial layer of maternal blood sinuses within the Lz and channel-TGCs (Ch-TGCs) surround the channels that traverse the Jz draining maternal blood from the placenta. Together with the S-TGCs, two layers of syncytiotrophoblast form the trilaminar structure of the murine labyrinth.

Based on lineage tracing experiments (Simmons & Cross 2005), GlyT cells share a common *Tpbpa*$^{+}$ EPC progenitor with SpT cells and arise early in development. Periodic acid-Schiff (PAS) staining, which is a general staining method used to detect polysaccharides (e.g. glycogen) in addition to mucosubstances (e.g. glycoproteins and glycolipids), demonstrates that small quantities of glycogen are stored by GlyT from ~E5.5 (Tesser *et al.* 2010), suggesting that GlyT are specified soon after implantation. An early lineage split of the GlyT and SpT populations is further suggested by expression of the GlyT-specific marker genes *Pcdh12* and *Aldh1a3* in a subset of EPC cells from ~E7.5 and ~E8.5, respectively (Rampon *et al.* 2005, Bouillot *et al.* 2006, Outhwaite *et al.* 2015). GlyT number expands nearly 300-fold between E12.5 and E16.5 before declining ~60% by E18.5 (Coan *et al.* 2006). This expansion coincides with a marked increase in PAS staining from E12.5 (Adamson *et al.* 2002), which distinguishes GlyT from the adjacent SpT cells (Fig. 2A, B and C). By comparison, the SpT population increases less than four-fold during the same period, with a modest ~20% reduction by E18.5 (Coan *et al.* 2006). During this developmental time frame, placental glycogen stores peak at ~E15.5 before declining by ~50% at E18.5 (Lopez *et al.* 1996) as the GlyT cell population diminishes. Even though both GlyT and SpT cells continue to express the Jz-marker gene *Tpbpa* in the mature placenta (Lescisin *et al.* 1988) (Fig. 2D and E), other GlyT-specific markers have been described, including the gap-junction genes *Gjb3* (*Cx31*) and *Gjb5* (*Cx31.1*), which are localised to GlyT cells from ~E12.5 (Coan *et al.* 2006) and E13.5 (Zheng-Fischhofer *et al.* 2007), respectively, alongside persistent expression of *Pcdh12* (Fig. 2F, G and H).

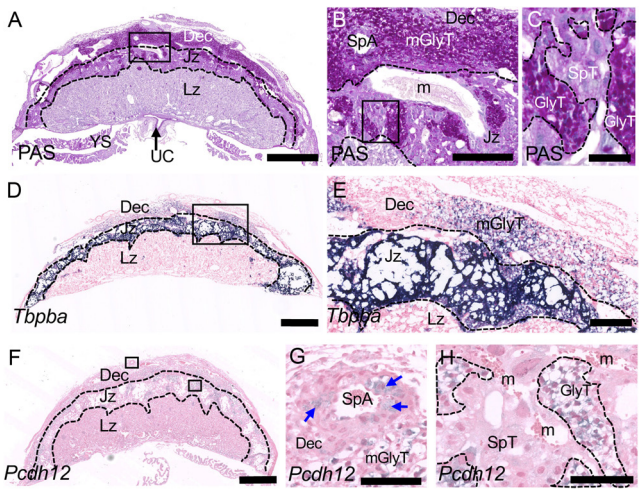

Figure 2 Histological methods for assessing glycogen trophoblast cells in the mouse placenta at E14.5. Histological sections of WT (C57BL/6J) mouse placentas at E14.5 stained using (A, B and C) Periodic acid-Schiff (PAS) stain (dark pink) that labels glycogen, glycoproteins and glycolipids, (D and E) *Tpbpa* RNA via an *in situ* hybridization probe (dark purple) that stains cells derived from the ectoplacental cone (e.g. spongiotrophoblast (SpT) and glycogen trophoblast (GlyT)) and (F, G and H) *Pcdh12* RNA via an *in situ* hybridization probe (dark purple) that stains GlyT. Nuclei are stained using (A, B and C) haematoxylin (blue) or (D, E, F, G, H) nuclear fast red (pink). Dec, maternal decidua; Jz, junctional zone; Lz, labyrinth zone; YS, yolk sac; UC, umbilicus (black arrow); SpA, spiral artery; m, maternal blood sinusoid; mGlyT, migratory GlyT (blue arrows). Dotted lines separate major placenta layers or trophoblast cell populations. Boxes represent regions shown in higher magnification to the right. Scale bars: A, D, F = 1 mm; B, E = 250 µm; C = 50 µm; G, H = 100 µm.

The putative role of placental glycogen

Studies in animal models have led to the hypothesis that placental glycogen stores provide a source of glucose to support fetal growth during late gestation (Coan *et al.* 2006). This hypothesis is supported by two observations: glycogen acts as the primary energy store in animals and placental glycogen diminishes toward the end of pregnancy coincident with a period of rapid fetal growth. However, little experimental evidence currently exists to directly support such a function. The location of GlyT cells next to maternal blood sinuses may provide some indication as to the ultimate destination of the glucose released from placental glycogen stores. For instance, from ~E12.5, some GlyT invade the decidua where they localize in close proximity to maternal spiral arteries and by E16.5 account for ~30% of the total GlyT population (Redline *et al.* 1993, Coan *et al.* 2006, Gasperowicz *et al.* 2013) (Fig. 1). Based on this association, it is possible that migratory GlyT cells metabolize glycogen into glucose for transport into the maternal blood entering the placenta for eventual fetal uptake. Indeed, large lacunae form in the decidua by E17.5 that are presumed to result from the lysis of multiple GlyT in the vicinity (Bouillot *et al.* 2006). In contrast, populations of non-migratory GlyT cells cluster in close proximity to channels in the Jz that drain maternal blood from the placenta (Gasperowicz *et al.* 2013) (Fig. 1). Whether these GlyT cells release glucose or other factors, such as hormones, into maternal blood to influence maternal physiology in preparation for parturition and lactation (Napso *et al.* 2018) to indirectly support fetal growth and well-being is yet to be determined. Beyond location, the migratory and non-migratory GlyT populations are distinguishable by expression of distinct *Prl* genes: migratory GlyT express *Prl7b1*, while non-migratory GlyT express *Prl6a1* (Simmons *et al.* 2008*b*). Further experiments are necessary to explore the functional differences between these GlyT sub-types. Simultaneously, it is also possible that placental glycogen stores might provide energy directly to the placenta, since it is a highly metabolic organ that consumes a considerable proportion of the glucose delivered to it (Hay 1995). Indeed, placental glycogen stores might fuel hormone production by the placenta (e.g. *Prl* and *Psg* gene family members), which

increases dramatically toward the term (McLellan *et al.* 2005, Simmons *et al.* 2008*b*).

How placental glycogen is metabolized is not well understood. Glucagon might play a role in stimulating the release of glycogen stores from GlyT (Coan *et al.* 2006). Yet, placental glycogen content is unaltered in mice deficient for the glucagon receptor (Ouhilal *et al.* 2012), indicating that an alternative pathway might be involved. However, only a small number of glucagon-receptor mutants were examined at a single developmental time-point with no consideration of fetal sex. Fetal sex was shown to influence the placental glycogen content, at least in the spiny mouse (O'Connell *et al.* 2013). Therefore, a role for glucagon in regulating glycogen metabolism cannot currently be excluded. Additional models are required to more fully understand the enzymes that are important for glycogen metabolism in the mouse placenta.

Common functionality of genes involved in GlyT development and function

Of the 47 mutant mouse lines that are known to exhibit GlyT phenotypes (with or without aberrant glycogen storage), 38 different genes are represented. As the list lengthens, common genetic pathways and gene classifications have emerged as important players in GlyT formation and function. The majority of these genes can be assigned to at least one of three functional groups (Table 1): imprinted and X-linked genes, cell signalling genes and genes involved in transcriptional regulation.

Imprinted and X-linked genes

Imprinted genes represent an unusual class of autosomal gene that are characterized by complete or partial parental-allele-biased expression. This means that gene expression is derived wholly, or predominantly, from either the maternally inherited allele (i.e. maternally expressed) or paternally inherited allele (i.e. paternally expressed) in one or more tissues. In excess of 100 imprinted genes have been reported in the mouse, many of which are known to regulate placental development and function (Tunster *et al.* 2013). A broad role for imprinted genes in regulating the GlyT lineage has been reviewed previously (Lefebvre 2012), whereby four genes (*Ascl2*, *Cdkn1c*, *Igf2* and *Phlda2*) that reside within a ~1 Mb imprinted region on mouse distal chromosome 7 were discussed in detail. In addition to these genes, the paternally expressed *Dlk1*, *Peg3* and *Peg10* genes are also implicated in regulating the GlyT lineage and/or placental glycogen stores (Table 1), further emphasizing the importance of imprinted genes in regulating placental glycogen stores.

A special class of imprinted genes is the X-chromosome-linked genes. To ensure similar gene dosage between males and females, one X chromosome in female cells is silenced. In extraembryonic lineages of female mouse conceptuses, the paternally inherited X-chromosome is preferentially silenced (Takagi & Sasaki 1975). A role for X-linked genes in regulating placental glycogen storage is demonstrated by mice that inherited only a single paternally inherited X chromosome (XpO) and display an expansion of the GlyT population (He *et al.* 2017). In support of this hypothesis, GlyT phenotypes were reported to varying degrees in loss-of-function models of four X-linked genes (i.e. *Plac1*, *Ldoc1*, *Wdr1* and *Esx1*) (Table 1). The functional convergence of imprinted and X-linked genes in regulating placental glycogen storage is consistent with the established role of imprinted genes in modulating fetal nutrient supply across the placenta (Angiolini *et al.* 2006, Tunster *et al.* 2013) and with the purported function of placental glycogen stores in providing an energy source to support fetal growth during late gestation. With the majority of imprinted genes exhibiting placental expression in the mouse, it will be of interest to explore whether other imprinted genes are involved in regulating placental glycogen storage.

Cell-cell signalling

Studies of mutant mouse lines that report glycogen storage defects that arise secondary to SpT phenotypes implicate a role for SpT-derived signals in the regulation of placental glycogen metabolism. For instance, genetic knockout or overexpression of the maternally expressed imprinted gene *Phlda2* causes an expansion or reduction of the SpT population, respectively (Tunster *et al.* 2010, 2014, 2015). Unaltered expression of the GlyT marker genes *Pcdh12* and *Gjb3* suggested that there was no overt effect on GlyT population size in either model. However, placental glycogen content closely paralleled the SpT phenotype, with a reduced SpT population leading to diminished glycogen content and an expanded SpT population associated with increased glycogen storage (Tunster *et al.* 2010, 2014, 2015). These studies implicate cell-to-cell signalling between SpT and GlyT, though the specific pathways that mediate this effect are unclear. A clue might lie within the *Crim1* knockout model. *Crim1* encodes for a transmembrane protein that mediates signal transduction by binding growth factors (e.g. vascular endothelial growth factor A; VEGF-A) to the cell surface. While *Crim1* mRNA expression is limited to SpT cells, the GlyT population expands in the absence of CRIM1 function (Pennisi *et al.* 2012). Therefore, CRIM1 might regulate a SpT-derived factor that signals to GlyT to regulate their proliferation (Pennisi *et al.* 2012).

Further evidence for a role of SpT-derived signals in modulating placental glycogen stores is provided by a comparison of *Phlda2* null conceptuses and their WT littermates with strain-matched control litters. Loss of

Table 1 Classification and expression of genes implicated in regulating GlyT differentiation and/or function.

Symbol	Gene name	Function	Spatial expression	GlyT differentiation†	Glycogen storage††	References
Imprinted and X-linked genes						
Ascl2	*Achaete-scute complex homolog 2*	Maternally expressed basic helix-loop helix transcription factor	Highly expressed in both EPC and ExE during early development. Expression declines toward the term	✓	✓	Guillemot *et al.* (1994), Rossant *et al.* (1998)
Cdkn1c	*Cyclin-dependent kinase inhibitor 1C*	Maternally expressed cyclin-dependent kinase inhibitor	Widely expressed with high expression in GlyT	×	✓	Matsuoka *et al.* (1995), Georgiades *et al.* (2002), Coan *et al.* (2006)
Dlk1	*Delta-like homolog 1*	Paternally expressed transmembrane glycoprotein. Encodes the precursor to fetal antigen 1 (FA1) that functions as a Notch antagonist.	Expressed in endothelial cells lining the fetal vasculature of the Lz. Not expressed in any trophoblast lineage.	✓	n/a	Laborda (2000), Baladron *et al.* (2005), Yevtodiyenko & Schmidt (2006)
Igf2	*Insulin-like growth factor 2*	Paternally expressed insulin-like growth factor 2	Highly expressed in Jz and Lz between E9.5 and E12.5. Expression subsequently declines dramatically in the Lz and SpT but remains highly expressed in GlyT.	✓	✓	Redline *et al.* (1993), Carter *et al.* (2006)
Peg3	*Paternally expressed gene 3*	Paternally expressed zinc finger transcriptional repressor	Widely expressed in the developing placenta	✓	✓	Relaix *et al.* (1996)
Peg10	*Paternally expressed gene 10*	Paternally expressed retrotransposon-derived gene	Widely expressed in all trophoblast lineages	✓	n/a	Ono *et al.* (2006)
Phlda2	*Pleckstrin homology-like domain family A member 2*	Maternally expressed pleckstrin-homology domain protein that inhibits the AKT pathway by competing for PIP targets	Highly expressed in the EPC and ExE. Restricted to the chorionic plate and syncytiotrophoblast layers of the Lz by E10.5. Expression declines dramatically from E12.5. Very few PHLDA2-positive cells remain at E14.5	n/a	✓	Frank *et al.* (1999), Frank *et al.* (2002), Saxena *et al.* (2002)
Esx1	*Extraembryonic, spermatogenesis, homeobox 1*	X-linked paired-like homeobox domain protein	Expressed in the EPC and chorion before becoming restricted to the Lz by mid-gestation	✓	n/a	Li *et al.* (1997), Li & Behringer (1998)
Plac1	*Placental specific protein 1*	X-linked putative signal peptide	Placentaspecific expression from early to mid-gestation. EPC and TGCs at E7.5; P-TGCs, Jz and Lz, by E11.5; restricted to P-TGCs by E14.5	n/a	✓	Cocchia *et al.* (2000)
Ldoc1	*Regulator of NFKB signalling*	X-linked gene derived from long terminal repeat retrotransposon	Highly expressed in TGCs, EPC and ExE at E8.5. Restricted to non-migratory GlyT by E15.5 and absent by E18.5.	×	n/a	Naruse *et al.* (2014)
Wdr13	*WD repeat domain 13*	X-linked member of WD-repeat protein family	Expressed predominantly in the Lz. Lower expression in the SpT and decidua. Not expressed in GlyT	n/a	✓	Singh *et al.* (2015)
Cell-cell signalling						
Akt1	*Thymoma viral proto-oncogene 1*	Encodes protein kinase Bα (PKBα), a downstream effector of the phosphatidylinositol 3-kinase (PI3K) signalling pathway	Widely expressed in all trophoblast lineages and fetal endothelium	n/a	✓	Yang *et al.* (2003)

(Continued)

Table 1 Continued.

