Homeobox genes and their down-stream transcription factors in normal and pathological human placental development

Padma Murthi^{ab}, Bill Kalionis^{ab}, Mélanie Cocquebert^{cde}, Gayathri Rajaraman^{ab}, Amy Chui^{ab}, Rosemary J. Keogh^{ab}, Danièle Evain-Brion^{cde}, Thierry Fournier^{cde}

^aDepartment of Perinatal Medicine Pregnancy Research Centre,

^bUniversity of Melbourne Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, 3052, Australia

^c Inserm, UMR-S 767, Paris, F-75006 France;

^d Université Paris Descartes, Sorbonne Paris Cité, Paris, F-75006 France;

^e PremUP, Foundation, Paris, F-75006 France;

Corresponding author to whom all correspondence should be addressed: Thierry Fournier, INSERM, UMR-S 767, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75006 Paris, France

Tel.: +33 1 53 73 96 03;

Fax: +33 1 44 07 39 92;

E-mail address: thierry.fournier@parisdescartes.fr

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Abstract

The placenta provides critical transport functions between the maternal and fetal circulations during intrauterine development. Formation of this interface is controlled by nuclear transcription factors including homeobox genes. Here we summarize current knowledge regarding the expression and function of homeobox genes in the placenta. We also describe the identification of target transcription factors, including PPAR γ , and biological pathways that are regulated by homeobox genes and their role in the formation of the functional placenta. The role of the nuclear receptor PPAR γ , ligands and target genes in human placental development is further discussed. A better understanding of these pathways will improve our knowledge of placental cell biology and has the potential to reveal new molecular targets for the early detection and diagnosis of pregnancy complications including human fetal growth restriction.

Introduction

During pregnancy, the placenta orchestrates multiple functions, which are required to initiate, maintain and control the outcome of gestation. In particular, generation of distinct trophoblast cell types within the placenta is required to fulfil the complex biological processes of implantation, maternal-fetal exchange and maternal tolerance of fetal-parental antigens. In some species, including human, the placenta performs hormonal functions required for the maintenance of gestation and fetal development.

Trophoblasts originate from the trophoctoderm, the outermost epithelial cell layer of the blastocyst, which triggers attachment and implantation in the receptive endometrium of the mother. In humans, the principal structures of the haemochorial placenta are apparent as early as the 21st day of pregnancy [1, 2]. Following blastocyst adhesion, trophoblast cells proliferate rapidly and undergo cell fusion to form the syncytium, which invades the maternal uterine stroma. By day 21 after ovulation, the chorionic villi, the definitive structural and functional units of the placenta, become established.

The trophoblast differentiates into either the villous or the extravillous trophoblast lineages. In the villous lineage, the cytotrophoblasts of the floating villi, which are present in the intervillous space, remain attached to the villous basement membrane and form a monolayer of epithelial cells. These cells proliferate and differentiate by fusion to form the syncytium, which covers the entire surface of the villus. Thus, the multinucleated syncytium of the definitive placenta is generated by continuous cell fusion of underlying mononuclear cytotrophoblasts. The syncytium is multi-functional, with primary functions that include absorption, exchange and transport of nutrients and gases from the maternal to the fetal circulation, and specific hormonal functions. Additionally, the multinuclear syncytium is the

primary site of major endocrine activity of the organ since it secretes hormones such as human chorionic gonadotropin (hCG), human placental lactogen (hPL) or placental growth hormone (PGH), which are important for placental growth and/or maternal adaptation to pregnancy [3-5].

In the extravillous phenotype, the cytotrophoblast cells of the anchoring villi in contact with the uterine wall proliferate, detach from the basement membrane and aggregate into multilayered columns of non-polarised cells that rapidly invade the uterus wall. This trophoblastic invasion is confined to the decidua, the first third of the myometrium, and the associated spiral arterioles. Interstitial trophoblasts invade the decidua and parts of the myometrium, whilst endovascular trophoblasts migrate and invade along the wall of the uterine spiral arteries, which results in remodelling of the uterine spiral arteries [6]. In particular, modification of the maternal vessels by trophoblasts, which replace the maternal endothelium, is thought to be critical for the successful progression of pregnancy since absence of the endovascular trophoblasts is associated with cases of pre-eclampsia (PE) and fetal growth restriction (FGR) [7, 8]. Thus, it is evident that elucidation of the key molecular mechanisms controlling commitment and regulating differentiation of the diverse trophoblast cell types in humans would be helpful to understand the pathogenesis of these pregnancy diseases.

During the past decade, transgenic mouse models that allow gene knock-out and gene targeting, have vastly improved our understanding of the genetic control of placental development in the mouse and have opened up new avenues of investigation in developmental biology. Despite considerable differences in placental development in mice and humans, some fundamental elements of placental development are similar in both species.

Furthermore, certain key molecular regulators such as transcription factors involved in the expression of various placental genes have been described in both human and mouse placenta [9-11].

Transcriptional control of placental development

Growth factors and signalling molecules represent the cue to which a cell responds by either maintaining or altering its state of differentiation. However, it is the transcription factors, located within the cell nucleus, that determine how this cue is interpreted and what the cellular response will be. Transcription factors achieve this by regulating expression of their target genes within the cell. Numerous transcription factors play essential roles in cellular development and differentiation of a variety of cell types, including the trophoblast cell type in the placenta [10, 11]. Transcription factors are categorised into a few large families such as the zinc finger, leucine zipper, helix-loop-helix, helix-turn-helix and homeobox genes [12, 13] and also include the ligand-activated nuclear receptor superfamily.

Homeobox genes

Homeobox genes (also known as homeotic genes) were originally discovered in the fruit fly *Drosophila melanogaster*, where they act as transcriptional regulators to control embryonic morphogenesis [14-17]. These genes contain a highly conserved 180 base pair homeobox sequence, which encodes a 60 amino acid homeodomain. Structural analyses have shown that the homeodomain consists of an evolutionarily conserved helix-turn-helix motif that binds to the DNA. The specificity of this binding allows homeodomain proteins to activate or repress the expression of batteries of down-stream target genes [18].

Homeobox genes are subdivided into the “clustered” homeobox genes known as “*HOX*” genes, the “non-clustered” divergent or orphan *HOX*-like genes, as well as several distinct classes of atypical homeodomain containing genes. The *HOX* family of homeobox genes plays a fundamental role in embryonic morphogenesis and was identified in mammals and vertebrates based on sequence homology to the genes of the *Drosophila* HOM-C complex [19, 20]. In humans the HOX complex is comprised of 39 genes that are arranged into four separate chromosomal clusters designated *HOX A, B, C* and *D* [21, 22]. Homeobox genes are grouped together into various subfamilies based on a variety of criteria such as their functional and structural characteristics and these subfamilies of homeobox genes are essential for the control of specific aspects of cellular growth and differentiation [23, 24].

Most important is that homeobox genes are directly or indirectly involved in a variety of developmental disorders, diseases and cancers [25]. Evidence for the deregulation of certain homeobox genes in cancer and other diseases provides support for the idea that homeobox genes are vital for normal mammalian development. Furthermore, characterisation of such homeobox genes may lead to a greater understanding of the developmental mechanisms, which are disrupted in a variety of disease states. There is evidence that normal homeobox gene expression can be altered during a disease state, such as decreased expression of *Cdx2* in the intestinal epithelium of patients with colorectal cancers and decreased *Meox2* expression in brain endothelial cells of patients affected by Alzheimer’s disease [25, 26]. Thus, homeobox genes could be used as disease markers or potential therapeutic targets of diseases, such as cancer, diabetic wound healing, lymphedema, Alzheimer’s disease, and stroke due to atherosclerosis [27, 28]. Mouse knockouts have provided genetic proof that homeobox genes regulate embryonic organogenesis and morphogenesis [28-31].