Symbol	Gene name	Function	Spatial expression	GlyT differentiation†	Glycogen storage††	References
Crim1	*Cysteine rich transmembrane BMP regulator 1*	Transmembrane protein that binds growth factors such as VEGF-A to the cell surface	Primarily expressed in SpT. Low expression in P-, C- and syncytiotrophoblast	n/a	×	Pennisi *et al.* (2007), Wilkinson *et al.* (2007), Pennisi *et al.* (2012)
Csf2	*Colony stimulating factor 2 (granulocyte-macrophage)*	Encodes the cytokine granulocyte-macrophage colony-stimulating factor 2	Expressed in the decidua and Jz	✓	n/a	Kanzaki *et al.* (1991), Robertson *et al.* (1999)
Egfr	*Epidermal growth factor receptor*	Encodes epidermal growth factor receptor (EGFR)	Expressed in Jz and Lz at E18.5	✓	✓	Dackor *et al.* (2007)
HtrA1	*HtrA serine peptidase 1*	Serine protease that functions as an antagonist of TGF-β signalling	Expressed in P-TGCs at E7.5. Expression is barely detectable by E13.5.	×	×	Oka *et al.* (2004), Nie *et al.* (2005), Hasan *et al.* (2015)
Hectd1	*HECT domain E3 ubiquitin protein ligase 1*	Ubiquitin ligase that functions as a negative regulator of Wnt signalling	Widely expressed in all regions of the placenta between E7.5 and E13.5.	✓	✓	Tran *et al.* (2013), Sarkar *et al.* (2014)
Igfbp1	*Insulin-like growth factor binding protein 1*	Carrier protein for insulin-like growth factors	Strongly expressed in the yolk sac endoderm but not expressed in trophoblast lineages	✓	n/a	Hwa *et al.* (1999), Carter *et al.* (2006)
Pcdh12	*Protocadherin 12*	Member of the protocadherin subgroup of the cadherin family of transmembrane calcium-dependent cell-adhesion proteins	Expressed exclusively in GlyT lineage throughout gestation	n/a	✓	Rampon *et al.* (2005)
Pgf	*Placental growth factor*	Encodes the pro-angiogenic placental growth factor (PlGF)	Widely expressed in the placenta at E10.5	✓	✓	Achen *et al.* (1997), Tayade *et al.* (2007)
Pik3ca	*Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha*	Encodes the ubiquitously expressed PI3-kinase catalytic subunit p110α	Ubiquitously expressed	n/a	✓	Foukas *et al.* (2006)
Prl7d1	*Prolactin family 7, subfamily d, member 1*	Member of the prolactin/placental lactogen (*Prl/Pl*) gene family	Widely expressed in all trophoblast subtypes; highest expression in GlyT	✓	✓	Simmons *et al.* (2008*a*)
Ptp4a2	*Protein tyrosine phosphatase 4a2*	Protein phosphatase	Widely expressed in all trophoblast lineages	✓	✓	Dong *et al.* (2012)
Pthlh	*Parathyroid hormone-like peptide*	Encodes the constitutively expressed parathyroid hormone-related peptide (PTHrP)	Constitutively expressed	n/a	✓	Wysolmerski & Stewart (1998), Duval *et al.* (2017)
sFlt-1	*Soluble fms related receptor tyrosine kinase 1*	An anti-angiogenic factor that sequesters placental growth factor (PGF) and vascular endothelial growth factor (VEGF)	Expressed in the EPC and later the Jz	✓	n/a	Kendall & Thomas (1993), Breier *et al.* (1995), Levine *et al.* (2006)
Transcriptional regulators						
Arnt	*Aryl hydrocarbon receptor nuclear translocator*	Encodes the HIF1β subunit of the heterodimeric transcription factor hypoxia inducible factor (HIF)	Constitutively expressed	✓	n/a	Adelman *et al.* (2000)

Bahd1	*Bromo adjacent homology domain containing 1*	Chromatin modifier that mediates heterochromatin-associated gene silencing. Implicated in the silencing of *IGF2* in humans	Not reported	n/a	✓	Bierne *et al.* (2009)
Cited2	*Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2*	Transcriptional co-factor	Expressed in all trophoblast lineages.	✓	n/a	Dunwoodie *et al.* (1998), Withington *et al.* (2006)
Hif1a	*Hypoxia inducible factor 1, alpha subunit*	Encodes the HIF1α subunit of the heterodimeric transcription factor hypoxia inducible factor (HIF)	Constitutively expressed but HIF1A protein is degraded in normoxia.	✓	n/a	Kozak *et al.* (1997), Cowden Dahl *et al.* (2005)
miR-126a	*microRNA 126a*	Endothelial-specific microRNA	Widely expressed in fetal endothelium of Lz, syncytiotrophoblast, P-TGC, SpT and GlyT.	✓	✓	Wang *et al.* (2008), Sharma *et al.* (2019)
Prdm1	*PR domain containing 1, with ZNF domain*	Zinc-finger transcriptional repressor	Expressed in GlyT progenitors at E7.5. Highly expressed in SpA-TGCs.	n/a	n/a	Mould *et al.* (2012)
Tfap2c	*Transcription factor AP-2, gamma*	Transcription Factor AP-2 Gamma	Expressed in the trophectoderm at E4.5. Restricted to Jz and TGCs by E11.5.	✓	✓	Kuckenberg *et al.* (2010)
Sp1 & Sp3	*Trans-acting transcription factor 1 & 3*	Zinc-finger transcription factors	Ubiquitously expressed	n/a	n/a	Kruger *et al.* (2007)
Miscellaneous						
Cts7	*Cathepsin 7*	Placenta-specific papain-like cysteine cathepsin protease that functions in lysosomal proteolysis	Highly expressed in TGCs at E7.5; expression declines and is restricted to the Lz by E15.5	✓	n/a	Hemberger *et al.* (2000), Deussing *et al.* (2002)
Fbxl12	*F-box and leucine-rich repeat protein 12*	Member of the F-box family of proteins, which function as the substrate recognition component in E3 ubiquitin ligase complexes	Jz specific at E15.5	n/a	✓	Nakayama & Nakayama (2006), Nishiyama *et al.* (2015), Zheng *et al.* (2016)
Pdcd5	*Programmed cell death 5*	Promoter of both apoptotic and the non-apoptotic programmed cell death pathway (paraptosis)	Not reported	✓	✓	Liu *et al.* (1999), Wang *et al.* (2004)
Tex19.1	*Testis expressed gene 19.1*	Mammalian-specific hypomethylation-sensitive genome-defence gene	Widely expressed in the EPC and Jz; some patchy expression in Lz	✓	n/a	Celebi *et al.* (2012), Reichmann *et al.* (2013)

[†]Changes in GlyT abundance or maturation implied an effect in GlyT differentiation. [††]Altered regulation of glycogen stores is suggested by an effect on total placental glycogen content (see Tables 2, 3, 4 and 5 for details and references).
E, embryonic day; EPC, ectoplacental cone; ExE, extraembryonic ectoderm; GlyT, glycogen trophoblast cells; Jz, junctional zone; Lz, labyrinth zone; n/a, data not available; P, postnatal day; PAS, Periodic Acid Schiff stain; SynT-II, syncytiotrophoblast cell layer II; TGC sub-types: C-TGC, canal TGC; P-TGC, parietal TGC; S-TGC, sinusoidal TGC; SpA-TGC, spiral artery associated TGC; TGC, trophoblast giant cell.

function of *Phlda2* results in expansion of the Jz (Frank *et al.* 2002), which is attributable to a disproportionate increase in the SpT population (Tunster *et al.* 2015). While there was no overt effect on GlyT cell number, an accumulation of glycogen in placentas from *Phlda2* null their WT littermates was apparent at E18.5. One explanation might be that the entire litter was exposed to an enhanced endocrine-signalling environment caused by SpT expansion in *Phlda2* null placentas resulting in excessive placental glycogen storage that potentially deprives the fetuses of nutrients (Tunster *et al.* 2015). However, it is not possible to exclude a role for broader effects on maternal physiology in impairing fetal growth in this model.

We have further identified fourteen genes that encode for specific components of cell-signalling pathways that might help to establish the importance of cell-cell signalling in the regulation of placental glycogen content (Table 1). These genes include signalling molecules such as cytokines (*Csf2*), hormones (*Pthlh, Prl7d1*) and growth factors (*Pgf*), receptors (*Egfr*) and downstream effectors, such as kinases (*Akt1*, *Pik3ca*) and phosphatases (*Ptp4a2*). Some of these genes encode for key intermediates in a diverse range of signalling pathways, namely VEGF (e.g. *Crim1*), epidermal growth factor (EGF) (e.g. *Egfr*) and transforming growth factor (TGF) (e.g. *HtrA1*) pathways.

In particular, aberrant AKT signalling is implicated in GlyT phenotypes associated with genetic knockouts of *Ptp4a2*, *Tfap2c*, *Igf2* or *Phlda2* (Frank *et al.* 1999, Saxena *et al.* 2002, Dong *et al.* 2012, Takao *et al.* 2012, Sharma *et al.* 2016, Sferruzzi-Perri *et al.* 2017). This, combined with the fact that loss of function of *Akt1* itself impairs placental glycogen storage (Yang *et al.* 2003), indicates that the AKT pathway is key to this process. AKT signalling is known to regulate specific cellular processes including growth, proliferation, metabolism and survival (Yu & Cui 2016, Hermida *et al.* 2017, Manning & Toker 2017). Specific to its role in glycogen storage, AKT also regulates glycogenesis through the inhibition of glycogen synthase kinase 3 (GSK3), a negative regulator of glycogen synthase (Cross *et al.* 1995, Diehl *et al.* 1998). Other models that show changes in the expression of *Igf2* or *Phlda2* (e.g. *Pcdh12* knockout (Rampon *et al.* 2008)) might also implicate AKT-signalling defects in their GlyT phenotypes. While aberrant AKT signalling was reported in the placentas of human FGR infants (Yung *et al.* 2008), placental glycogen storage has not yet been investigated in the context of human FGR to date.

Ablation of *Akt1*, which encodes for protein kinase Bα (PKBα), causes fetal and placental growth restriction (Yang *et al.* 2003). A near complete loss of glycogen-containing trophoblast cells was reported (Yang *et al.* 2003), though this result was based solely on examination of PAS-stained placenta sections. The absence of PAS staining is more likely to indicate a failure to accumulate glycogen, rather than ablation of the GlyT lineage. Therefore, this model should be revisited for a more detailed analysis of the GlyT phenotype, including determination of spatiotemporal expression of markers such as *Pcdh12* (Bouillot *et al.* 2006), *Gjb3* (Coan *et al.* 2006), *Aldh1a3* (Outhwaite *et al.* 2015) and biochemical analysis of glycogen content (Lo *et al.* 1970). Regardless, the result is consistent with the role of AKT in regulating GSK3 and, indirectly, glycogen synthase (Yang *et al.* 2003).

Upstream regulators of AKT signalling include PRL2 and IGF2, both of which have been implicated in placental development. The *Ptp4a2* gene encodes for the protein phosphatase PRL2 and, when knocked out, the placentas were small in size due to a substantially reduced Jz defined by a near complete loss of SpT with only a few non-migratory GlyT remaining (Dong *et al.* 2012). While PAS staining was reduced, placental glycogen content was not directly quantified (Dong *et al.* 2012). *Ptp4a2*$^{-/-}$ placentas also exhibited reduced AKT phosphorylation (Dong *et al.* 2012), indicating that PRL2 is important for this process. The associated *Ptp4a2*$^{-/-}$ fetuses were growth restricted at E16.5. In fetal tissues, AKT and MAPK (mitogen activated protein kinase) pathways are activated via IGF2 signalling mediated primarily through binding IGF1R (insulin-like growth factor 1 receptor) (Forbes & Westwood 2008, Sferruzzi-Perri *et al.* 2017). However, the absence of a placental phenotype in *Igf1r* null mice suggests that IGF2 acts through an alternative, unknown receptor in the placenta (Baker *et al.* 1993, Efstratiadis 1998). Whether IGF2 activates AKT signalling in the placenta is unclear, and a detailed assessment of AKT signalling in the placenta of *Igf2* mutants is required. Additionally, further work is warranted to explore the broad role of AKT signalling in regulating the placental glycogen stores by utilizing mouse models with known GlyT phenotypes.

Transcriptional regulators

The GlyT population is highly dynamic, with the number of GlyT cells increasing by nearly 300-fold between E12.5 and E16.5, and switching from an apparently glycogenic state to a glycogenolytic state (Lopez *et al.* 1996). These changes implicate major shifts in transcriptional regulation to modulate placental glycogen metabolism. A broad role for transcriptional regulators in modulating placental glycogen storage and/or the GlyT lineage has not been previously recognized. We identified eight genes encoding specific transcriptional regulators that are implicated in the development and/or function of the GlyT lineage (Table 1). One example is the endothelial-specific microRNA *miR-126a* (*miR-126*), which when ablated leads to global hypermethylation associated with dysregulated expression of placenta-

specific genes (e.g. *Prl6a1*, *Pcdh12* and *Tpbpa*) and imprinted genes (e.g. *Igf2*, *Phlda2* and *Cdkn1c*) (Sharma *et al.* 2019). Genetic ablation of *miR-126a* results in Jz hyperplasia, attributable to a specific expansion of the GlyT population, without a change in SpT abundance. Consequently *miR-126a*$^{-/-}$ placentas accumulated ~50% more glycogen than controls and were associated with fetal growth restriction at E15.5 (Sharma *et al.* 2019). The specific targets of *miR-126a* are yet to be determined. Further work is required to fully elucidate the transcriptional networks involved in regulating the GlyT lineage and glycogen storage.

Using genetic mouse models to understand the function of placental glycogen

The association of altered placental glycogen storage with human pregnancy complications supports an important role for glycogen in achieving a successful pregnancy outcome (reviewed in Akison *et al.* 2017). The majority of mouse models with GlyT phenotypes show evidence of fetal growth restriction (FGR), which is consistent with the putative role of placental glycogen in supporting fetal growth (Tables 2 and 3). In contrast, the remaining models showed GlyT phenotypes that were either associated with normal, enhanced or undetermined effects on fetal growth (Table 4) or embryonic lethality around mid-gestation (Table 5) that precluded a meaningful assessment of fetal growth.

In our evaluation of the literature, we observed that the extent to which GlyT phenotypes are characterized varies considerably between studies. Indeed, only a minority of these models were assessed using a direct biochemical determination of placental glycogen content. Instead, reduced glycogen storage or mobilization was inferred from one or more of the following methods: reduced (or absent) PAS staining; reduced GlyT abundance as determined by histology and/or reduced genetic marker expression for GlyT cells and mislocalization of GlyT within the Lz or a failure of GlyT cells to migrate to the decidua. As a result, there is a need for established criteria in describing GlyT phenotypes and glycogen storage and metabolic defects. We identified four key parameters for characterizing GlyT phenotypes: (1.) GlyT lineage specification and differentiation by assessing lineage marker expression, (2.) GlyT cell number, (3.) GlyT localization and degree of migration into the decidua and (4.) quantification of total placental glycogen content using a biochemical assay (Lo *et al.* 1970). A collective evaluation of these genetic mutants using these criteria will help to separate GlyT developmental defects from metabolic/storage defects and improve our understanding of how placental glycogen stores support growth.

Reduced placental glycogen content is frequently associated with fetal growth restriction

Several mouse models that demonstrate a glycogen storage deficit are also associated with FGR (Table 2). It is well known that IGF2 is an important regulator of fetal and placental growth (DeChiara *et al.* 1990, Constância *et al.* 2005). *Igf2* mRNA is highly expressed in the GlyT lineage (Redline *et al.* 1993, Georgiades *et al.* 2002, Coan *et al.* 2006), and constitutive deletion of *Igf2* results in reduced GlyT abundance and placental glycogen content (Lopez *et al.* 1996). Deletion of the placenta-specific *Igf2* transcript (*Igf2P0*) results in a similar reduction of GlyT cell number (Sferruzzi-Perri *et al.* 2011), even though the *Igf2P0* transcript accounts for only 10% of total placental *Igf2* (Moore *et al.* 1997). While constitutive deletion of *Igf2* results in a 50% reduction in fetal weight (DeChiara *et al.* 1990), *Igf2P0* mutants are 25% lighter than controls (Constância *et al.* 2005), at least partially attributing FGR to a placental defect. However, the contribution of placental glycogen is unclear, as glycogen content was not directly assessed in the *Igf2P0* mutants.

Human placentas exhibiting elevated expression of *PHLDA2* are frequently linked with FGR (reviewed in Jensen *et al.* 2014), although placental glycogen stores have not been investigated in these placentas. Analysis of a mouse model with overexpression of *Phlda2* demonstrates that placental glycogen content is reduced by 50% of controls. However, the effect on glycogen storage is likely secondary to a reduction in the *Prl8a8*$^{+}$ SpT population, since there was no overt effect on expression of the GlyT markers *Pcdh12* or *Gjb3* (Tunster *et al.* 2010, 2014, 2015). The effect on fetal growth in the *Phlda2* overexpression model is complex and dependent upon the genetic background of the mouse, even though similar placental defects are apparent between strains. For instance, fetal growth was asymmetrically restricted when on the 129S2/SvHsd background (Salas *et al.* 2004, Tunster *et al.* 2010), but was unaffected on the C57BL/6 background (Tunster *et al.* 2014). Since genetically WT placentas on the C57BL/6 background naturally accumulate more than twice the glycogen content of 129S2/SvHsd placentas (Tunster *et al.* 2012), fetal growth might be protected on the C57BL/6 background.

Even though GlyT are important for fetal growth, these cells are not essential to fetal survival. GlyT cells were absent in mutant mouse lines, whereby the expression of *Ascl2* was at ~50% of endogenous levels (*Ascl2*$^{LacZ/Del7AI}$ or *Del*^{7AI}/+ mutants). Placentas either demonstrated a lack of PAS stain and/or *Pcdh12* expression at E15.5. The associated fetuses survived to term, albeit exhibiting growth restriction of 15–20% (Lefebvre *et al.* 2009, Oh-McGinnis *et al.* 2011, Bogutz *et al.* 2018). In contrast, *Ascl2* null placentas lack the EPC progenitor cells that give rise to the SpT and GlyT resulting in embryonic

Table 2 Genetic mouse models with fetal growth restriction and placentas with reduced glycogen content and/or GlyT phenotypes.

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content	GlyT number	GlyT markers	GlyT localization	Lz phenotype	References
Akt$^{-/-}$	Knockout	↓ (E14.5, E16.5)	↓↓ (E14.5, E16.5)	↓ PAS	n/a	n/a	n/a	Reduced Lz size	Yang *et al.* (2003)
Del^{7Al}/+	*Ascl2* expressed reduced by 40%	↓ (P0)	↓↓ (E15.5)	n/a	↓↓↓↓ (E15.5)	↓ *Tpbpa* ↓↓↓↓ *Pcdh12*	n/a	Disorganized structure	Lefebvre *et al.* (2009), Oh-McGinnis *et al.* (2011)
Ascl2$^{LacZ/Del7Al}$	*Ascl2* expressed reduced by 60%	↓↓ (E15.5)	↓↓ (E15.5)	↓↓↓↓ PAS	↓↓↓↓ (E15.5)	↓ *Tpbpa* ↓↓↓↓ *Pcdh12*	n/a	Disorganized structure	Bogutz *et al.* (2018)
Bahd1$^{-/-}$	Knockout	↓↓ (E18.5)	n/a (↓ circumference and area at E18.5)	↓ PAS	n/a	n/a	n/a	Reduced Lz size	Lakisic *et al.* (2016)
Csf2$^{-/-}$	Mixed 129/Sv x C57BL/6 background	↓ (E16.5)	Normal (E16.5)	n/a	↓↓	n/a	n/a	Reduced Lz size	Kanzaki *et al.* (1991), Robertson *et al.* (1999)
Dlk1$^{+/-}$	Knockout*	↓ (E18.5, P7)	Normal (E12.5, E14.5, E16.5)	n/a	↑ progenitors ↓ mature GlyT	n/a	n/a	Reduced Lz size; vascularization defect	Yevtodiyenko & Schmidt (2006), Appelbe *et al.* (2013), Cleaton *et al.* (2016)
Egfr$^{-/-}$	Knockout	↓ (from E13.5)	↓ (from E11.5)	n/a	n/a	↓ *Tpbpa* (E13.5)	n/a	n/a	Sibilia & Wagner (1995)
Egfr^{wa2}	*Egfr* hypomorph	↓ (E18.5) (Effects are genetic background dependent)	↓↓ (variable, E15.5, E18.5)	↓ PAS	n/a	↓ *Tpbpa* ↓ *Pcdh12*	n/a	Increased expression of *Gcm1, Dlx3, Tcfeb*	Dackor *et al.* (2009*b*)
Fbxl12$^{-/-}$	Knockout	↓ (E17.5)	↓↓↓ (E17.5)	↓ PAS	n/a	↓ *Tpbpa* ↓ *Pcdh12* ↓ *Gjb3*	n/a	Vascularization defect	Nishiyama *et al.* (2015)
hIGFBP1-Tg	Over-expression of human *IGFBP1*	↓ (E11.5) Normal (E14.5)	↑ (E11.5)	n/a	↓ GlyT:SpT ratio (in females)	n/a	n/a	Increased Lz size	Crossey *et al.* (2002)
HtrA1$^{-/-}$	Knockout	↓ (E14.5)	↓ (E14.5)	n/a	n/a	↓ *Tpbpa*	Mislocalization of *Tpbpa*$^{+}$; PAS^{+} cells within Lz	n/a	Nie *et al.* (2005), Hasan *et al.* (2015)
Igf2$^{-/-}$ or *Igf2*$^{+/-}$	Fetal and placental knockout*	↓ (E16.0)	↓↓↓ (E15.5) ↓↓ (E18.5)	↓↓↓ glycogen (E15.5, E18.5)	↓↓↓ (E15.5, E18.5)	n/a	n/a	n/a	DeChiara *et al.* (1990), Redline *et al.* (1993), Lopez *et al.* (1996), Carter *et al.* (2006)
Igf2P0	Loss of placental-specific *Igf2* transcripts*	↓ (from E16.5)	↓ (E13.5) ↓↓ (E15.5, E18.5)	n/a	↓↓ (E15.5)	n/a	n/a	Reduced Lz size, increased trophoblast barrier thickness, reduced diffusional capacity	Sibley *et al.* (2004), Constância *et al.* (2005), Sferruzzi-Perri *et al.* (2011)
LV-hsFLT-1	Overexpression of human *sFLT-1*	↓ (E18.5)	↓ (E18.5)	n/a	↓↓ GlyT proportion of Jz	n/a	n/a	Reduced Lz size	Kuhnel *et al.* (2017)
Peg3$^{+/-}$	Knockout*	↓ (E17.5) ↓ (P0)	↓↓ (E14.5, E17.5)	↓↓ glycogen (E14.5)	↓↓ (males more affected than females)	↓ *Tpbpa* (in males only) *Pcdh12* and *Gjb3* normal	n/a	n/a	Kuroiwa *et al.* (1996), Relaix *et al.* (1996), Li *et al.* (1999), Curley *et al.* (2004), Thiaville *et al.* (2013), Lee *et al.* (2015), He *et al.* (2016), Tunster *et al.* (2018)

Phlda2$^{+/+BACx1}$ or *Phlda2*$^{+/+BACx3}$	2–4-fold overexpression of *Phlda2*	↓ (E18.5)	↓ (variable, from E12.5)	↓↓↓ glycogen ↓ PAS	n/a	↓↓↓ *Tpbpa* *Pcdh12* normal *Gjb3* normal	Impaired migration of PAS$^+$ cells to decidua	n/a	Tunster *et al.* (2010), Tunster *et al.* (2014)
Pik3ca$^{-/+}$ or *Pik3ca*$^{+/-}$	Knockout	↓ (E15.5)	↓ (E15.5, E18.5)	↓↓ glycogen (if dam is *Pik3ca*$^{+/-}$)	n/a	n/a	n/a	Reduction in Lz size, fetal capillary volume, exchange surface area and diffusion capacity dependent upon maternal genotype	Foukas *et al.* (2006), Sferruzzi-Perri *et al.* (2016)
Ptp4a2$^{-/-}$	Knockout	↓ (E16.5)	↓↓ (E16.5)	↓ PAS	↓ Non-migratory GlyT ↓↓↓↓ Migratory GlyT	↓ *Tpbpa*	Failure of *Tpbpa*$^+$; PAS$^+$ cells to migrate to decidua	Reduced Lz size, reduced transport capacity	Dong *et al.* (2012)
Pthlh$^{-/-}$	Knockout	↓ (from E16.5)	Normal (E12.5, E14.5, E16.5, E18.5)	↓ glycogen (E12.5, E14.5, E18.5) normal glycogen (E16.5)	n/a	↓ *Tpbpa*	n/a	Reduced Lz size, reduced transport capacity	Duval *et al.* (2017)
Ldoc1$^{-/+}$ or *Ldoc1*$^{-/-}$	Knockout	↓ (E16.5) Normal (E18.5)	Normal (E12.5) ↑ (E16.5, E18.5)	n/a	Normal	*Pcdh12* normal	Mislocalization of *Prl6a1*$^+$ cells in Lz	n/a	Naruse *et al.* (2014)
Sp1$^{+/-}$;*Sp3*$^{+/-}$	Embryonic lethal between E16.5 and birth	↓↓ (E16.5)	↓ (E14.5)	n/a	n/a	↓ *Tpbpa* ↓ *Pcdh12*	n/a	Disorganized structure	Kruger *et al.* (2007)
Tex19.1$^{-/-}$	Knockout	↓ (E18.5)	↓↓ (E18.5)	n/a	↓↓↓ (E18.5)	↓ *Tpbpa* ↓ *Pcdh12* ↓ *Gjb3*	n/a	Fewer S-TGCs	Celebi *et al.* (2012), Reichmann *et al.* (2013)
Tfap2c$^{+/-}$	Knockout	↓ (E14.5) ↓ (E18.5)	↓ (E14.5, E18.5; trend only)	n/a	n/a	n/a	Mislocalization of PAS$^+$ cells within Lz	Disrupted and hemorrhagic Lz	Kuckenberg *et al.* (2010), Kaiser *et al.* (2015)
Cre-Tpbpa: Tfap2c $^{-/-}$	Conditional knockout of *Tfap2c* in *Tpbpa*$^+$ cells	↓ (from E16.5)	n/a	↓↓ glycogen (E14.5)	↓↓↓↓ (E16.5)	↓ *Tpbpa* (E12.5) ↓↓↓↓ *Tpbpa* (E16.5) ↓ *Gjb3*	n/a	n/a	Sharma *et al.* (2016)
Wdr13$^{-/+}$	Knockout*	↓ (from E17.5)	↓ (E17.5, E18.5)	↓ PAS staining	n/a	n/a	n/a	Reduced Lz size, fewer S-TGCs, Increased MBS	Singh *et al.* (2015)

Developmental stage in brackets is the time assessed. Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining.
* denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.
↓ = <20% reduction; ↓↓ = 20–50% reduction; ↓↓↓ = >50% reduction; ↓↓↓↓ = undetected; ↑ = <20% increase; ↑↑ = 20–50% increase; ↑↑↑ = >50% increase. E, embryonic day; GlyT, glycogen trophoblast cells; Jz, junctional zone; Lz, labyrinth zone; MBS, maternal blood sinusoids; P, postnatal day; n/a, not available; SpT, spongiotrophoblast cells; S-TGCs, sinusoidal trophoblast giant cells; Tg, transgenic.

Table 3 Genetic mouse models with fetal growth restriction and increased placental glycogen content and/or GlyT phenotypes.