Homeobox genes in placental development

Given the highly important role of homeobox genes in embryonic and adult development, it is not surprising that homeobox genes also play major roles in controlling extraembryonic development of the placenta. Homeobox genes regulate mouse placental cell functions and targeted gene mutations of homeobox genes in the mouse produce FGR-like effects [29, 32-42].

Homeobox genes in human placental development

Understanding the role of homeobox genes in human placental development and their functional role in pregnancy pathologies including fetal growth restriction and preeclampsia is highly important. A strategy for understanding the molecular mechanisms of placental function in normal and pathological human placentas requires (i) determining the spatio-temporal expression pattern of homeobox genes during placental development that have an “evolutionary history” of regulating cell fate decisions during embryonic or adult development, (ii) determining whether specific homeobox gene expression levels are changed in pathological placentas compared with gestation matched controls, (iii) creating *in vitro* models of placental cultured cells that “mimic” homeobox gene expression changes observed in pathological placentas by the use of loss- or gain-of function phenotypes using RNA interference systems or gene overexpression plasmids and (iv) defining the biological functions of the target genes using *in vitro* models. Similar approaches have been proven very successful in identifying transcriptional control of endocrine functions during murine placental development [reviewed in [9-11]]. Therefore, identification of the homeobox target genes in specialised cell types of the human placenta could reveal the molecular pathways responsible for important placental cell functions. These pathways are likely to be affected in

placental pathologies. Using the strategy described above, we undertook comprehensive and extensive analyses of homeobox genes in the normal and pathological placenta.

Homeobox genes in human placental trophoblast cells

(i) Spatio-temporal expression patterns of homeobox genes in the placenta

Studies in the human placenta have focused mainly on identifying homeobox genes expressed in the normal placenta [43, 44], and those showing altered expression in trophoblastic cancers [45]. The homeobox genes we and others have identified to be of potential importance in the human placenta are *DLX3*, *DLX4*, *GAX*, *ESX1L* and *HLX* [33, 46-49]. These genes are also expressed in the embryo and play major roles in embryonic development [50, 51]. More specifically, we have demonstrated that the homeobox gene *HLX* is expressed primarily in the proliferating cytotrophoblast cell types in early placental development [52]. Recently, we also demonstrated the expression profile of homeobox gene *DLX3* in the human placenta [53]. In the first trimester and term placenta, *DLX3* was localised to the nuclei of villous cytotrophoblast cells, the syncytiotrophoblast layer and the proliferating extravillous trophoblast cells in the proximal regions of the cell columns [53].

Few functional studies have been carried out on human placental homeobox genes. Inactivation of homeobox gene *DLX4* resulted in altered rates of trophoblast cell apoptosis and a potential role in epithelial-mesenchymal transition [54, 55]. Homeobox gene *DLX3* regulates the expression of the alpha subunit of hCG [56] and of 3- β HSD [35], both of which are important for placental trophoblast function.

In the following section we summarise our current understanding of homeobox gene regulation in human placental development, more specifically in human trophoblast function,

as well as give insights into novel mechanisms of trophoblast dysfunction observed in the important pregnancy pathology of idiopathic fetal growth restriction (FGR).

(ii) *Homeobox gene expression levels are changed in FGR placentas compared with gestation-matched controls.*

The cohort of FGR-affected pregnancies used for our studies was carefully defined in clinical terms and represented the severe end of spectrum of idiopathic FGR. FGR pregnancies were identified using the general inclusion criterion of a birth weight less than the 10th centile for gestation age, using Australian growth charts. FGR cases were classified as idiopathic if there was evidence of an underlying pathology, judged by the presence of at least two of the following antenatal ultrasound diagnostic criteria; abnormal umbilical artery Doppler flow velocimetry, oligohydramnios as determined by amniotic fluid index (AFI) <7, or asymmetric growth of the fetus as measured from the HC (head circumference) to AC (abdominal circumference) ratio (>1.2). Fetuses showed reduced growth by the late second and early third trimester. Reduced placental villous tree elaboration, diminished surface area of the placenta and abnormal end-diastolic blood flow in the umbilical artery are associated with pregnancies with severely growth-restricted infants [57, 58].

The pattern of normal human fetal growth is complex. Increases in the rates of fetal weight gain and length increase are not parallel throughout pregnancy. Evidence suggests that the maximal growth rate for length is seen in the second trimester, whereas the maximal rate of weight gain is early in the third trimester [59-61]. Guihard-Costa et al. [59], in a longitudinal study of human fetal growth, have reported a linear growth rate until 26 weeks, and thereafter the growth rate decreased. Homeobox genes *HLX* [62] and *ESX1L* [63] showed decreased expression in FGR-affected placentas compared with matched controls. There was a rapid

decline in the levels of both *HLX* and *ESX1L* expression from 27-week gestation, which may correspond to the decline in the growth rate of the fetus [59-61] seen in the third trimester.

However, homeobox genes *DLX4* and *DLX3* showed increased expression in FGR-affected placentas [64, 65]. Our observation of altered homeobox gene expression levels, *i.e.* decreased (*HLX*, *ESX1L*) or increased (*DLX3* and *DLX4*) expression in FGR-placentas compared with gestation matched controls, prompted us to model these effects in cell culture.

(iii) Creating in vitro models of placental cultured cells that “mimic” homeobox gene expression changes observed on FGR

To identify the functional role homeobox genes we altered homeobox gene expression to “mimic” changes observed in FGR using techniques of gene inactivation or over-expression in trophoblast-derived cell lines *in vitro*. We used transformed trophoblast-derived cell lines that represent first trimester human villous and extravillous cytotrophoblast cells and which are capable of proliferation, differentiation, migration and invasion. To determine the functional role of decreased *HLX* levels observed in FGR, we reduced *HLX* expression levels in trophoblast-derived cell lines SGHPL4 and HTR8-SV/neo using short-interference RNAs (siRNA) specific for *HLX* [66-68].

To identify the functional role of *DLX3*, which showed increased expression in FGR, we increased the expression of *DLX3* in trophoblast-derived cell line, BeWo using a *DLX3*-specific over-expressing plasmid construct [69]. Over-expression of *DLX3* in BeWo cells increased BeWo cell differentiation, as measured by markers of differentiation: 3 β -HSD, syncytin and β -hCG protein. The complementary result of decreased BeWo cell

differentiation was obtained when *DLX3* expression was reduced in the presence of *DLX3*-specific siRNA [65].

Alterations in the rate of trophoblast differentiation, proliferation and invasion are involved in the placental pathologies of fetal growth restriction and pre-eclampsia. From the above studies, we have concluded that homeobox genes may have several potentially important roles in the regulation of fundamental functions of trophoblast proliferation and differentiation during placental development in humans and there may be significant deleterious consequences to decreased *HLX* or increased *DLX3* expression levels in the FGR-affected placenta.