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localization	Lz phenotype	References
Ascl2-Tg	*Ascl2* over-expression	↓ (E18.5)	↓ (E12.5, E14.5) Normal (E16.5, E18.5)	↑↑ glycogen (E16.5) ↑↑↑ glycogen (E18.5)	n/a	↓↓↓ *Tpbpa* ↑↑ *Prl7b1* Normal *Pcdh12* Normal *Gjb3*	Mislocalisation of *Tpbpa*$^{+}$; PAS^{+} cells within Lz	n/a	Tunster *et al.* (2016)
Crim1$^{KST264/KST264}$	Hypomorphic generated by gene-trap insertion	↓ (E17.5)	↓ (from E13.5)	Normal PAS	n/a	↑ *Pcdh12*$^{+}$ cell area	n/a	Fewer S-TGCs	Pennisi *et al.* (2007), Pennisi *et al.* (2012)
Csf2$^{-/-}$	C57BL/6 background	↓ (E14.5) Normal (E17.5)	↓ (E17.5)	n/a	↑↑↑	↑ *Tpbpa*	n/a	n/a	Sferruzzi-Perri *et al.* (2009)
Esx1$^{-/+}$ or *Esx1*$^{-/-}$	Knockout*	↓↓ (P0)	↑↑↑ (E14.5) ↑↑ (E18.5)	n/a	↑	↑ *Tpbpa*	n/a	Vascularization defect. Failure of SynT-II formation	Li *et al.* (1997), Li & Behringer (1998)
miR-126a$^{-/-}$	Knockout, embryonic lethal (from E15.5)	↓ (E15.5)	Normal (E12.5, E15.5)	↑↑↑ glycogen (E15.5)	↑ (E13.5, E15.5)	↑ *Tpbpa* ↑ *Cdkn1c* (E15.5)	n/a	Increased Jz:Lz ratio (no effect on gross Lz size)	Sharma *et al.* (2019)
Pcdh12$^{-/-}$	Knockout	↓ (E12.5, E17.5)	↓ (E17.5)	↑↑↑ glycogen (E12.5) ↑↑ glycogen (E17.5)	n/a	n/a	Mislocalization of PAS^{+} cells within Lz	Reduced cell density	Rampon *et al.* (2005), Rampon *et al.* (2008)
Plac1$^{-/+}$ or *Plac1*$^{-/-}$	Knockout*	↓ (E16.5)	↑↑↑ (E16.5)	↑ PAS	n/a	n/a	n/a	n/a	Cocchia *et al.* (2000), Jackman *et al.* (2012)
Phlda2$^{-/+}$	Knockout*	Normal (vs *Phlda2*$^{+/+}$ littermates) ↓ (vs WT litters)	↑↑ (from E12.5)	↑↑ glycogen	n/a	↑↑ *Tpbpa* Normal *Pcdh12* Normal *Gjb3*	n/a	n/a	Frank *et al.* 1999, 2002, Tunster *et al.* (2015)
Prl7d1$^{-/-}$	Knockout	↓ (E12.5)	↓ (E12.5 in males)	↑↑↑ PAS (males only)	↑↑ (males only)	n/a	n/a	Increased Jz:Lz ratio. impaired remodelling of maternal spiral arteries.	Zhang *et al.* (2019)
Pthlh$^{-/-}$	Knockout	↓ (from E16.5)	Normal	↓ glycogen (E12.5, E14.5) Normal glycogen (E16.5) ↑ glycogen (E18.5)	n/a	↓ *Tpbpa*	n/a	Reduced Lz size, reduced transport capacity	Duval *et al.* (2017)

Developmental stage in brackets is the time assessed.
‡Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.
↓=<20% reduction; ↓↓=20–50% reduction; ↓↓↓=>50% reduction; ↑=<20% increase; ↑↑=20–50% increase; ↑↑↑=>50% increase. E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; P, postnatal day; n/a, not available; S-TGCs, sinusoidal trophoblast giant cells; SynT-II, syncytiotrophoblast cell layer II; Tg, transgenic.

Table 4 Genetic mouse models with normal or enhanced fetal growth and increased placental glycogen content and/or GlyT phenotypes.

Mouse Model	Characteristics	Fetal weight or size	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localization	Lz phenotype	References
Cdkn1c$^{-/+}$	Knockout*	↑ (E15.5, E18.5)=(P0)	↑↑↑ (from E13.5)	↓↓ glycogen (E15.5, E18.5)	Normal	↓ *Tpbpa* ↓ *Gjb3* *Pcdh12* normal	n/a	Increased Lz cell number, fewer S-TGCs	Takahashi *et al.* (2000), Georgiades *et al.* (2002), Coan *et al.* (2006), Tunster *et al.* (2011)
Cts7 ^{Tg}	*Cts7* over-expression	n/a	n/a	n/a	↓↓ (E12.5)	↓ *Tpbpa* (E10.5, E12.5)	n/a	Fewer S-TGCs	Hemberger *et al.* (2000), Screen *et al.* (2008)
Egfr^{Dsk5}	*Egfr* hypomorph	Normal (E15.5)	↑ to ↑↑↑ (variable, E15.5) Genetic background dependent	↑ PAS	↑	↑ *Tpbpa* ↑ *Pcdh12*	n/a	↓ *Gcm1* ↓ *Dlx3*	Dackor *et al.* (2007), Dackor *et al.* (2009*b*)
H19$^{-/-}$	Knockout*	↑ (from E15.5)	↑ (E15.5, E18.5)	↑ glycogen (E15.5)	↑↑ (E15.5)	n/a	n/a	n/a	Leighton *et al.* (1995), Esquiliano *et al.* (2009)
Pgf$^{-/-}$	Knockout	Normal (P0)	↑ (P0)	↑↑ glycogen ↑ Best's Carmine staining	↑	n/a	n/a	Reduced Lz size	Achen *et al.* (1997), Tayade *et al.* (2007), Parchem *et al.* (2018)

Developmental stage in brackets is the time assessed.
‡Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. * denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.
↓=<20% reduction; ↓↓=20–50% reduction; ↓↓↓=>50% reduction; ↑=<20% increase; ↑↑=20–50% increase; ↑↑↑=>50% increase. E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; n/a, not available; P, postnatal day; S-TGCs, sinusoidal trophoblast giant cells; Tg, transgenic.

Table 5 Genetic mouse models with glycogen content or GlyT phenotypes associated with embryonic lethality at mid-gestation.

Mouse model	Characteristics	Stage of embryonic lethality	Placental weight	Glycogen content‡	GlyT number	GlyT markers	GlyT localization	Lz phenotype	References
Arnt$^{-/-}$	Knockout	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E9.5)	↓↓↓↓ *Tpbpa* (E9.5)	n/a	Reduced Lz size, Labyrinth trophoblast defect, decreased VEGF expression	Kozak *et al.* (1997), Maltepe *et al.* (1997), Adelman *et al.* (2000), Watson & Cross (2005)
Ascl2$^{-/-}$ or *Ascl2*$^{-/+}$	Knockout*	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E8.5)	↓↓↓↓ *Tpbpa* (E8.5)	n/a	Vascularization defect	Guillemot *et al.* (1994), Guillemot *et al.* (1995), Rossant *et al.* (1998)
Cited2$^{-/-}$	Knockout; associated with congenital malformations (e.g. NTD)	By E13.5	↓↓ (E12.5)	n/a	↓ Migratory GlyT	↓ *Tpbpa*	Impaired migration to decidua	Vascularization defect	Dunwoodie *et al.* (1998), Yin *et al.* (2002), Withington *et al.* (2006), Barbera *et al.* (2002)
Hectd1$^{-/-}$	Knockout; associated with congenital malformations (e.g. heart defects)	By E12.5	↓ (incomplete penetrance)	↓↓ glycogen	↓	↓ *Tpbpa* ↓ PAS	n/a	Disrupted structure, Vascularization defect	Sarkar *et al.* (2014), Sarkar *et al.* (2016)
Hif1a$^{-/-}$	Knockout; associated with congenital malformations (e.g. NTD, heart defects)	By E10.5	n/a	n/a	↓↓↓ progenitors	↓ *Tpbpa*	n/a	Impaired vascularization of chorion	Kozak *et al.* (1997), Compernolle *et al.* (2003), Cowden Dahl *et al.* (2005)
Pdcd5$^{-/-}$	Knockout; associated with congenital malformations (e.g. heart defects)	By E13.5	n/a	↓↓↓↓ PAS (E13.5)	↓↓↓↓ (E13.5)	n/a	n/a	Disorganizd structure, Necrotic and hemorrhagic	Li *et al.* (2017)
Peg10$^{+/-}$	Knockout*	By E10.5	n/a	n/a	↓↓↓↓ progenitors (E9.5)	↓↓↓↓ *Tpbpa* (E9.5)	n/a	Impaired development	Ono *et al.* (2006)
Prdm1$^{-/-}$	Knockout; associated with congenital malformations (e.g. heart defects)	By E10.5	n/a	n/a	n/a	Normal *Tpbpa*	Failure to migrate to decidua	Disrupted structure	Vincent *et al.* (2005), Mould *et al.* (2012)

Developmental stage in brackets is the time assessed.
‡Total placental glycogen content quantified by biochemical or enzymatic methods or estimated from comparison of PAS (Periodic Acid Schiff) staining. *Denotes imprinted or X-linked gene; heterozygous inheritance of a null allele from the normally active parental lineage effectively ablates gene expression.
↓=<20% reduction; ↓↓=20–50% reduction; ↓↓↓=>50% reduction; ↓↓↓↓=undetected; E, embryonic day; GlyT, glycogen trophoblast cells; Lz, labyrinth zone; n/a, not available.

lethality at mid-gestation (Guillemot *et al.* 1994). Therefore, *Ascl2* is likely required for the formation of GlyT cells and might also be associated with glycogen storage or metabolism.

Beyond GlyT differentiation, several mouse strains demonstrate abnormal localization of GlyT (Table 2). Normally, GlyT are present in the Jz with a subpopulation of GlyT migrating into the decidua (Coan *et al.* 2006). *Ldoc1*, *HtrA1* and *Tfap2c* single knockout mutations lead to placentas with GlyT that have mislocalized to the Lz (Naruse *et al.* 2014, Hasan *et al.* 2015, Kaiser *et al.* 2015). The Jz naturally interdigitates with the Lz (Soares & Hunt 2014), the extent of which depends upon gestational stage and genetic background (Tunster *et al.* 2012). Therefore, it is important to be cautious when histologically characterizing the clusters of GlyT located in the Lz. Serial sections of placenta can be assessed to differentiate a GlyT cluster from a Jz finger protruding in to the Lz. Alternatively, failure of GlyT to migrate into the decidua, as in *Ptp4a2*$^{-/-}$ or *Phlda2* overexpression mice (Tunster *et al.* 2010, Dong *et al.* 2012), might affect maternal-fetal interactions. Whether defective GlyT migration in these models results from altered cell-signalling pathways associated with adhesion and cytoskeleton or an inappropriate response to chemotactic signals remains to be determined. However, appropriate localization of GlyT appears to be important for normal fetal growth.

Increased placental glycogen storage is also associated with impaired fetal growth

Unexpectedly, FGR can also occur in the context of increased placental glycogen storage. For example, when the number of GlyT cells exceeded 35% of the Jz, as in interspecific mouse hybrids, fetal growth was inversely correlated with GlyT abundance (Kurz *et al.* 1999). Additionally, several genetic mouse lines exist that demonstrate increased placental glycogen storage associated with FGR (Table 3). One potential explanation for these counterintuitive findings is that the mutant placentas are able to store glycogen but cannot effectively mobilize and release the glycogen. Further work is necessary to elucidate the mechanism, with a specific focus on whether glycogenesis is increased, leading to enhanced glycogen storage, whether glycogenolysis is impaired, leading to failed glucose release, or whether transport mechanisms are defective. Alternatively, FGR in models associated with increased placental glycogen storage might be caused by a mechanism independent of the GlyT or placental glycogen. Of note, some models (e.g. *Ascl2*-Tg, *Pcdh12*$^{-/-}$ and *Plac1*$^{-/-}$) with increased glycogen storage also display mislocalization of GlyT clusters within the Lz (Rampon *et al.* 2008, Jackman *et al.* 2012, Tunster *et al.* 2016). This result is consistent with a role for the normal microenvironment of the Jz in modulating the mobilization of glycogen stores by the GlyT.

Enhanced fetal growth might be independent of a GlyT phenotype

It is possible for enhanced fetal growth to associate with aberrant placental glycogen storage, as occurs in *Cdkn1c*$^{-/+}$ and *H19*$^{-/-}$ mice (Esquiliano *et al.* 2009, Tunster *et al.* 2011) (Table 4). However, it is unlikely that these placental phenotypes directly cause fetal overgrowth, since these genes exert an intrinsic effect on fetal growth. For instance, conditional overexpression of *Cdkn1c* in a subset of fetal, but not placental, tissues restricts fetal growth (John *et al.* 2001, Andrews *et al.* 2007). In contrast, while *Cdkn1c* knockout mice exhibit fetal overgrowth and placentomegaly at E15.5 and E18.5, fetal weights are normalized at birth (Tunster *et al.* 2011). This failure to sustain fetal overgrowth may be attributable to the manifestation of severe defects in *Cdkn1c*$^{-/+}$ placentas including substantially diminished glycogen stores (Tunster *et al.* 2011), although GlyT abundance is unaffected (Takahashi *et al.* 2000). Similarly, ablation of *H19* drives overexpression of the growth-promoting gene *Igf2*, resulting in neonates that are 30% heavier than their littermates (Leighton *et al.* 1995), and placentas with increased GlyT number and placental glycogen content (Esquiliano *et al.* 2009). However, since the *H19*-driven fetal overgrowth phenotype persists to adulthood, the cause is likely independent of increased placental glycogen stores.

GlyT phenotypes associated with early embryonic lethality

Several genetic mouse models display a GlyT phenotype and result in embryonic lethality at or around mid-gestation (Table 5). This outcome occurs before GlyT cells mature and begin accumulating glycogen stores in earnest. Early fetal demise prevents a meaningful assessment of the effect of placental glycogen stores on fetal growth in this context. It is clear that embryonic lethality is attributable to a placental defect in the case of *Ascl2* and *Peg10* knockouts, since lethality was rescued using a tetraploid aggregation approach (Guillemot *et al.* 1994, 1995, Ono *et al.* 2006). This method generates a chimeric conceptus in which a mutant fetus is supported by a placenta containing WT tetraploid cells (Tarkowski *et al.* 1977, Nagy *et al.* 1990). In this context, rescue of embryonic lethality indicates a placental cause. Embryonic lethality in the remaining models coincides with the appearance of congenital malformations (Table 5). A conditional knockout approach is required to elucidate the role of these genes in later stage placentas, including in GlyT development and function. A recent study demonstrated that defects in placental

development and/or function might contribute to the formation of congenital malformations in the fetus, particularly those affecting heart, brain and vascular development (Perez-Garcia *et al.* 2018). The extent to which GlyT and placental glycogen stores play a role in this phenomenon is yet to be determined.

Limitations

While the studies presented here broadly support a role for placental glycogen in regulating fetal growth, a major limitation to understanding the true cause-effect relationship is that the Lz structure is also disrupted in many of these models (Tables 2, 3, 4 and 5). The Lz facilitates the exchange of nutrients, gases and waste products between maternal and fetal circulations (Watson & Cross 2005). Reduced branching morphogenesis in the Lz decreases the surface area for nutrient transport, compromising placental function (Cross *et al.* 2006). Consequently, it is difficult to directly attribute the adverse fetal growth outcomes specifically to aberrant placental glycogen storage for most genes. One model in which fetal growth and placental glycogen content might show a direct link is the *Egfr* hypomorph mutation. These mutant mice have placentas with a GlyT-specific phenotype characterized by reduced PAS staining and diminished GlyT-marker expression but no other placental defect. Associated fetuses were growth restricted in a background-specific manner, with *Egfr* hypomorphs growth restricted on the 129Sv and BTBR/J backgrounds but not the C57BL/6J background (Dackor *et al.* 2009*a*). Crucially, while PAS staining of *Egfr* hypomorphic placentas was reduced on the 129Sv and BTBR/J backgrounds, abundant PAS staining was evident on the C57BL/6 genetic background (Dackor *et al.* 2009*b*). This emphasizes a potential relationship between glycogen storage and fetal growth.