(iv) Identification of down-stream target genes of homeobox genes

Understanding the precise regulatory mechanisms through which homeobox genes achieve molecular control during placental development requires the identification of target genes within the downstream developmental pathways. Genes involved in regulating cellular mechanisms such as mitotic rate, cell-cell adhesion, and cell migration during morphogenesis have been identified as target genes for many homeobox genes [70-79]. Thus, homeobox genes act as “master regulators” of development and control transcription by binding to regulatory elements in the promoter regions of target genes [20].

Few studies of target genes regulated by transcription factors in the human placenta have been reported. Therefore, we undertook the identification of down-stream targets of human placental homeobox genes. We used *in vitro* models of trophoblast-derived cell lines that represent villous and extravillous trophoblasts. We mimicked changes in expression of

homeobox genes observed in FGR- affected placentae either by gene inactivation or gene overexpression and then determined the target genes that were affected by these changes.

Using siRNA-mediated inactivation of *HLX*, we investigated the target genes by which *HLX* mediates extravillous trophoblast function in normal and FGR-affected placentae. Previous studies in other systems showed that inhibition of *HLX* by antisense oligonucleotide methods impaired CD34+ bone marrow cell proliferation in response to stimulation by cytokines, whilst inducing differentiation of these cells. Moreover, *HLX* inhibition also reduced the levels of c-myc, c-fos, cyclin B and p34cdc2 mRNA expression [80]. These cell cycle regulatory genes were predicted to be involved in the function of trophoblast cells [81].

We used siRNA to inactivate *HLX* in the SGHPL-4 and HTR-8/SVneo trophoblast cell line models and detected changes in gene expression using pathway-specific low density PCR arrays for the MAP (mitogen activated signaling)- kinase signaling pathways. The downstream target genes of *HLX* were identified as *RBI*, *MYC*, *EGRI*, *CDKN1C*, *ELK1*, *CCNB1* and *JUN* [67]. We identified four *HLX* downstream target genes: *CCNB1*, *MYC*, *CDKN1C* and *JUN*, which as described above were also previously identified as *HLX* target genes in haematopoietic progenitor cells [81]. Thus, *HLX* homeobox gene targets were cell cycle regulatory genes in two independent cell types *i.e* trophoblast and haematopoietic cells.

Further analyses of candidate down-stream target genes of *HLX*, and their level of expression and potential contribution to functional abnormalities, was investigated in FGR-affected placental tissues. Retinoblastoma-1 (*RBI*, also known as Rb) is a tumor suppressor gene that was first discovered in genetic studies of hereditary retinoblastoma and plays an important role in regulating cell proliferation and differentiation [82]. *RBI* is expressed in the proximal

region of proliferating trophoblast cells in the trophoblast cell column [83], where *HLX* is also expressed [52]. We also demonstrated that of the target genes that showed increased expression following siRNA mediated *HLX* inactivation in cell culture, *RBI* showed the highest relative increase in expression levels in FGR-affected placental tissues compared with controls [67]. These data suggest *RBI* is a negative regulator of cell proliferation and that increased *RBI* expression levels in FGR may reduce trophoblast proliferation and result in a depleted pool of trophoblast cells available to migrate and invade into the maternal decidua. This reduction in trophoblast proliferation may contribute to the shallow, inadequate remodeling of the maternal spiral arteries associated with FGR.

MYC is a proto-oncogene and is expressed in the actively proliferating extravillous trophoblast cells of the human placenta [84], where we have shown *HLX* to be highly expressed [52]. Our findings showed that *MYC* expression was significantly increased in FGR-affected human placental tissue, consistent with the increase in *MYC* expression in *HLX* inactivated cultured trophoblast cells [67]. This suggested that *MYC*, as a downstream target gene of *HLX*, is a molecular target associated with idiopathic human FGR. Therefore, *HLX* mediated increase in *MYC* expression may contribute to increased cell apoptosis that is frequently associated with FGR.

CDKN1C (p57/kip2) is a member of the CIP/KIP family of cyclin dependent kinase inhibitors that inhibits several cyclin dependent kinase kinase/cyclin complexes and is a regulator of cell proliferation [85]. In the normal human placenta, strong nuclear *CDKN1C* expression was observed in extravillous trophoblast, cytotrophoblast, and implantation-site interstitial trophoblast, but was absent in syncytiotrophoblast [86]. This expression of *CDKN1C* in the human placenta is consistent with the expression pattern of *HLX* from our study [52]. Results

from our own findings showed that *CDKN1C* expression is significantly reduced in cultured trophoblast cells and therefore is a direct or indirect target candidate gene of *HLX* in cultured trophoblast cells [67]. This suggests that *HLX*-mediated reduction of *CDKN1C* expression reduces trophoblast proliferation. Further confirmation of reduced *CDKN1C* mRNA expression was obtained in FGR-affected human placental tissue.

ELK1 is a member of the *ETS* family and acts as a transcription factor for the *MET* gene [87], which is expressed in placental cytotrophoblasts [88]. As with *HLX*, *ELK1* is also expressed in human extravillous trophoblast cells [89], and is suggested to play a role in the regulation of cell proliferation and migration [90]. Consistent with our *in vitro* results suggesting *ELK1* is a target for *HLX*, we showed that *ELK1* expression was also significantly decreased in FGR-affected human placental tissue.

CCNB1 is a regulatory gene expressed predominantly during the G2/M phase of the cell cycle, functionally involved in cell mitosis. Studies have shown expression of *CCNB1* in the villous trophoblast and the extravillous trophoblast cell types of the human placenta [91, 92], which also correlates with placental *HLX* expression from our findings [52]. We showed that *CCNB1* mRNA expression was significantly reduced in cultured trophoblast cells with *HLX* inactivation, suggesting that *CCNB1* is a downstream target gene of *HLX*. *CCNB1* expression was also significantly reduced in FGR-affected placental tissues compared with controls. Therefore, our findings showed that the cell culture model was consistent with the observed changes seen in *HLX* levels in FGR and changes in *CCNB1* levels, and suggested an association between reduced *HLX* levels and reduced *CCNB1* levels in FGR.

JUN is a member of the *AP-1* family of transcription factors and is implicated as a key regulator of human extravillous trophoblast proliferation, invasion and differentiation [93]. Not surprisingly, *JUN* is also strongly expressed in the highly proliferative extravillous trophoblast cells of the human placenta [93], consistent with *HLX* expression in the human placenta [52]. We showed that *JUN* expression was also significantly decreased in FGR-affected human placental tissues compared with controls. Therefore, decreased *JUN* expression, either directly or indirectly by *HLX*, may result in aberrant trophoblast functions associated with FGR.

Our findings demonstrate that *in vitro* models of siRNA-mediated knockdown of *HLX* expression in placental trophoblast cells show consistent changes to those observed in human FGR, where *HLX* levels are reduced. These results suggest that reduced levels of *HLX* seen in FGR cause direct or indirect effects on target genes that are altered in FGR. Therefore, reduced *HLX* levels directly or indirectly cause gene expression changes in targets that result in deleterious effects on trophoblast function.

Identification of targets of DLX3

Recent studies in our laboratory reported that *DLX3* is expressed in the differentiating villous cytotrophoblasts of term placentae and the localisation of 3 β HSD to syncytiotrophoblast in term placentae, suggesting that in the human placenta, *DLX3* may have a regulatory role on 3 β HSD expression and differentiation of villous cytotrophoblasts to syncytiotrophoblast [65]. We have also shown that *DLX3* was detected in villous cytotrophoblasts and syncytiotrophoblast in both term control and term FGR-affected placentae [65]. Therefore, we investigated the possible role of *DLX3* in villous trophoblast differentiation.

Villous trophoblast differentiation is marked by increased production of 3 β HSD [94], β hCG [95] and syncytin [96]. Our study demonstrated that inactivation of *DLX3* in the trophoblast derived BeWo cell line, and in primary villous cytotrophoblasts, resulted in decreased expression of differentiation markers 3 β HSD, β hCG and syncytin. This suggests that 3 β HSD, β hCG and syncytin are targets of *DLX3* and that *DLX3* is required for trophoblast differentiation. In another study from our laboratory, we observed forskolin treatment of BeWo cells was used to induce differentiation and resulted in increased mRNA expression of *DLX3* as well as *3BHSD* (3 β HSD), *CGB* (β hCG) and *syncytin* [69]. Furthermore, we observed that over-expression of *DLX3*, using a *DLX3* over-expressing plasmid construct in BeWo cells, significantly increased *3BHSD*, *CGB* and *syncytin* expression [69]. Together these studies provide strong evidence that 3 β HSD, β hCG and syncytin are targets of *DLX3* in cultured trophoblast cells.

Expression of 3 β HSD, β hCG and syncytin and *DLX3* was increased in FGR-affected placental tissues compared with controls, providing evidence that these genes are targets of *DLX3* *in vivo* as well as *in vitro* [65]. Together, these suggest that *DLX3* may have several potentially important roles in the regulation of trophoblast differentiation during placental development in humans and there may be significant deleterious consequences to increased *DLX3* in FGR, through the action of target genes 3 β HSD, β hCG and syncytin, on trophoblast function.

Recent studies from our laboratory have identified the nuclear receptor, peroxisome proliferator-activated receptors (PPAR) as a candidate downstream target gene of *DLX3* (Chui et al., submitted to *J Pathology*). This is potentially significant, since PPAR γ is an important gene in placental development and its expression is significantly altered in human FGR [97].

The peroxisome proliferators-activated receptor family

Peroxisome proliferators-activated receptors (PPARs; for review see [98]) are transcription factors belonging to the large ligand-activated nuclear receptor superfamily including the retinoic acid receptors (RAR, RXR), the thyroid hormone receptors (TR), the liver X receptors (LXR), the vitamin D3 receptors (VDR), and the steroid receptors (SR) [99]. In 1990, PPARs were first identified as receptors for fibrates, a class of hypolipidemic drugs used in humans since the late 1960s [100]. The name “PPAR” was initially chosen because of their ability to induce the proliferation of peroxisomes in rodents [101], but since the initial cloning in the mouse by Issemann and Green of the first PPAR isotype called PPAR α , and the discovery of two additional isotypes (PPAR β / δ and PPAR γ) by Dreyer et al. in 1992 [102], numerous studies have revealed that PPARs exhibit distinct tissue distribution patterns and control the expression of a large array of genes involved in lipid metabolism but also in the control of a broad range of cellular responses. Thus, PPAR α is mainly expressed in the liver where it represents a major regulator of lipid catabolism and transport, whereas PPAR γ is highly expressed in white and brown adipose tissue and is an important determinant of adipocyte differentiation, lipid storage and glucose homeostasis. PPAR β / δ is ubiquitously expressed throughout the body and is involved in numerous biological processes including cholesterol transport and wound healing. In addition to these specific functions, the three PPARs isotypes share common functional features such as anti-inflammatory action and their implication in normal cellular differentiation and carcinogenesis. PPARs thus represent critical sensors of environmental/dietary stimuli that are crucial in the regulation of mammalian metabolism.

To date, three PPAR isotypes encoded by distinct single copy genes have been identified: PPAR α (NR1C1), PPAR β or PPAR δ (NR1C2) and PPAR γ (NR1C3), which are located on

407 chromosomes 15, 17 and 6 in mouse and on chromosomes 22, 6 and 4 in human, respectively
408 [103-106]. The human and mouse PPAR γ genes generate three transcripts by alternate
409 splicing and promoter usage: PPAR γ 1, PPAR γ 2 and PPAR γ 3. However, up to seven
410 transcripts for PPAR γ were more recently described in human [107]. The translation of
411 human PPAR γ 1, PPAR γ 3 and PPAR γ 4 lead to an identical protein [108], whereas
412 PPAR γ 2 has 30 additional amino acids in the N-terminal region due to the translation of an
413 additional exon [109]. Interestingly, this additional exon is affected by a common
414 polymorphism (Pro12Ala) and might be associated with type 2 diabetes [110] and the
415 polycystic ovary syndrome [111]. The general structure of PPARs genes revealed a
416 conserved genomic organisation of six coding exons that probably derive from a common
417 ancestor [108, 109, 112, 113]. PPARs are proteins of 49 - 56 kDa that contain multiple
418 structural and functional domains, which are common to most nuclear receptors [114, 115].
419 PPARs consist of four main domains coded by six exons. The N-terminal A/B domain is the
420 least conserved among the nuclear receptors and contains a ligand-independent transactivation
421 domain called activation function 1 (AF-1). This domain was shown to interact with
422 cofactors and contain conserved Map-kinase phosphorylation serine sites. The highly
423 conserved C domain contains the DNA binding domain (DBD) that fold into two zinc fingers.
424 The DBD is responsible for the recognition of the core hexanucleotide motif AGGTCA. DNA
425 binding of PPAR to its response element PPRE (PPAR response element) requires obligatory
426 heterodimerization with another nuclear receptor, the retinoid X receptor (RXR). PPRE
427 consists of two copies of the core motif organized as a direct repeat spaced by one nucleotide
428 (DR-1) [116] and a 5' -extension A(A/T)CT. Thus, PPAR/RXR heterodimer recognizes
429 response elements (PPRE) exhibiting the following consensus sequence 5' -A(A/T)CT
430 (A/G)GGNCA A AG(G/T) TCA-3'. The D or hinge domain linking the DBD to the ligand
431 binding domain (LBD) is highly conserved and may be involved in nuclear localisation [117].

It interacts with regulatory proteins [118] and contains consensus PKC phosphorylation sites [119]. The C-terminal E/F domain or LBD contains a large hydrophobic pocket that can bind a broad range of lipophilic ligands and a ligand-dependent transactivation function (AF-2). This region also includes dimerization interfaces and is involved in interaction with RXR, its obligate heterodimerization partner [120]. The D and E/F regions of PPAR α and PPAR β / δ were also described to interact with heat shock protein 90 and to modulate their activity [121].