The ability to ascertain the mechanism driving aberrant glycogen storage from existing studies is difficult given that GlyT abundance and total placental glycogen content are not always assessed together. Therefore, it is unclear whether an early defect in GlyT progenitor maintenance or differentiation leading to a reduction in mature GlyT cells is the main cause of glycogen storage defects. This appears to be the case for at least 11 mutant mouse lines where both parameters have been assessed (Table 1). The majority of studies have assessed only a single parameter: GlyT abundance or placental glycogen content. This will affect the conclusions drawn by each study and not necessarily reflect whether the mechanism is developmental or metabolic in origin. The only known model whereby placental glycogen stores are independent of GlyT differentiation and abundance is the *Cdkn1c* null line (Tunster *et al.* 2011, Takahashi *et al.* 2000). From this model, we might better understand how glycogen metabolism occurs in the placenta and how fetal growth responds.

Future directions

Considerable work is required to fully elucidate the normal physiological role of placental glycogen stores in humans given that we are only beginning to understand its role during pregnancy. To improve our understanding, a systematic analysis of the GlyT population is required in known mouse models with placenta phenotypes and as part of the routine assessment of new mouse placenta phenotypes. We have proposed four key parameters to assess the GlyT lineage to better understand their development and function: (1.) quantification of GlyT cell number as determined by nuclear counts on histological sections; (2.) assessment of GlyT-specific marker gene expression (e.g. *Pcdh12*, *Aldh1a3* and *Gjb3*) either by *in situ* hybridization on histological sections (Fig. 2) or by qPCR analysis at multiple stages of development. This should occur in concert with assessment of other trophoblast lineage marker expression; (3.) analysis of the localization of GlyT cells by undergoing *in situ* hybridization for the migratory (*Prl7b1*) and non-migratory (*Prl6a1*) markers. This will indicate the migratory capacity of these cells and whether placental structure is affected and (4.) exploring placental glycogen content by performing PAS staining of histological sections (Fig. 2) alongside quantification of total placental glycogen content using a biochemical assay (Lo *et al.* 1970). Altogether, these criteria will help to direct subsequent phenotype evaluation and ultimately identify further molecules and pathways important for GlyT formation and function and for the regulation of placental glycogen stores.

Given the highly dynamic nature of the GlyT cells over the course of pregnancy, it is important to assess placental glycogen-related phenotypes (and arguably other placental phenotypes) at multiple gestational stages. Characterization of a single developmental stage might misrepresent the full extent of the phenotype. One model that exemplifies this issue is the *Pthlh* knockout mouse (Duval *et al.* 2017), whereby placental glycogen content was reduced at E12.5 and E14.5. By E16.5, glycogen had normalized to control levels. However, glycogen content was elevated at E18.5 relative to controls, failing to exhibit the anticipated decline toward term that is normally attributed to the mobilisation of glycogen stores in late gestation. Coinciding with the glycogen phenotype, fetal growth was restricted from E16.5 (Duval *et al.* 2017). Although *Pthlh*-null mice exhibit severe skeletal abnormalities and die soon after birth (Karaplis *et al.* 1994, Bond *et al.* 2008), the late-onset FGR observed in this model implicates a causal role for placental dysfunction. However, it is unclear whether FGR can be attributed to impaired glycogen storage during mid-gestation or the apparent failure to mobilize placental glycogen stores at term. Assessing phenotypes at multiple stages of development will

potentially allow for better separation of developmental defects from functional defects.

The regulation of glycogenesis and glycogenolysis pathway activity by GlyT will provide further insight into placental glycogen metabolism. Central to glucose release from a cell is the enzyme glucose 6-phosphatase, which is responsible for generating free glucose by hydrolyzing the polar phosphate group from glucose 6-phosphate and allowing its transport out of the cell (van Schaftingen & Gerin 2002). The absence of glucose 6-phosphatase activity in GlyT cells would prevent the release of glucose for consumption by the fetus, mother or trophoblast cells. While expression of glucose 6-phosphatase has not been investigated in the mouse placenta to date, its activity in the human placenta is attributed to the *G6PC3* isoform (Guionie *et al.* 2003). This isoform is distinct from the liver-specific *G6PC1* isoform and is expressed in the placenta from at least week 28 of human pregnancy (Matsubara *et al.* 1999) and may be associated with the production of glucose in the placenta at term (Prendergast *et al.* 1999). However, the ultimate destination of glucose derived from placental glycogen stores remains unclear. It is possible that it is directed for fetal use and/or placental consumption. Signals from the fetus that release placental glycogen stores have not been identified.

It is also possible that placental glycogen stores support maternal physiology. The localization of non-migratory GlyT around channels that drain maternal blood from the placenta suggests that some of the glucose released from placental glycogen stores may first be available for uptake by the mother. GlyT express several *Prl* genes and the localization of non-migratory GlyT suggest the encoded hormones may be released into maternal blood. While placental hormones are known to adapt maternal physiology to pregnancy, there is currently no evidence that the glucose metabolized from placental glycogen stores acts to the benefit of maternal physiology. Future studies should assess the effects of aberrant placental glycogen storage on maternal physiology. Indeed, genetic perturbations of several genes that regulate the GlyT lineage also have effects on maternal behavior, including *Peg3* (Li *et al.* 1999, McNamara *et al.* 2018) and *Phlda2* (Creeth *et al.* 2018). Little is known about changes to maternal physiology in these models. *Dlk1*$^{+/-}$ mice are the only model in which maternal physiology was characterized in association with a placental GlyT phenotype (Appelbe *et al.* 2013, Cleaton *et al.* 2016). However, the effects on maternal physiology are more likely attributable to the loss of circulating DLK1 (also known as fetal antigen-1; FA1), rather than a consequence of altered placental glycogen metabolism.

Conversely, there is evidence that maternal physiology can regulate placental glycogen stores. A loss-of-function allele of *Pik3ca*, which encodes for the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), showed a maternal zygotic effect resulting in diminished glycogen stores (Sferruzzi-Perri *et al.* 2016), although the underlying mechanism is not well understood. While manipulation of maternal diet modulates fetal growth in mouse models (Zhang *et al.* 2005, 2009, Jones *et al.* 2009), only a few studies have investigated the effect of maternal diet upon placental glycogen storage. Both calorie and protein restriction in dams impair placental glycogen storage (Coan *et al.* 2010, Sferruzzi-Perri *et al.* 2011, Gonzalez *et al.* 2016), presumably as a consequence of diminished nutrient availability. A high sugar, high fat (HSHF) maternal diet results in FGR at E15.5, a phenotype that is normalized by E18.5 (Sferruzzi-Perri *et al.* 2013), potentially due to increased utilization of glycogen to support accelerated fetal growth during late gestation. Further work is necessary to explore the relationship between maternal diet, placental glycogen metabolism and fetal growth.

Attempts to infer the function of placental glycogen in models where the GlyT lineage is ablated or diminished might be confounded by additional functions of GlyT cells, which perform at least two additional functions to glycogen storage. First, GlyT cells contribute to placental endocrine function as evidenced by their expression of a subset of the *Prl* gene cluster (Simmons *et al.* 2008*b*). Secondly, GlyT expression of ALDH1A3, an enzyme that oxidises retinal to retinoic acid, implies that GlyT might be a source of retinoic acid, which is an important regulator of trophoblast differentiation (Outhwaite *et al.* 2015). Therefore, alterations in the GlyT population might have profound effects on placental structure and function independent of glycogen storage.

Overall, better characterization of GlyT phenotypes in mice will allow us to evaluate the glycogenesis and glycogenolysis pathways involved in storing and metabolizing glycogen in normal pregnancies and pathological contexts. More sophisticated approaches to constitutive gene knockout models will be necessary to elucidate the specific physiological role(s) of placental glycogen. These analyses should include single-cell sequencing approaches to better understand genetic and metabolic pathways within GlyT cells together with a more holistic approach that relates placental structure and glycogen metabolism to fetal growth and development and to maternal physiology. Only then will the normal function and relevance of aberrant placental glycogen stores to human pregnancy complications, such as PE and GDM, be clarified.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

S J T conceived the study. S J T, E D W, A L F and G J B wrote and edited the manuscript. All authors have read and approved the final version of this manuscript.

References

Achen MG, Gad JM, Stacker SA & Wilks AF 1997 Placenta growth factor and vascular endothelial growth factor are co-expressed during early embryonic development. *Growth Factors* **15** 69–80. (https://doi.org/10.3109/08977199709002113)

Adamson SL, Lu Y, Whiteley KJ, Holmyard D, Hemberger M, Pfarrer C & Cross JC 2002 Interactions between trophoblast cells and the maternal and fetal circulation in the mouse placenta. *Developmental Biology* **250** 358–373. (https://doi.org/10.1016/s0012-1606(02)90773-6)

Adelman DM, Gertsenstein M, Nagy A, Simon MC & Maltepe E 2000 Placental cell fates are regulated in vivo by HIF-mediated hypoxia responses. *Genes and Development* **14** 3191–3203. (https://doi.org/10.1101/gad.853700)

Akison LK, Nitert MD, Clifton VL, Moritz KM & simmons DG 2017 Review: Alterations in placental glycogen deposition in complicated pregnancies: current preclinical and clinical evidence. *Placenta* **54** 52–58. (https://doi.org/10.1016/j.placenta.2017.01.114)

Andrews SC, Wood MD, Tunster SJ, Barton SC, Surani MA & John RM 2007 Cdkn1c (p57Kip2) is the major regulator of embryonic growth within its imprinted domain on mouse distal chromosome 7. *BMC Developmental Biology* **7** 53. (https://doi.org/10.1186/1471-213X-7-53)

Angiolini E, Fowden A, Coan P, Sandovici I, Smith P, Dean W, Burton G, Tycko B, Reik W, Sibley C *et al*. 2006 Regulation of placental efficiency for nutrient transport by imprinted genes. *Placenta* **27** (Supplement A) S98–102. (https://doi.org/10.1016/j.placenta.2005.12.008)

Appelbe OK, Yevtodiyenko A, Muniz-Talavera H & Schmidt JV 2013 Conditional deletions refine the embryonic requirement for Dlk1. *Mechanisms of Development* **130** 143–159. (https://doi.org/10.1016/j.mod.2012.09.010)

Arkwright PD, Rademacher TW, Dwek RA & Redman CW 1993 Pre-eclampsia is associated with an increase in trophoblast glycogen content and glycogen synthase activity, similar to that found in hydatidiform moles. *Journal of Clinical Investigation* **91** 2744–2753. (https://doi.org/10.1172/JCI116515)

Baker J, Liu JP, Robertson EJ & Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75** 73–82. (https://doi.org/10.1016/S0092-8674(05)80085-6)

Baladron V, Ruiz-Hidalgo MJ, Nueda ML, Diaz-Guerra MJ, Garcia-Ramirez JJ, Bonvini E, Gubina E & Laborda J 2005 dlk acts as a negative regulator of Notch1 activation through interactions with specific EGF-like repeats. *Experimental Cell Research* **303** 343–359. (https://doi.org/10.1016/j.yexcr.2004.10.001)

Barash V & Shafrir E 1990 Mobilization of placental glycogen in diabetic rats. *Placenta* **11** 515–521. (https://doi.org/10.1016/s0143-4004(05)80197-3)

Barbera JP, Rodriguez TA, Greene ND, Weninger WJ, Simeone A, Copp AJ, Beddington RS & Dunwoodie S 2002 Folic acid prevents exencephaly in Cited2 deficient mice. *Human Molecular Genetics* **11** 283–293. (https://doi.org/10.1093/hmg/11.3.283)

Bierne H, Tham TN, Batsche E, Dumay A, Leguillou M, Kerneis-Golsteyn S, Regnault B, Seeler JS, Muchardt C, Feunteun J *et al*. 2009 Human BAHD1 promotes heterochromatic gene silencing. *PNAS* **106** 13826–13831. (https://doi.org/10.1073/pnas.0901259106)

Bogutz AB, Oh-Mcginnis R, Jacob KJ, Ho-Lau R, Gu T, Gertsenstein M, Nagy A & Lefebvre L 2018 Transcription factor ASCL2 is required for development of the glycogen trophoblast cell lineage. *PLoS Genetics* **14** e1007587. (https://doi.org/10.1371/journal.pgen.1007587)

Bond H, Dilworth MR, Baker B, Cowley E, Jimenez AR, Boyd RDH, Husain SM, Ward BS, Sibley CP & Glazier JD 2008 Increased maternofetal calcium flux in parathyroid hormone-related protein-null mice. *Journal of Physiology* **586** 2015–2025. (https://doi.org/10.1113/jphysiol.2007.149104)

Bouillot S, Rampon C, Tillet E & Huber P 2006 Tracing the glycogen cells with protocadherin 12 during mouse placenta development. *Placenta* **27** 882–888. (https://doi.org/10.1016/j.placenta.2005.09.009)

Boyd J 1959 *Glycogen in Early Human Implantation Sites*.

Breier G, Clauss M & Risau W 1995 Coordinate expression of vascular endothelial growth factor receptor-1 (flt-1) and its ligand suggests a paracrine regulation of murine vascular development. *Developmental Dynamics* **204** 228–239. (https://doi.org/10.1002/aja.1002040303)

Burton GJ & Fowden AL 2015 The placenta: a multifaceted, transient organ. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **370** 20140066. (https://doi.org/10.1098/rstb.2014.0066)

Carter AM, Nygard K, Mazzuca DM & Han VK 2006 The expression of insulin-like growth factor and insulin-like growth factor binding protein mRNAs in mouse placenta. *Placenta* **27** 278–290. (https://doi.org/10.1016/j.placenta.2005.01.014)

Celebi C, Van Montfoort A, Skory V, Kieffer E, Kuntz S, Mark M & Viville S 2012 Tex 19 paralogs exhibit a gonad and placenta-specific expression in the mouse. *Journal of Reproduction and Development* **58** 360–365. (https://doi.org/10.1262/jrd.11-047k)

Cleaton MA, Dent CL, Howard M, Corish JA, Gutteridge I, Sovio U, Gaccioli F, Takahashi N, Bauer SR, Charnock-Jones DS *et al*. 2016 Fetus-derived DLK1 is required for maternal metabolic adaptations to pregnancy and is associated with fetal growth restriction. *Nature Genetics* **48** 1473–1480. (https://doi.org/10.1038/ng.3699)

Coan PM, Conroy N, Burton GJ & Ferguson-Smith AC 2006 Origin and characteristics of glycogen cells in the developing murine placenta. *Developmental Dynamics* **235** 3280–3294. (https://doi.org/10.1002/dvdy.20981)

Coan PM, Vaughan OR, Sekita Y, Finn SL, Burton GJ, Constancia M & Fowden AL 2010 Adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice. *Journal of Physiology* **588** 527–538. (https://doi.org/10.1113/jphysiol.2009.181214)

Cocchia M, Huber R, Pantano S, Chen EY, Ma P, Forabosco A, Ko MS & Schlessinger D 2000 PLAC1, an Xq26 gene with placenta-specific expression. *Genomics* **68** 305–312. (https://doi.org/10.1006/geno.2000.6302)

Compernolle V, Brusselmans K, Franco D, Moorman A, Dewerchin M, Collen D & Carmeliet P 2003 Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1alpha. *Cardiovascular Research* **60** 569–579. (https://doi.org/10.1016/j.cardiores.2003.07.003)

Constância M, Angiolini E, Sandovici I, Smith P, Smith R, Kelsey G, Dean W, Ferguson-Smith A, Sibley CP, Reik W *et al*. 2005 Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. *PNAS* **102** 19219–19224. (https://doi.org/10.1073/pnas.0504468103)

Cowden Dahl KD, Fryer BH, Mack FA, Compernolle V, Maltepe E, Adelman DM, Carmeliet P & Simon MC 2005 Hypoxia-inducible factors 1alpha and 2alpha regulate trophoblast differentiation. *Molecular and Cellular Biology* **25** 10479–10491. (https://doi.org/10.1128/MCB.25.23.10479-10491.2005)

Creeth HDJ, Mcnamara GI, Tunster SJ, Boque-Sastre R, Allen B, Sumption L, Eddy JB, Isles AR & John RM 2018 Maternal care boosted by paternal imprinting in mammals. *PLoS Biology* **16** e2006599. (https://doi.org/10.1371/journal.pbio.2006599)

Cross DA, Alessi DR, Cohen P, Andjelkovich M & Hemmings BA 1995 Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378** 785–789. (https://doi.org/10.1038/378785a0)

Cross JC, Nakano H, Natale DR, Simmons DG & Watson ED 2006 Branching morphogenesis during development of placental villi. *Differentiation: Research in Biological Diversity* **74** 393–401. (https://doi.org/10.1111/j.1432-0436.2006.00103.x)

Crossey PA, Pillai CC & Miell JP 2002 Altered placental development and intrauterine growth restriction in IGF binding protein-1 transgenic mice. *Journal of Clinical Investigation* **110** 411–418. (https://doi.org/10.1172/JCI10077)

Curley JP, Barton S, Surani A & Keverne EB 2004 Coadaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proceedings: Biological Sciences* **271** 1303–1309. (https://doi.org/10.1098/rspb.2004.2725)

Dackor J, Strunk KE, Wehmeyer MM & Threadgill DW 2007 Altered trophoblast proliferation is insufficient to account for placental dysfunction in egfr null embryos. *Placenta* **28** 1211–1218. (https://doi.org/10.1016/j.placenta.2007.07.005)

Dackor J, Caron KM & Threadgill DW 2009*a* Placental and embryonic growth restriction in mice with reduced function epidermal growth factor receptor alleles. *Genetics* **183** 207–218. (https://doi.org/10.1534/genetics.109.104372)

Dackor J, Li M & Threadgill DW 2009*b* Placental overgrowth and fertility defects in mice with a hypermorphic allele of epidermal growth factor receptor. *Mammalian Genome* **20** 339–349. (https://doi.org/10.1007/s00335-009-9189-2)

Dechiara TM, Efstratiadis A & Robertson EJ 1990 A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345** 78–80. (https://doi.org/10.1038/345078a0)

Desoye G, Hofmann HH & Weiss PA 1992 Insulin binding to trophoblast plasma membranes and placental glycogen content in well-controlled gestational diabetic women treated with diet or insulin, in well-controlled overt diabetic patients and in healthy control subjects. *Diabetologia* **35** 45–55. (https://doi.org/10.1007/bf00400851)

Desoye G, Korgun ET, Ghaffari-Tabrizi N & Hahn T 2002 Is fetal macrosomia in adequately controlled diabetic women the result of a placental defect? A hypothesis. *Journal of Maternal-Fetal and Neonatal Medicine* **11** 258–261. (https://doi.org/10.1080/jmf.11.4.258.261)

Deussing J, Kouadio M, Rehman S, Werber I, Schwinde A & Peters C 2002 Identification and characterization of a dense cluster of placenta-specific cysteine peptidase genes and related genes on mouse chromosome 13. *Genomics* **79** 225–240. (https://doi.org/10.1006/geno.2002.6696)

Diamant YZ, Metzger BE, Freinkel N & Shafrir E 1982 Placental lipid and glycogen content in human and experimental diabetes mellitus. *American Journal of Obstetrics and Gynecology* **144** 5–11. (https://doi.org/10.1016/0002-9378(82)90385-4)

Diehl JA, Cheng M, Roussel MF & Sherr CJ 1998 Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes and Development* **12** 3499–3511. (https://doi.org/10.1101/gad.12.22.3499)

Dong Y, Zhang L, Zhang S, Bai Y, Chen H, Sun X, Yong W, Li W, Colvin SC, Rhodes SJ *et al*. 2012 Phosphatase of regenerating liver 2 (PRL2) is essential for placental development by down-regulating PTEN (phosphatase and tensin homologue deleted on chromosome 10) and activating Akt protein. *Journal of Biological Chemistry* **287** 32172–32179. (https://doi.org/10.1074/jbc.M112.393462)

Driessen LF 1907 Ueber glykogen in der placenta. *Archiv für Gynaekologie* **82** 278–301. (https://doi.org/10.1007/BF01785087)

Dunwoodie SL, Rodriguez TA & Beddington RS 1998 Msg1 and Mrg1, founding members of a gene family, show distinct patterns of gene expression during mouse embryogenesis. *Mechanisms of Development* **72** 27–40. (https://doi.org/10.1016/s0925-4773(98)00011-2)

Duval C, Dilworth MR, Tunster SJ, Kimber SJ & Glazier JD 2017 PTHrP is essential for normal morphogenetic and functional development of the murine placenta. *Developmental Biology* **430** 325–336. (https://doi.org/10.1016/j.ydbio.2017.08.033)

Efstratiadis A 1998 Genetics of mouse growth. *International Journal of Developmental Biology* **42** 955–976.

Esquiliano DR, Guo W, Liang L, Dikkes P & Lopez MF 2009 Placental glycogen stores are increased in mice with H19 null mutations but not in those with insulin or IGF type 1 receptor mutations. *Placenta* **30** 693–699. (https://doi.org/10.1016/j.placenta.2009.05.004)

Forbes K & Westwood M 2008 The IGF axis and placental function. a mini review. *Hormone Research* **69** 129–137. (https://doi.org/10.1159/000112585)

Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ & Vanhaesebroeck B 2006 Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. *Nature* **441** 366–370. (https://doi.org/10.1038/nature04694)

Frank D, Mendelsohn CL, Ciccone E, Svensson K, Ohlsson R & Tycko B 1999 A novel pleckstrin homology-related gene family defined by Ipl/Tssc3, TDAG51, and Tih1: tissue-specific expression, chromosomal location, and parental imprinting. *Mammalian Genome* **10** 1150–1159. (https://doi.org/10.1007/s003359901182)

Frank D, Fortino W, Clark L, Musalo R, Wang W, Saxena A, Li CM, Reik W, Ludwig T & Tycko B 2002 Placental overgrowth in mice lacking the imprinted gene Ipl. *PNAS* **99** 7490–7495. (https://doi.org/10.1073/pnas.122039999)

Frost JM & Moore GE 2010 The importance of imprinting in the human placenta. *PLoS Genetics* **6** e1001015. (https://doi.org/10.1371/journal.pgen.1001015)

Gabbe SG, Demers LM, Greep RO & Villee CA 1972 Placental glycogen metabolism in diabetes mellitus. *Diabetes* **21** 1185–1191. (https://doi.org/10.2337/diab.21.12.1185)

Gasperowicz M, Surmann-Schmitt C, Hamada Y, Otto F & Cross JC 2013 The transcriptional co-repressor TLE3 regulates development of trophoblast giant cells lining maternal blood spaces in the mouse placenta. *Developmental Biology* **382** 1–14. (https://doi.org/10.1016/j.ydbio.2013.08.005)

Georgiades P, Ferguson-Smith AC & Burton GJ 2002 Comparative developmental anatomy of the murine and human definitive placentae. *Placenta* **23** 3–19. (https://doi.org/10.1053/plac.2001.0738)

Gheorman V, Gheorman L, Ivanus C, Pana RC, Goganau AM & Patrascu A 2013 Comparative study of placenta acute fetal distress and diabetes associated with pregnancy. *Romanian Journal of Morphology and Embryology* **54** 505–511.

Gonzalez PN, Gasperowicz M, Barbeito-Andres J, Klenin N, Cross JC & Hallgrimsson B 2016 Chronic protein restriction in mice impacts placental function and maternal body weight before fetal growth. *PLoS ONE* **11** e0152227. (https://doi.org/10.1371/journal.pone.0152227)

Gude NM, Roberts CT, Kalionis B & King RG 2004 Growth and function of the normal human placenta. *Thrombosis Research* **114** 397–407. (https://doi.org/10.1016/j.thromres.2004.06.038)

Guillemot F, Nagy A, Auerbach A, Rossant J & Joyner AL 1994 Essential role of Mash-2 in extraembryonic development. *Nature* **371** 333–336. (https://doi.org/10.1038/371333a0)

Guillemot F, Caspary T, Tilghman SM, Copeland NG, Gilbert DJ, Jenkins NA, Anderson DJ, Joyner AL, Rossant J & Nagy A 1995 Genomic imprinting of Mash2, a mouse gene required for trophoblast development. *Nature Genetics* **9** 235–242. (https://doi.org/10.1038/ng0395-235)

Guionie O, Clottes E, Stafford K & Burchell A 2003 Identification and characterisation of a new human glucose-6-phosphatase isoform. *FEBS Letters* **551** 159–164. (https://doi.org/10.1016/s0014-5793(03)00903-7)

Hasan MZ, Ikawati M, Tocharus J, Kawaichi M & Oka C 2015 Abnormal development of placenta in HtrA1-deficient mice. *Developmental Biology* **397** 89–102. (https://doi.org/10.1016/j.ydbio.2014.10.015)

Hay Jr WW 1995 Regulation of placental metabolism by glucose supply. *Reproduction, Fertility, and Development* **7** 365–375. (https://doi.org/10.1071/rd9950365)

He H, Perera BP, Ye A & Kim J 2016 Parental and sexual conflicts over the Peg3 imprinted domain. *Scientific Reports* **6** 38136. (https://doi.org/10.1038/srep38136)

He N, Lim SJ, Moreira De Mello JC, Navarro I, Bialecka M, Salvatori DCF, Van Der Westerlaken LAJ, Pereira LV & Chuva De Sousa Lopes SM 2017 At term, XmO and XpO mouse placentas show differences in glucose metabolism in the trophectoderm-derived outer zone. *Frontiers in Cell and Developmental Biology* **5** 63. (https://doi.org/10.3389/fcell.2017.00063)

Heijkenskjold F & Gemzell CA 1957 Glycogen content in the placenta in diabetic mothers. *Acta Paediatrica* **46** 74–80. (https://doi.org/10.1111/j.1651-2227.1957.tb08633.x)

Hemberger M, Himmelbauer H, Ruschmann J, Zeitz C & Fundele R 2000 cDNA subtraction cloning reveals novel genes whose temporal and spatial expression indicates association with trophoblast invasion. *Developments in Biologicals* **222** 158–169.

Hermida MA, Dinesh Kumar J & Leslie NR 2017 GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation* **65** 5–15. (https://doi.org/10.1016/j.jbior.2017.06.003)

Hwa V, Oh Y & Rosenfeld RG 1999 The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocrine Reviews* **20** 761–787. (https://doi.org/10.1210/edrv.20.6.0382)

Jackman SM, Kong X & Fant ME 2012 Plac1 (placenta-specific 1) is essential for normal placental and embryonic development. *Molecular*

Reproduction and Development **79** 564–572. (https://doi.org/10.1002/mrd.22062)

Jensen AB, Tunster SJ & John RM 2014 The significance of elevated placental PHLDA2 in human growth restricted pregnancies. *Placenta* **35** 528–532. (https://doi.org/10.1016/j.placenta.2014.04.018)

John RM, Ainscough JF, Barton SC & Surani MA 2001 Distant cis-elements regulate imprinted expression of the mouse p57(Kip2) (Cdkn1c) gene: implications for the human disorder, Beckwith–Wiedemann syndrome. *Human Molecular Genetics* **10** 1601–1609. (https://doi.org/10.1093/hmg/10.15.1601)

Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL & Jansson T 2009 High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB Journal* **23** 271–278. (https://doi.org/10.1096/fj.08-116889)

Kaiser S, Koch Y, Kuhnel E, Sharma N, Gellhaus A, Kuckenberg P, Schorle H & Winterhager E 2015 Reduced gene dosage of Tfap2c impairs trophoblast lineage differentiation and alters maternal blood spaces in the mouse placenta. *Biology of Reproduction* **93** 31. (https://doi.org/10.1095/biolreprod.114.126474)

Kanzaki H, Crainie M, Lin H, Yui J, Guilbert LJ, Mori T & Wegmann TG 1991 The in situ expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA at the maternal-fetal interface. *Growth Factors* **5** 69–74. (https://doi.org/10.3109/08977199109000272)

Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM & Mulligan RC 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes and Development* **8** 277–289. (https://doi.org/10.1101/gad.8.3.277)

Kendall RL & Thomas KA 1993 Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *PNAS* **90** 10705–10709. (https://doi.org/10.1073/pnas.90.22.10705)

Kozak KR, Abbott B & Hankinson O 1997 ARNT-deficient mice and placental differentiation. *Developmental Biology* **191** 297–305. (https://doi.org/10.1006/dbio.1997.8758)

Kruger I, Vollmer M, Simmons DG, Elsasser HP, Philipsen S & Suske G 2007 Sp1/Sp3 compound heterozygous mice are not viable: impaired erythropoiesis and severe placental defects. *Developmental Dynamics* **236** 2235–2244. (https://doi.org/10.1002/dvdy.21222)

Kuckenberg P, Buhl S, Woynecki T, Van Furden B, Tolkunova E, Seiffe F, Moser M, Tomilin A, Winterhager E & Schorle H 2010 The transcription factor TCFAP2C/AP-2gamma cooperates with CDX2 to maintain trophectoderm formation. *Molecular and Cellular Biology* **30** 3310–3320. (https://doi.org/10.1128/MCB.01215-09)