The mechanisms of action of PPARs

RXR natural ligand is 9-cis retinoic acid, whereas PPARs are receptors for various endogenous small lipophilic molecules such as medium and long-chain fatty acids and eicosanoids. Some long chain polyunsaturated fatty acids including linoleic acid, linolenic acid and arachidonic acid were shown to bind to the three PPAR isotypes in the mM range [122, 123]. Natural oxidized lipids were identified as more selective ligands. Eicosanoids such as 8(S)-hydroxy-eicosatetraenoic acid (HETE) and leukotriene B₄ [123, 124] were shown to be PPAR α ligands, whereas prostacyclin [125] and 15-deoxy- Δ 12,14 prostaglandin J₂ [123, 126] were identified as specific ligands of PPAR β / δ and PPAR γ , respectively. PPAR γ /RXR heterodimers can be activated by either selective RXR (9-cis retinoic acid) or PPAR γ ligands, the combination resulting in an additive or synergistic effect. PPAR γ is bound and activated by natural ligands such as eicosanoids, fatty acids and oxidized low-density lipoprotein compounds. 15deoxy-PGJ₂ is considered as a potent natural PPAR γ agonist [123, 126]. Oxidized metabolites of linoleic acid such as 9- and 13-hydroxy-octadecadienoic acid (HODE) are found in oxidized low-density lipoproteins and were identified as PPAR γ specific ligands [127]. However, the physiological relevance of these natural ligands remains unclear because of their relatively weak affinity (in the submicromolar to the micromolar range) and because their intracellular concentrations cannot

be accurately measured. Hexadecyl azelaoyl phosphatidylcholine, an abundant oxidatively fragmented alkyl phospholipid present in oxidized LDL, was identified as a high affinity ligand and agonist for PPAR γ with a K_d of about 40 nM [128]. In addition, synthetic agonists of PPAR γ have been developed. The thiazolidinedione class of drugs, including rosiglitazone or BRL49653, were the first synthetic drugs showing a high selectivity for one PPAR isotype (PPAR γ), with high affinity in the nanomolar range [129]. Thiazolidinediones are used in humans to treat non-insulin-dependent-diabetes mellitus because it was initially shown that they improve insulin sensitivity in both animals and in humans.

The ability of nuclear receptors to initiate or suppress the transcription process is mediated through their interaction with negatively or positively acting cofactors. Models for this nuclear receptor activation propose that in the absence of ligand they recruit co-repressors, while ligand binding results in conformational changes leading to the recruitment of co-activators. co-repressor are part of the multiprotein complex containing histone deacetylase (HDAC) activity. The deacetylated state of histones does not allow gene transcription. Ligand binding induces conformational change in the transactivation domain (AF-2) of the LBD resulting in local release of co-repressors and recruitment of co-activators via interaction with LXXLL-motifs present in the co-activators [130, 131].

Posttranslational regulation such as phosphorylation on serines located in the N-terminal A/B domain was shown to modulate transcription activity of PPARs but in the opposite way depending on the isotype. Thus, insulin-induced phosphorylation of Ser12 and/or Ser21 [132, 133] enhances transcriptional activity of PPAR α , whereas phosphorylation of Ser 112 by ERKs and p38 MAP kinases decreases PPAR γ 2 transcriptional activity [134-136].

PPAR γ , placental development and trophoblast differentiation (for review see [137, 138])

In 1999, genetic studies performed in mice established that RXRs and PPAR γ are essential for placental development and vasculature. Indeed, RXR $\alpha^{-/-}$ /RXR $\beta^{-/-}$ conceptuses fail to develop a normal chorioallantoic placenta with a functional labyrinthine zone, resulting in compromised maternal-fetal exchanges and therefore in early embryonic death at embryonic day E9.5 [139]. Likewise, PPAR $\gamma^{-/-}$ conceptuses exhibited similar placental agenesis with defects in trophoblast differentiation and vascular processes [140, 141]. Unexpectedly, these studies demonstrated that PPAR γ /RXR heterodimers are essential for the formation of a functional placenta in mice. More recently, fully viable PPAR γ -null mice were obtained through specific and total epiblastic gene deletion, demonstrating that the placental defect is the unique cause of PPAR $\gamma^{-/-}$ embryonic lethality [142].

In human placenta PPAR γ is located in the nuclei of early and term villous trophoblast and in extravillous trophoblast from first trimester placentas [143-145]. Interestingly, PPAR γ is abundantly and specifically expressed in the trophoblast [137, 146], whereas its obligate dimerization partner RXR α is ubiquitously present, including trophoblasts, the mesenchymal core of the villi and decidual cells [146, 147]. PPAR γ is present in the villous trophoblast including mononucleated VCT and ST, and in the EVCT along their differentiation pathway, including proliferative and intermediate EVCT in the proximal and distal columns of the anchoring villi, invasive EVCT located in the decidua, endovascular and perivascular EVCT, and giant cells (for review see [137]). Thus, these histological studies demonstrate that at the maternal fetal interface, PPAR γ /RXR α heterodimers are exclusively located in the trophoblasts.

Few studies have described the effect of PPAR γ activation on human trophoblast differentiation and invasion. In term villous cytotrophoblasts, activation of PPAR γ by the

synthetic agonist troglitazone (a drug belonging to the thiazolidinedione) induces the formation of syncytiotrophoblast *in vitro* [148]. Interestingly, differentiation of mononucleated villous cytotrophoblasts into syncytiotrophoblast *in vitro* is accompanied by activation of PPAR γ as demonstrated by accumulation of neutral lipids revealed by oil red O staining [149]. PPAR γ stimulation of villous trophoblast differentiation is associated with increase in hCG expression and secretion [149]. In EVCT primary cultures obtained from first trimester chorionic villi as well as in the EVCT cell line (HIPEC), activation of PPAR γ /RXR α heterodimers by either natural (15deoxy-prostaglandin J₂, oxidized lipids, components from oxidized LDL) or synthetic (rosiglitazone) PPAR γ ligands markedly decreased the cell invasion and migration process in a concentration-dependent manner [146, 150]. Activation of RXR α by 9 cis retinoic acid has no effect by itself but increased PPAR γ -induced inhibition of trophoblast invasiveness [146]. In contrast, incubation of EVCT with either PPAR γ or pan RXR antagonists promote cell invasion [146].

It has been recently shown that when infecting the trophoblasts, the cytomegalovirus (CMV) activates and uses trophoblastic PPAR γ for its own replication, thus upsetting some of the cellular mechanisms such as invasion and migration involved in the physiological process of anchoring the placenta to the uterine wall. Because infection of the fetus is always preceded by infection of the placenta, the authors hypothesized that infection of the placenta by CMV might explain miscarriage or low birth weight observed in infected pregnant women [151].

PPAR γ target genes in human trophoblasts and involvement in gestational diseases

It was shown that like in other PPAR γ target cells, PPAR γ and its heterodimeric nuclear receptor partner RXR α regulate fatty acid uptake by trophoblast [152]. Analysis of PPAR γ -target genes in the human trophoblast led to the discovery of known and new factors involved in the regulation of human trophoblast invasion [153, 154]. Among those, the human

532 placental growth hormone (hPGH), the pregnancy-associated plasma protein A (PAPP-A)
533 [155] and more recently the human chorionic gonadotropin hormone (hCG) [156, 157].
534
535 Human chorionic gonadotropin (hCG) is the major pregnancy glycoprotein hormone whose
536 maternal concentration and glycan structure change all along pregnancy. It is well established
537 that hCG is secreted by the endocrine trophoblast (the syncytiotrophoblast) into the maternal
538 compartment. HCG also plays an essential role in trophoblast differentiation and stimulates
539 *in vitro* the differentiation of cytotrophoblasts to syncytiotrophoblast by an autocrine and
540 paracrine mechanism [158, 159]. It was reported *in situ* and *in vitro* that invasive EVCT also
541 produce and secrete hCG suggesting an autocrine/paracrine role at the implantation site [157].
542 Surprisingly, secretion of hCG as well as expression of α and β transcripts was inversely
543 regulated by PPAR γ in endocrine villous and invasive extravillous cytotrophoblasts primary
544 cultures from first trimester placentas [156]. In primary villous trophoblast PPAR γ activation
545 increases hCG production at first [156] and third trimester [149, 160], whereas in primary
546 EVCT, hCG was decreased [156]. Furthermore, hCG from invasive EVCT stimulated the
547 trophoblastic cell invasion process as assayed using Matrigel-coated transwell inserts,
548 whereas hCG from the syncytiotrophoblast had no effect. These results offer evidence that
549 glycoforms of hCG secreted *in vitro* by the invasive EVCT, but not by the
550 syncytiotrophoblast, promotes trophoblast invasion and may participate as a PPAR γ -target
551 gene to the control of the trophoblastic cell invasion process in an autocrine manner [161].
552 Recently, we have demonstrated that invasive EVCT and endovascular EVCT in particular
553 produced a hyperglycosylated form of hCG (hCG-H, the one produced by the
554 choriocarcinoma JEG-3) that was able to promote cell invasion through a LH/CG receptor
555 independent pathway. Moreover, this hyperglycosylated hCG was found in maternal serum
556 from early pregnancy and might therefore provide a good serum marker of physiological early

placentation process such as human trophoblast invasion [162].

Despite the key role of PPARs in trophoblast differentiation and function, few studies have been conducted in pregnancy diseases and results are difficult to interpret probably because gestational diseases are highly heterogeneous (for review see [163-165]). PPAR γ immunostaining is reduced in trophoblastic diseases such as hydatidiform moles and choriocarcinoma [166], whereas Toth et al. (2008) [167] observed increased expression of PPAR β/δ and PPAR γ in hydatidiform mole. An increased PPAR γ labeling was reported in EVCT from miscarriage patients [168]. No modification of PPAR β/δ , PPAR γ and RXR α expression studied by immunohistochemistry is observed in placentas from pregnancies associated with FGR and preeclampsia (PE) [169]. Similarly, it was recently reported that PPAR γ and RXR α mRNA and protein levels remain unchanged in PE [170]. In contrast, these authors found increases in PPAR γ and RXR α RNA and protein expression when PE was associated with FGR. Moreover, higher PPAR γ DNA binding activity was observed in placental tissues from FGR with or without PE. Gestational diabetes mellitus, was in contrast associated with lower placental PPAR γ expression [170, 171]. Recently, a novel missense heterozygous mutation of PPAR γ was identified during pregnancy in a young diabetic woman with a familial partial lipodystrophy type 3 (FPLD3). She prematurely delivered a baby who died 24 hours later. Histopathological analysis revealed important placental vascular abnormalities. The incidence of the PPAR γ mutation in placental development remained to be determined [172].

All together, these data underscore a major role for PPAR γ and its target genes such as hCG, in the control of human cytotrophoblast differentiation and the pathophysiology of certain gestational diseases.

Conclusions and future directions

Our current understanding of how homeobox genes regulate trophoblast functions reveals that important aspects of transcriptional regulation are conserved between the extraembryonic placenta and embryonic morphogenetic and differentiation events. The strategy we have employed has resulted in the identification of homeobox genes, which are expressed in normal placental development and that show altered expression in FGR. Functional assays following target gene inactivation in cultured cells reveal that homeobox genes control important trophoblast properties. The discovery of targets of homeobox genes has revealed down-stream transcription factors, and biological pathways, not previously implicated in FGR. In future, target genes, and genes within novel biological pathways, will be further assessed for their potential use in the early detection and diagnosis of FGR.

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Figure legends

Figure 1: Role of DLX3, PPAR γ and its target gene hCG in the differentiation of human cytotrophoblasts along the endocrine villous or the invasive extravillous pathways.

This scheme describes how DLX3 and PPAR γ may control trophoblast invasion and syncytiotrophoblast formation through the control of hCG gene expression. hCG stimulates ST formation, whereas hCG-H, mainly produced by the invasive EVCT, promotes trophoblastic cell invasion.

Figure 2: Role of homeobox genes in human placental development.

This flowchart depicts how the regulation of homeobox gene expression by growth factors and cytokines controls basic cellular functions such as proliferation, migration, invasion, differentiation and apoptosis of placental cells. In fetal growth restriction-affected pregnancies, differential expression of homeobox genes could cause abnormal trophoblast function that is characteristic of FGR with placental insufficiency.

References

- [1] Loke Y, King A. Human Implantation: Cell Biology and Immunology. Cambridge: Cambridge University Press; 1993.
- [2] Benirschke K, Kaufmann P. Pathology of the Human Placenta. New York: Springer-Verlag; 2000.
- [3] Morrish DW, Dakour J, Li H. Functional regulation of human trophoblast differentiation. *J Reprod Immunol.* 1998;39:179-95.
- [4] Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J Pediatr Endocrinol Metab.* 2000;13:343-56.
- [5] Lacroix MC, Guibourdenche J, Frenzo JL, Pidoux G, Evain-Brion D. Placental growth hormones. *Endocrine.* 2002;19:73-9.
- [6] Pijnenborg R, Vercruysse L, Brosens I. Deep placentation. *Best Pract Res Clin Obstet Gynaecol.* 2011;25:273-85.
- [7] Knofler M, Pollheimer J. IFPA Award in Placentology lecture: molecular regulation of human trophoblast invasion. *Placenta.* 2012;33 Suppl:S55-62.
- [8] Whitley GS, Cartwright JE. Cellular and molecular regulation of spiral artery remodelling: lessons from the cardiovascular field. *Placenta.* 2010;31:465-74.
- [9] Cross JC, Anson-Cartwright L, Scott IC. Transcription factors underlying the development and endocrine functions of the placenta. *Recent Prog Horm Res.* 2002;57:221-34.
- [10] Cross JC, Baczyk D, Dobric N, Hemberger M, Hughes M, Simmons DG, et al. Genes, development and evolution of the placenta. *Placenta.* 2003;24:123-30.
- [11] Hemberger M, Cross JC. Genes governing placental development. *Trends Endocrinol Metab.* 2001;12:162-8.

644 [12] Johnson PF, McKnight SL. Eukaryotic transcriptional regulatory proteins. *Annu Rev*
645 *Biochem.* 1989;58:799-839.

646 [13] Woodside KJ, Shen H, Muntzel C, Daller JA, Sommers CL, Love PE. Expression of
647 *Dlx* and *Lhx* family homeobox genes in fetal thymus and thymocytes. *Gene Expr Patterns.*
648 2004;4:315-20.

649 [14] McGinnis W, Krumlauf R. Homeobox genes and axial patterning. *Cell.* 1992;68:283-
650 302.

651 [15] Favier B, Dolle P. Developmental functions of mammalian *Hox* genes. *Mol Hum*
652 *Reprod.* 1997;3:115-31.

653 [16] Prince V. The *Hox* Paradox: More complex(es) than imagined. *Dev Biol.* 2002;249:1-
654 15.

655 [17] Scott MP, Carroll SB. The segmentation and homeotic gene network in early
656 *Drosophila* development. *Cell.* 1987;51:689-98.

657 [18] Levine M, Hoey T. Homeobox proteins as sequence-specific transcription factors.
658 *Cell.* 1988;55:537-40.

659 [19] Bachiller D, Macias A, Duboule D, Morata G. Conservation of a functional hierarchy
660 between mammalian and insect *Hox/HOM* genes. *EMBO J.* 1994;13:1930-41.