Kuhnel E, Kleff V, Stojanovska V, Kaiser S, Waldschutz R, Herse F, Plosch T, Winterhager E & Gellhaus A 2017 Placental-specific overexpression of sFlt-1 alters trophoblast differentiation and nutrient transporter expression in an IUGR mouse model. *Journal of Cellular Biochemistry* **118** 1316–1329. (https://doi.org/10.1002/jcb.25789)

Kuroiwa Y, Kaneko-Ishino T, Kagitani F, Kohda T, Li LL, Tada M, Suzuki R, Yokoyama M, Shiroishi T, Wakana S *et al*. 1996 Peg3 imprinted gene on proximal chromosome 7 encodes for a zinc finger protein. *Nature Genetics* **12** 186–190. (https://doi.org/10.1038/ng0296-186)

Kurz H, Zechner U, Orth A & Fundele R 1999 Lack of correlation between placenta and offspring size in mouse interspecific crosses. *Anatomy and Embryology* **200** 335–343. (https://doi.org/10.1007/s004290050284)

Laborda J 2000 The role of the epidermal growth factor-like protein dlk in cell differentiation. *Histology and Histopathology* **15** 119–129. (https://doi.org/10.14670/HH-15.119)

Lakisic G, Lebreton A, Pourpre R, Wendling O, Libertini E, Radford EJ, Le Guillou M, Champy MF, Wattenhofer-Donze M, Soubigou G *et al*. 2016 Role of the BAHD1 chromatin-repressive complex in placental development and regulation of steroid metabolism. *PLoS Genetics* **12** e1005898. (https://doi.org/10.1371/journal.pgen.1005898)

Lee S, Ye A & Kim J 2015 DNA-binding motif of the imprinted transcription factor PEG3. *PLoS ONE* **10** e0145531. (https://doi.org/10.1371/journal.pone.0145531)

Lefebvre L 2012 The placental imprintome and imprinted gene function in the trophoblast glycogen cell lineage. *Reproductive Biomedicine Online* **25** 44–57. (https://doi.org/10.1016/j.rbmo.2012.03.019)

Lefebvre L, Mar L, Bogutz A, Oh-Mcginnis R, Mandegar MA, Paderova J, Gertsenstein M, Squire JA & Nagy A 2009 The interval between Ins2 and Ascl2 is dispensable for imprinting centre function in the murine Beckwith-Wiedemann region. *Human Molecular Genetics* **18** 4255–4267. (https://doi.org/10.1093/hmg/ddp379)

Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A & Tilghman SM 1995 Disruption of imprinting caused by deletion of the H19 gene region in mice. *Nature* **375** 34–39. (https://doi.org/10.1038/375034a0)

Lescisin KR, Varmuza S & Rossant J 1988 Isolation and characterization of a novel trophoblast-specific cDNA in the mouse. *Genes and Development* **2** 1639–1646. (https://doi.org/10.1101/gad.2.12a.1639)

Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R *et al*. 2006 Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *New England Journal of Medicine* **355** 992–1005. (https://doi.org/10.1056/NEJMoa055352)

Li Y & Behringer RR 1998 Esx1 is an X-chromosome-imprinted regulator of placental development and fetal growth. *Nature Genetics* **20** 309–311. (https://doi.org/10.1038/3129)

Li Y, Lemaire P & Behringer RR 1997 Esx1, a novel X chromosome-linked homeobox gene expressed in mouse extraembryonic tissues and male germ cells. *Developmental Biology* **188** 85–95. (https://doi.org/10.1006/dbio.1997.8640)

Li L, Keverne EB, Aparicio SA, Ishino F, Barton SC & Surani MA 1999 Regulation of maternal behavior and offspring growth by paternally expressed Peg3. *Science* **284** 330–333. (https://doi.org/10.1126/science.284.5412.330)

Li G, Xu C, Lin X, Qu L, Xia D, Hongdu B, Xia Y, Wang X, Lou Y, He Q *et al*. 2017 Deletion of Pdcd5 in mice led to the deficiency of placenta development and embryonic lethality. *Cell Death and Disease* **8** e2811. (https://doi.org/10.1038/cddis.2017.124)

Liu H, Wang Y, Zhang Y, Song Q, Di C, Chen G, Tang J & Ma D 1999 TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal. *Biochemical and Biophysical Research Communications* **254** 203–210. (https://doi.org/10.1006/bbrc.1998.9893)

Lo S, Russell JC & Taylor AW 1970 Determination of glycogen in small tissue samples. *Journal of Applied Physiology* **28** 234–236. (https://doi.org/10.1152/jappl.1970.28.2.234)

Lopez MF, Dikkes P, Zurakowski D & Villa-Komaroff L 1996 Insulin-like growth factor II affects the appearance and glycogen content of glycogen cells in the murine placenta. *Endocrinology* **137** 2100–2108. (https://doi.org/10.1210/endo.137.5.8612553)

Maltepe E, Schmidt JV, Baunoch D, Bradfield CA & Simon MC 1997 Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* **386** 403–407. (https://doi.org/10.1038/386403a0)

Manning BD & Toker A 2017 AKT/PKB signaling: navigating the network. *Cell* **169** 381–405. (https://doi.org/10.1016/j.cell.2017.04.001)

Matsubara S, Takizawa T & Sato I 1999 Glucose-6-phosphatase is present in normal and pre-eclamptic placental trophoblasts: ultrastructural enzyme-histochemical evidence. *Placenta* **20** 81–85. (https://doi.org/10.1053/plac.1998.0346)

Matsuoka S, Edwards MC, Bai C, Parker S, Zhang P, Baldini A, Harper JW & Elledge SJ 1995 p57KIP2, a structurally distinct member of the p21Cip1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes and Development* **9** 650–662. (https://doi.org/10.1101/gad.9.6.650)

Mclellan AS, Fischer B, Dveksler G, Hori T, wynne F, Ball M, Okumura K, Moore T & Zimmermann W 2005 Structure and evolution of the mouse pregnancy-specific glycoprotein (Psg) gene locus. *BMC Genomics* **6** 4. (https://doi.org/10.1186/1471-2164-6-4)

Mcnamara GI, Creeth HDJ, Harrison DJ, Tansey KE, Andrews RM, Isles AR & John RM 2018 Loss of offspring Peg3 reduces neonatal ultrasonic vocalizations and increases maternal anxiety in wild-type mothers. *Human Molecular Genetics* **27** 440–450. (https://doi.org/10.1093/hmg/ddx412)

Moore T, Constância M, Zubair M, Bailleul B, Feil R, Sasaki H & Reik W 1997 Multiple imprinted sense and antisense transcripts, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse Igf2. *PNAS* **94** 12509–12514. (https://doi.org/10.1073/pnas.94.23.12509)

Mould A, Morgan MA, Li L, Bikoff EK & Robertson EJ 2012 Blimp1/Prdm1 governs terminal differentiation of endovascular trophoblast giant cells and defines multipotent progenitors in the developing placenta.

Genes and Development **26** 2063–2074. (https://doi.org/10.1101/gad.199828.112)

Nagy A, Gocza E, Diaz EM, Prideaux VR, Ivanyi E, Markkula M & Rossant J 1990 Embryonic stem cells alone are able to support fetal development in the mouse. *Development* **110** 815–821.

Nakayama KI & Nakayama K 2006 Ubiquitin ligases: cell-cycle control and cancer. *Nature Reviews: Cancer* **6** 369–381. (https://doi.org/10.1038/nrc1881)

Napso T, Yong HEJ, Lopez-Tello J & Sferruzzi-Perri AN 2018 The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. *Frontiers in Physiology* **9** 1091. (https://doi.org/10.3389/fphys.2018.01091)

Naruse M, Ono R, Irie M, Nakamura K, Furuse T, Hino T, Oda K, Kashimura M, Yamada I, Wakana S *et al*. 2014 Sirh7/Ldoc1 knockout mice exhibit placental P4 overproduction and delayed parturition. *Development* **141** 4763–4771. (https://doi.org/10.1242/dev.114520)

Nie G, Li Y & Salamonsen LA 2005 Serine protease HtrA1 is developmentally regulated in trophoblast and uterine decidual cells during placental formation in the mouse. *Developmental Dynamics* **233** 1102–1109. (https://doi.org/10.1002/dvdy.20399)

Nishiyama M, Nita A, Yumimoto K & Nakayama KI 2015 FBXL12-mediated degradation of ALDH3 is essential for trophoblast differentiation during placental development. *Stem Cells* **33** 3327–3340. (https://doi.org/10.1002/stem.2088)

O'Connell BA, Moritz KM, Walker DW & Dickinson H 2013 Treatment of pregnant spiny mice at mid gestation with a synthetic glucocorticoid has sex-dependent effects on placental glycogen stores. *Placenta* **34** 932–940. (https://doi.org/10.1016/j.placenta.2013.06.310)

Oh-Mcginnis R, Bogutz AB & Lefebvre L 2011 Partial loss of Ascl2 function affects all three layers of the mature placenta and causes intrauterine growth restriction. *Developmental Biology* **351** 277–286. (https://doi.org/10.1016/j.ydbio.2011.01.008)

Oka C, Tsujimoto R, Kajikawa M, Koshiba-Takeuchi K, Ina J, Yano M, Tsuchiya A, Ueta Y, Soma A, Kanda H *et al*. 2004 HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins. *Development* **131** 1041–1053. (https://doi.org/10.1242/dev.00999)

Ono R, Nakamura K, Inoue K, Naruse M, Usami T, Wakisaka-Saito N, Hino T, Suzuki-Migishima R, Ogonuki N, Miki H *et al*. 2006 Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. *Nature Genetics* **38** 101–106. (https://doi.org/10.1038/ng1699)

Ouhilal S, Vuguin P, Cui L, Du XQ, Gelling RW, Reznik SE, Russell R, Parlow AF, Karpovsky C, Santoro N *et al*. 2012 Hypoglycemia, hyperglucagonemia, and fetoplacental defects in glucagon receptor knockout mice: a role for glucagon action in pregnancy maintenance. *American Journal of Physiology: Endocrinology and Metabolism* **302** E522–E531. (https://doi.org/10.1152/ajpendo.00420.2011)

Outhwaite JE, Natale BV, Natale DR & Simmons DG 2015 Expression of aldehyde dehydrogenase family 1, member A3 in glycogen trophoblast cells of the murine placenta. *Placenta* **36** 304–311. (https://doi.org/10.1016/j.placenta.2014.12.002)

Parchem JG, Kanasaki K, Kanasaki M, Sugimoto H, Xie L, Hamano Y, Lee SB, Gattone VH, Parry S, Strauss JF *et al*. 2018 Loss of placental growth factor ameliorates maternal hypertension and preeclampsia in mice. *Journal of Clinical Investigation* **128** 5008–5017. (https://doi.org/10.1172/JCI99026)

Pennisi DJ, Wilkinson L, Kolle G, Sohaskey ML, Gillinder K, Piper MJ, Mcavoy JW, Lovicu FJ & Little MH 2007 Crim1KST264/KST264 mice display a disruption of the Crim1 gene resulting in perinatal lethality with defects in multiple organ systems. *Developmental Dynamics* **236** 502–511. (https://doi.org/10.1002/dvdy.21015)

Pennisi DJ, Kinna G, Chiu HS, Simmons DG, Wilkinson L & Little MH 2012 Crim1 has an essential role in glycogen trophoblast cell and sinusoidal-trophoblast giant cell development in the placenta. *Placenta* **33** 175–182. (https://doi.org/10.1016/j.placenta.2011.12.014)

Perez-Garcia V, Fineberg E, Wilson R, Murray A, Mazzeo CI, Tudor C, Sienerth A, White JK, Tuck E, Ryder EJ *et al*. 2018 Placentation defects are highly prevalent in embryonic lethal mouse mutants. *Nature* **555** 463–468. (https://doi.org/10.1038/nature26002)

Prendergast CH, Parker KH, Gray R, Venkatesan S, Bannister P, Castro-Soares J, Murphy KW, Beard RW, Regan L, Robinson S *et al*. 1999 Glucose production by the human placenta in vivo. *Placenta* **20** 591–598. (https://doi.org/10.1053/plac.1999.0419)

Rampon C, Prandini MH, Bouillot S, Pointu H, Tillet E, Frank R, Vernet M & Huber P 2005 Protocadherin 12 (VE-cadherin 2) is expressed in endothelial, trophoblast, and mesangial cells. *Experimental Cell Research* **302** 48–60. (https://doi.org/10.1016/j.yexcr.2004.08.024)

Rampon C, Bouillot S, Climescu-Haulica A, Prandini MH, Cand F, Vandenbrouck Y & Huber P 2008 Protocadherin 12 deficiency alters morphogenesis and transcriptional profile of the placenta. *Physiological Genomics* **34** 193–204. (https://doi.org/10.1152/physiolgenomics.00220.2007)

Redline RW, Chernicky CL, Tan HQ, Ilan J & Ilan J 1993 Differential expression of insulin-like growth factor-II in specific regions of the late (post day 9.5) murine placenta. *Molecular Reproduction and Development* **36** 121–129. (https://doi.org/10.1002/mrd.1080360202)

Reichmann J, Reddington JP, Best D, Read D, Ollinger R, Meehan RR & Adams IR 2013 The genome-defence gene Tex19.1 suppresses LINE-1 retrotransposons in the placenta and prevents intra-uterine growth retardation in mice. *Human Molecular Genetics* **22** 1791–1806. (https://doi.org/10.1093/hmg/ddt029)

Relaix F, Weng X, Marazzi G, Yang E, Copeland N, Jenkins N, Spence SE & Sassoon D 1996 Pw1, a novel zinc finger gene implicated in the myogenic and neuronal lineages. *Developmental Biology* **177** 383–396. (https://doi.org/10.1006/dbio.1996.0172)

Robb SA & Hytten FE 1976 Placental glycogen. *British Journal of Obstetrics and Gynaecology* **83** 43–53. (https://doi.org/10.1111/j.1471-0528.1976.tb00729.x)

Robertson SA, Roberts CT, Farr KL, Dunn AR & Seamark RF 1999 Fertility impairment in granulocyte-macrophage colony-stimulating factor-deficient mice. *Biology of Reproduction* **60** 251–261. (https://doi.org/10.1095/biolreprod60.2.251)

Rossant J & Cross JC 2001 Placental development: lessons from mouse mutants. *Nature Reviews: Genetics* **2** 538–548. (https://doi.org/10.1038/35080570)

Rossant J, Guillemot F, Tanaka M, Latham K, Gertenstein M & Nagy A 1998 Mash2 is expressed in oogenesis and preimplantation development but is not required for blastocyst formation. *Mechanisms of Development* **73** 183–191. (https://doi.org/10.1016/s0925-4773(98)00051-3)

Salas M, John R, Saxena A, Barton S, Frank D, Fitzpatrick G, Higgins MJ & Tycko B 2004 Placental growth retardation due to loss of imprinting of Phlda2. *Mechanisms of Development* **121** 1199–1210. (https://doi.org/10.1016/j.mod.2004.05.017)