661 [20] Gehring WJ, Affolter M, Burglin T. Homeodomain proteins. *Annu Rev Biochem.*
662 1994;63:487-526.

663 [21] Cillo C, Cantile M, Faiella A, Boncinelli E. Homeobox genes in normal and malignant
664 cells. *J Cell Physiol.* 2001;188:161-9.

665 [22] Cillo C, Faiella A, Cantile M, Boncinelli E. Homeobox genes and cancer. *Exp Cell*
666 *Res.* 1999;248:1-9.

667 [23] Lemons D, McGinnis W. Genomic evolution of *Hox* gene clusters. *Science.*
668 2006;313:1918-22.

669 [24] Jagla K, Bellard M, Frasch M. A cluster of *Drosophila* homeobox genes involved in
670 mesoderm differentiation programs. *Bioessays*. 2001;23:125-33.

671 [25] Nunes FD, de Almeida FC, Tucci R, de Sousa SC. Homeobox genes: a molecular link
672 between development and cancer. *Pesqui Odontol Bras*. 2003;17:94-8.

673 [26] Choi BJ, Kim CJ, Cho YG, Song JH, Kim SY, Nam SW, et al. Altered expression of
674 CDX2 in colorectal cancers. *APMIS*. 2006;114:50-4.

675 [27] Wu Z, Guo H, Chow N, Sallstrom J, Bell RD, Deane R, et al. Role of the MEOX2
676 homeobox gene in neurovascular dysfunction in Alzheimer disease. *Nat Med*. 2005;11:959-
677 65.

678 [28] Myers C, Charboneau A, Boudreau N. Homeobox B3 promotes capillary
679 morphogenesis and angiogenesis. *J Cell Biol*. 2000;148:343-51.

680 [29] Gorski DH, Walsh K. Control of vascular cell differentiation by homeobox
681 transcription factors. *Trends Cardiovasc Med*. 2003;13:213-20.

682 [30] Gorski DH, Leal AJ. Inhibition of endothelial cell activation by the homeobox gene
683 Gax. *J Surg Res*. 2003;111:91-9.

684 [31] Myers C, Charboneau A, Cheung I, Hanks D, Boudreau N. Sustained expression of
685 homeobox D10 inhibits angiogenesis. *Am J Pathol*. 2002;161:2099-109.

686 [32] Hentsch B, Lyons I, Li R, Hartley L, Lints TJ, Adams JM, et al. Hlx homeo box gene
687 is essential for an inductive tissue interaction that drives expansion of embryonic liver and
688 gut. *Genes Dev*. 1996;10:70-9.

689 [33] Li Y, Behringer RR. Esx1 is an X-chromosome-imprinted regulator of placental
690 development and fetal growth. *Nat Genet*. 1998;20:309-11.

691 [34] Rossant J, Cross JC. Placental development: lessons from mouse mutants. *Nat Rev*
692 *Genet*. 2001;2:538-48.

693 [35] Peng L, Payne AH. AP-2 gamma and the homeodomain protein distal-less 3 are
694 required for placental-specific expression of the murine 3 beta-hydroxysteroid dehydrogenase
695 VI gene, Hsd3b6. *J Biol Chem.* 2002;277:7945-54.

696 [36] Beck F, Erler T, Russell A, James R. Expression of Cdx-2 in the mouse embryo and
697 placenta: possible role in patterning of the extra-embryonic membranes. *Dev Dyn.*
698 1995;204:219-27.

699 [37] Chawengsaksophak K, James R, Hammond VE, Kontgen F, Beck F. Homeosis and
700 intestinal tumours in Cdx2 mutant mice. *Nature.* 1997;386:84-7.

701 [38] Han YJ, Park AR, Sung DY, Chun JY. Psx, a novel murine homeobox gene expressed
702 in placenta. *Gene.* 1998;207:159-66.

703 [39] Chun JY, Han YJ, Ahn KY. Psx homeobox gene is X-linked and specifically
704 expressed in trophoblast cells of mouse placenta. *Dev Dyn.* 1999;216:257-66.

705 [40] Lints TJ, Hartley L, Parsons LM, Harvey RP. Mesoderm-specific expression of the
706 divergent homeobox gene Hlx during murine embryogenesis. *Dev Dyn.* 1996;205:457-70.

707 [41] Boudreau NJ, Varner JA. The homeobox transcription factor Hox D3 promotes
708 integrin alpha5beta1 expression and function during angiogenesis. *J Biol Chem.*
709 2004;279:4862-8.

710 [42] Boudreau N, Andrews C, Srebrow A, Ravanpay A, Cheresch DA. Induction of the
711 angiogenic phenotype by Hox D3. *J Cell Biol.* 1997;139:257-64.

712 [43] Knofler M, Saleh L, Bauer S, Vasicek R, Griesinger G, Strohmer H, et al. Promoter
713 elements and transcription factors involved in differentiation-dependent human chorionic
714 gonadotrophin-alpha messenger ribonucleic acid expression of term villous trophoblasts.
715 *Endocrinology.* 2000;141:3737-48.

716 [44] Loregger T, Pollheimer J, Knofler M. Regulatory transcription factors controlling
717 function and differentiation of human trophoblast--a review. *Placenta*. 2003;24 Suppl
718 A:S104-10.

719 [45] Asanoma K, Kato H, Inoue T, Matsuda T, Wake N. Analysis of a candidate gene
720 associated with growth suppression of choriocarcinoma and differentiation of trophoblasts. *J*
721 *Reprod Med*. 2004;49:617-26.

722 [46] Berghorn KA, Clark PA, Encarnacion B, Deregis CJ, Folger JK, Morasso MI, et al.
723 Developmental expression of the homeobox protein Distal-less 3 and its relationship to
724 progesterone production in mouse placenta. *J Endocrinol*. 2005;186:315-23.

725 [47] Quinn LM, Latham SE, Kalionis B. Homeobox gene HB24, a regulator of
726 haematopoiesis, is a candidate for regulating differentiation of the extra-embryonic
727 trophoblast cell lineage. *Reprod Fertil Dev*. 1997;9:617-23.

728 [48] Quinn LM, Latham SE, Kalionis B. The homeobox genes MSX2 and MOX2 are
729 candidates for regulating epithelial-mesenchymal cell interactions in the human placenta.
730 *Placenta*. 2000;21 Suppl A:S50-4.

731 [49] Quinn LM, Johnson BV, Nicholl J, Sutherland GR, Kalionis B. Isolation and
732 identification of homeobox genes from the human placenta including a novel member of the
733 Distal-less family, DLX4. *Gene*. 1997;187:55-61.

734 [50] Kraus P, Lufkin T. Mammalian Dlx homeobox gene control of craniofacial and inner
735 ear morphogenesis. *J Cell Biochem*. 1999;Suppl 32-33:133-40.

736 [51] Maas R, Bei M. The genetic control of early tooth development. *Crit Rev Oral Biol*
737 *Med*. 1997;8:4-39.

738 [52] Rajaraman G, Murthi P, Quinn L, Brennecke SP, Kalionis B. Homeodomain protein
739 HLX is expressed primarily in cytotrophoblast cell types in the early pregnancy human
740 placenta. *Reprod Fertil Dev*. 2008;20:357-67.