Sarkar AA, Nuwayhid SJ, Maynard T, Ghandchi F, Hill JT, Lamantia AS & Zohn IE 2014 Hectd1 is required for development of the junctional zone of the placenta. *Developmental Biology* **392** 368–380. (https://doi.org/10.1016/j.ydbio.2014.05.007)

Sarkar AA, Sabatino JA, Sugrue KF & Zohn IE 2016 Abnormal labyrinthine zone in the Hectd1-null placenta. *Placenta* **38** 16–23. (https://doi.org/10.1016/j.placenta.2015.12.002)

Saxena A, Morozov P, Frank D, Musalo R, Lemmon MA, Skolnik EY & Tycko B 2002 Phosphoinositide binding by the pleckstrin homology domains of Ipl and Tih1. *Journal of Biological Chemistry* **277** 49935–49944. (https://doi.org/10.1074/jbc.M206497200)

Screen M, Dean W, Cross JC & Hemberger M 2008 Cathepsin proteases have distinct roles in trophoblast function and vascular remodelling. *Development* **135** 3311–3320. (https://doi.org/10.1242/dev.025627)

Sferruzzi-Perri AN, Macpherson AM, Roberts CT & Robertson SA 2009 Csf2 null mutation alters placental gene expression and trophoblast glycogen cell and giant cell abundance in mice. *Biology of Reproduction* **81** 207–221. (https://doi.org/10.1095/biolreprod.108.073312)

Sferruzzi-Perri AN, Vaughan OR, Coan PM, Suciu MC, Darbyshire R, Constancia M, Burton GJ & Fowden AL 2011 Placental-specific Igf2 deficiency alters developmental adaptations to undernutrition in mice. *Endocrinology* **152** 3202–3212. (https://doi.org/10.1210/en.2011-0240)

Sferruzzi-Perri AN, Vaughan OR, Haro M, Cooper WN, Musial B, Charalambous M, Pestana D, Ayyar S, Ferguson-Smith AC, Burton GJ *et al*. 2013 An obesogenic diet during mouse pregnancy modifies maternal nutrient partitioning and the fetal growth trajectory. *FASEB Journal* **27** 3928–3937. (https://doi.org/10.1096/fj.13-234823)

Sharma A, Lacko LA, Argueta LB, Glendinning MD & Stuhlmann H 2019 miR-126 regulates glycogen trophoblast proliferation and DNA

methylation in the murine placenta. *Developmental Biology* **449** 21–34. (https://doi.org/10.1016/j.ydbio.2019.01.019)

Sferruzzi-Perri AN, Lopez-Tello J, Fowden AL & Constancia M 2016 Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)-p110alpha signaling to modify placental resource allocation. *PNAS* **113** 11255–11260. (https://doi.org/10.1073/pnas.1602012113)

Sferruzzi-Perri AN, Sandovici I, Constancia M & Fowden AL 2017 Placental phenotype and the insulin-like growth factors: resource allocation to fetal growth. *Journal of Physiology* **595** 5057–5093. (https://doi.org/10.1113/JP273330)

Sharma N, Kubaczka C, Kaiser S, Nettersheim D, Mughal SS, Riesenberg S, Holzel M, Winterhager E & Schorle H 2016 Tpbpa-Cre-mediated deletion of TFAP2C leads to deregulation of Cdkn1a, Akt1 and the ERK pathway, causing placental growth arrest. *Development* **143** 787–798. (https://doi.org/10.1242/dev.128553)

Sibilia M & Wagner EF 1995 Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* **269** 234–238. (https://doi.org/10.1126/science.7618085)

Sibley CP, Coan PM, Ferguson-Smith AC, Dean W, Hughes J, Smith P, Reik W, Burton GJ, Fowden AL & Constância M 2004 Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *PNAS* **101** 8204–8208. (https://doi.org/10.1073/pnas.0402508101)

Simmons DG & Cross JC 2005 Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Developmental Biology* **284** 12–24. (https://doi.org/10.1016/j.ydbio.2005.05.010)

Simmons DG, Fortier AL & Cross JC 2007 Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta. *Developmental Biology* **304** 567–578. (https://doi.org/10.1016/j.ydbio.2007.01.009)

Simmons DG, Natale DR, Begay V, Hughes M, Leutz A & Cross JC 2008*a* Early patterning of the chorion leads to the trilaminar trophoblast cell structure in the placental labyrinth. *Development* **135** 2083–2091. (https://doi.org/10.1242/dev.020099)

Simmons DG, Rawn SM, Davies A, Hughes M & Cross JC 2008*b* Spatial and temporal expression of the 23 murine prolactin/placental lactogen-related genes is not associated with their position in the locus. *BMC Genomics* **9** 352. (https://doi.org/10.1186/1471-2164-9-352)

Singh VP, Alex JL, Lakshmi BJ, Sailasree SP, Raj TA & Kumar S 2015 Role of mouse Wdr13 in placental growth; a genetic evidence for lifetime body weight determination by placenta during development. *Scientific Reports* **5** 13371. (https://doi.org/10.1038/srep13371)

Soares MJ & Hunt JS 2014 *Placenta and Trophoblast: Methods and Protocols*. Humana Press.

Takagi N & Sasaki M 1975 Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature* **256** 640–642. (https://doi.org/10.1038/256640a0)

Takahashi K, Kobayashi T & Kanayama N 2000 p57(Kip2) regulates the proper development of labyrinthine and spongiotrophoblasts. *Molecular Human Reproduction* **6** 1019–1025. (https://doi.org/10.1093/molehr/6.11.1019)

Takao T, Asanoma K, Tsunematsu R, Kato K & Wake N 2012 The maternally expressed gene Tssc3 regulates the expression of MASH2 transcription factor in mouse trophoblast stem cells through the AKT-Sp1 signaling pathway. *Journal of Biological Chemistry* **287** 42685–42694. (https://doi.org/10.1074/jbc.M112.388777)

Tarkowski AK, Witkowska A & Opas J 1977 Development of cytochalasin in B-induced tetraploid and diploid/tetraploid mosaic mouse embryos. *Journal of Embryology and Experimental Morphology* **41** 47–64.

Tayade C, Hilchie D, He H, Fang Y, Moons L, Carmeliet P, Foster RA & Croy BA 2007 Genetic deletion of placenta growth factor in mice alters uterine NK cells. *Journal of Immunology* **178** 4267–4275. (https://doi.org/10.4049/jimmunol.178.7.4267)

Tesser RB, Scherholz PL, Do Nascimento L & Katz SG 2010 Trophoblast glycogen cells differentiate early in the mouse ectoplacental cone: putative role during placentation. *Histochemistry and Cell Biology* **134** 83–92. (https://doi.org/10.1007/s00418-010-0714-x)

Thiaville MM, Huang JM, Kim H, Ekram MB, Roh TY & Kim J 2013 DNA-binding motif and target genes of the imprinted transcription factor PEG3. *Gene* **512** 314–320. (https://doi.org/10.1016/j.gene.2012.10.005)

Tran H, Bustos D, Yeh R, Rubinfeld B, Lam C, Shriver S, Zilberleyb I, Lee MW, Phu L, Sarkar AA *et al*. 2013 HectD1 E3 ligase modifies adenomatous polyposis coli (APC) with polyubiquitin to promote the APC-axin interaction. *Journal of Biological Chemistry* **288** 3753–3767. (https://doi.org/10.1074/jbc.M112.415240)

Tsoi SC, Cale JM, Bird IM & Kay HH 2003 cDNA microarray analysis of gene expression profiles in human placenta: up-regulation of the transcript encoding muscle subunit of glycogen phosphorylase in preeclampsia. *Journal of the Society for Gynecologic Investigation* **10** 496–502. (https://doi.org/10.1016/s1071-5576(03)00154-0)

Tunster SJ, Tycko B & John RM 2010 The imprinted Phlda2 gene regulates extraembryonic energy stores. *Molecular and Cellular Biology* **30** 295–306. (https://doi.org/10.1128/MCB.00662-09)

Tunster SJ, Van De Pette M & John RM 2011 Fetal overgrowth in the Cdkn1c mouse model of Beckwith-Wiedemann syndrome. *Disease Models and Mechanisms* **4** 814–821. (https://doi.org/10.1242/dmm.007328)

Tunster SJ, Van De Pette M & John RM 2012 Impact of genetic background on placental glycogen storage in mice. *Placenta* **33** 124–127. (https://doi.org/10.1016/j.placenta.2011.11.011)

Tunster SJ, Jensen AB & John RM 2013 Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* **145** R117–R137. (https://doi.org/10.1530/REP-12-0511)

Tunster SJ, Van De Pette M & John RM 2014 Isolating the role of elevated Phlda2 in asymmetric late fetal growth restriction in mice. *Disease Models and Mechanisms* **7** 1185–1191. (https://doi.org/10.1242/dmm.017079)

Tunster SJ, Creeth HD & John RM 2015 The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. *Developments in Biologicals*.

Tunster SJ, Mcnamara GI, Creeth HDJ & John RM 2016 Increased dosage of the imprinted Ascl2 gene restrains two key endocrine lineages of the mouse Placenta. *Developmental Biology* **418** 55–65. (https://doi.org/10.1016/j.ydbio.2016.08.014)

Tunster SJ, Boque-Sastre R, Mcnamara GI, Hunter SM, Creeth HDJ & John RM 2018 Peg3 deficiency results in sexually dimorphic losses and gains in the normal repertoire of placental hormones. *Frontiers in Cell and Developmental Biology* **6** 123. (https://doi.org/10.3389/fcell.2018.00123)

Van Schaftingen E & Gerin I 2002 The glucose-6-phosphatase system. *Biochemical Journal* **362** 513–532. (https://doi.org/10.1042/0264-6021:3620513)

Villee CA 1953 The metabolism of human placenta in vitro. *Journal of Biological Chemistry* **205** 113–123.

Vincent SD, Dunn NR, Sciammas R, Shapiro-Shalef M, Davis MM, Calame K, Bikoff EK & Robertson EJ 2005 The zinc finger transcriptional repressor Blimp1/Prdm1 is dispensable for early axis formation but is required for specification of primordial germ cells in the mouse. *Development* **132** 1315–1325. (https://doi.org/10.1242/dev.01711)

Wang Y, Li X, Wang L, Ding P, Zhang Y, Han W & Ma D 2004 An alternative form of paraptosis-like cell death, triggered by TAJ/TROY and enhanced by PDCD5 overexpression. *Journal of Cell Science* **117** 1525–1532. (https://doi.org/10.1242/jcs.00994)

Wang SS, Aurora AB, Johnson BA, Qi XX, Mcanally J, Hill JA, Richardson JA, Bassel-Duby R & Olson EN 2008 The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. *Developmental Cell* **15** 261–271. (https://doi.org/10.1016/j.devcel.2008.07.002)

Watson ED & Cross JC 2005 Development of structures and transport functions in the mouse placenta. *Physiology* **20** 180–193. (https://doi.org/10.1152/physiol.00001.2005)

Wilkinson L, Gilbert T, Kinna G, Ruta LA, Pennisi D, Kett M & Little MH 2007 Crim1KST264/KST264 mice implicate Crim1 in the regulation of vascular endothelial growth factor-A activity during glomerular vascular development. *Journal of the American Society of Nephrology* **18** 1697–1708. (https://doi.org/10.1681/ASN.2006091012)

Wislocki GB & Bennett HS 1943 The histology and cytology of the human and monkey placenta, with special reference to the trophoblast. *American Journal of Anatomy* **73** 335–449. (https://doi.org/10.1002/aja.1000730303)

Withington SL, Scott AN, Saunders DN, Lopes Floro K, Preis JI, Michalicek J, Maclean K, Sparrow DB, Barbera JP & Dunwoodie SL 2006 Loss of Cited2 affects trophoblast formation and vascularization

of the mouse placenta. *Developmental Biology* **294** 67–82. (https://doi.org/10.1016/j.ydbio.2006.02.025)

Wysolmerski JJ & Stewart AF 1998 The physiology of parathyroid hormone-related protein: an emerging role as a developmental factor. *Annual Review of Physiology* **60** 431–460. (https://doi.org/10.1146/annurev.physiol.60.1.431)

Yang ZZ, Tschopp O, Hemmings-Mieszczak M, Feng J, Brodbeck D, Perentes E & Hemmings BA 2003 Protein kinase B alpha/Akt1 regulates placental development and fetal growth. *Journal of Biological Chemistry* **278** 32124–32131. (https://doi.org/10.1074/jbc.M302847200)

Yevtodiyenko A & Schmidt JV 2006 Dlk1 expression marks developing endothelium and sites of branching morphogenesis in the mouse embryo and placenta. *Developmental Dynamics* **235** 1115–1123. (https://doi.org/10.1002/dvdy.20705)

Yin Z, Haynie J, Yang X, Han B, Kiatchoosakun S, Restivo J, Yuan S, Prabhakar NR, Herrup K, Conlon RA *et al*. 2002 The essential role of Cited2, a negative regulator for HIF-1alpha, in heart development and neurulation. *PNAS* **99** 10488–10493. (https://doi.org/10.1073/pnas.162371799)

Yu JS & Cui W 2016 Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development* **143** 3050–3060. (https://doi.org/10.1242/dev.137075)

Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS & Burton GJ 2008 Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *American Journal of Pathology* **173** 451–462. (https://doi.org/10.2353/ajpath.2008.071193)

Zhang J, Wang C, Terroni PL, Cagampang FR, Hanson M & Byrne CD 2005 High-unsaturated-fat, high-protein, and low-carbohydrate diet during pregnancy and lactation modulates hepatic lipid metabolism in female adult offspring. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **288** R112–R118. (https://doi.org/10.1152/ajpregu.00351.2004)

Zhang J, Zhang F, Didelot X, Bruce KD, Cagampang FR, Vatish M, Hanson M, Lehnert H, Ceriello A & Byrne CD 2009 Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring. *BMC Genomics* **10** 478. (https://doi.org/10.1186/1471-2164-10-478)

Zhang Q, Hao J & Li G 2019 Deletion of Prl7d1 causes placental defects at mid-pregnancy in mice. *Molecular Reproduction and Development* **86** 696–713. (https://doi.org/10.1002/mrd.23148)

Zheng-Fischhofer Q, Kibschull M, Schnichels M, Kretz M, Petrasch-Parwez E, Strotmann J, Reucher H, Lynn BD, Nagy JI, Lye SJ *et al*. 2007 Characterization of connexin31.1-deficient mice reveals impaired placental development. *Developmental Biology* **312** 258–271. (https://doi.org/10.1016/j.ydbio.2007.09.025)

Zheng N, Wang Z & Wei W 2016 Ubiquitination-mediated degradation of cell cycle-related proteins by F-box proteins. *International Journal of Biochemistry and Cell Biology* **73** 99–110. (https://doi.org/10.1016/j.biocel.2016.02.005)

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