741 [53] Chui A, Pathirage NA, Johnson B, Cocquebert M, Fournier T, Evain-Brion D, et al.
742 Homeobox gene distal-less 3 is expressed in proliferating and differentiating cells of the
743 human placenta. *Placenta*. 2010;31:691-7.

744 [54] Sun Y, Lu X, Yin L, Zhao F, Feng Y. Inhibition of DLX4 promotes apoptosis in
745 choriocarcinoma cell lines. *Placenta*. 2006;27:375-83.

746 [55] Sun YY, Lu M, Xi XW, Qiao QQ, Chen LL, Xu XM, et al. Regulation of epithelial-
747 mesenchymal transition by homeobox gene DLX4 in JEG-3 trophoblast cells: a role in
748 preeclampsia. *Reprod Sci*. 2011;18:1138-45.

749 [56] Roberson MS, Meermann S, Morasso MI, Mulvaney-Musa JM, Zhang T. A role for
750 the homeobox protein Distal-less 3 in the activation of the glycoprotein hormone alpha
751 subunit gene in choriocarcinoma cells. *J Biol Chem*. 2001;276:10016-24.

752 [57] Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental
753 villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol*.
754 2000;92:35-43.

755 [58] Chen CP, Bajoria R, Aplin JD. Decreased vascularization and cell proliferation in
756 placentas of intrauterine growth-restricted fetuses with abnormal umbilical artery flow
757 velocity waveforms. *Am J Obstet Gynecol*. 2002;187:764-9.

758 [59] Guihard-Costa AM, Larroche JC. Growth velocity of some fetal parameters. II. Body
759 weight, body length and head circumference. *Biol Neonate*. 1992;62:317-24.

760 [60] Morris JM, Trudinger BJ. Sonographic evaluation of intrauterine growth retardation.
761 *Curr Opin Radiol*. 1992;4:102-10.

762 [61] Guaran RL, Wein P, Sheedy M, Walstab J, Beischer NA. Update of growth percentiles
763 for infants born in an Australian population. *Aust N Z J Obstet Gynaecol*. 1994;34:39-50.

764 [62] Murthi P, Doherty V, Said J, Donath S, Brennecke SP, Kalionis B. Homeobox gene
765 HLX1 expression is decreased in idiopathic human fetal growth restriction. *Am J Pathol.*
766 2006;168:511-8.

767 [63] Murthi P, Doherty VL, Said JM, Donath S, Brennecke SP, Kalionis B. Homeobox
768 gene ESX1L expression is decreased in human pre-term idiopathic fetal growth restriction.
769 *Mol Hum Reprod.* 2006;12:335-40.

770 [64] Murthi P, Said JM, Doherty VL, Donath S, Nowell CJ, Brennecke SP, et al.
771 Homeobox gene DLX4 expression is increased in idiopathic human fetal growth restriction.
772 *Mol Hum Reprod.* 2006;12:763-9.

773 [65] Chui A, Tay C, Cocquebert M, Sheehan P, Pathirage NA, Donath S, et al. Homeobox
774 gene Distal-less 3 is a regulator of villous cytotrophoblast differentiation and its expression is
775 increased in human idiopathic foetal growth restriction. *J Mol Med (Berl).* 2012;90:273-84.

776 [66] Rajaraman G, Murthi P, Leo B, Brennecke SP, Kalionis B. Homeobox gene HLX1 is a
777 regulator of colony stimulating factor-1 dependent trophoblast cell proliferation. *Placenta.*
778 2007;28:991-8.

779 [67] Rajaraman G, Murthi P, Pathirage N, Brennecke SP, Kalionis B. Downstream targets
780 of homeobox gene HLX show altered expression in human idiopathic fetal growth restriction.
781 *Am J Pathol.* 2010;176:278-87.

782 [68] Rajaraman G, Murthi P, Brennecke SP, Kalionis B. Homeobox gene HLX is a
783 regulator of HGF/c-met-mediated migration of human trophoblast-derived cell lines. *Biol*
784 *Reprod.* 2010;83:676-83.

785 [69] Chui A, Evseenko DA, Brennecke SP, Keelan JA, Kalionis B, Murthi P. Homeobox
786 gene Distal-less 3 (DLX3) is a regulator of villous cytotrophoblast differentiation. *Placenta.*
787 2011;32:745-51.

788 [70] Valarche I, Tissier-Seta JP, Hirsch MR, Martinez S, Goridis C, Brunet JF. The mouse
789 homeodomain protein Phox2 regulates Ncam promoter activity in concert with Cux/CDP and
790 is a putative determinant of neurotransmitter phenotype. *Development*. 1993;119:881-96.

791 [71] Edelman GM, Jones FS. Developmental control of N-CAM expression by Hox and
792 Pax gene products. *Philos Trans R Soc Lond B Biol Sci*. 1995;349:305-12.

793 [72] Edelman GM, Jones FS. Gene regulation of cell adhesion: a key step in neural
794 morphogenesis. *Brain Res Brain Res Rev*. 1998;26:337-52.

795 [73] Jones FS, Kioussi C, Copertino DW, Kallunki P, Holst BD, Edelman GM. Barx2, a
796 new homeobox gene of the Bar class, is expressed in neural and craniofacial structures during
797 development. *Proc Natl Acad Sci U S A*. 1997;94:2632-7.

798 [74] McWhirter JR, Goulding M, Weiner JA, Chun J, Murre C. A novel fibroblast growth
799 factor gene expressed in the developing nervous system is a downstream target of the
800 chimeric homeodomain oncoprotein E2A-Pbx1. *Development*. 1997;124:3221-32.

801 [75] Meier BC, Price JR, Parker GE, Bridwell JL, Rhodes SJ. Characterization of the
802 porcine Lhx3/LIM-3/P-Lim LIM homeodomain transcription factor. *Mol Cell Endocrinol*.
803 1999;147:65-74.

804 [76] van Oostveen J, Bijl J, Raaphorst F, Walboomers J, Meijer C. The role of homeobox
805 genes in normal hematopoiesis and hematological malignancies. *Leukemia*. 1999;13:1675-90.

806 [77] Boncinelli E, Morgan R. Downstream of Otx2, or how to get a head. *Trends Genet*.
807 2001;17:633-6.

808 [78] Dailey L, Basilico C. Coevolution of HMG domains and homeodomains and the
809 generation of transcriptional regulation by Sox/POU complexes. *J Cell Physiol*.
810 2001;186:315-28.

811 [79] Akin ZN, Nazarali AJ. Hox genes and their candidate downstream targets in the
812 developing central nervous system. *Cell Mol Neurobiol*. 2005;25:697-741.

813 [80] Kehrl JH, Deguchi Y. Potential roles for two human homeodomain containing proteins
814 in the proliferation and differentiation of human hematopoietic progenitors. *Leuk Lymphoma*.
815 1993;10:173-6.

816 [81] Morrish DW, Dakour J, Li H. Life and death in the placenta: new peptides and genes
817 regulating human syncytiotrophoblast and extravillous cytotrophoblast lineage formation and
818 renewal. *Curr Protein Pept Sci*. 2001;2:245-59.

819 [82] Deshpande A, Hinds PW. The retinoblastoma protein in osteoblast differentiation and
820 osteosarcoma. *Curr Mol Med*. 2006;6:809-17.

821 [83] Quenby S, Brazeau C, Drakeley A, Lewis-Jones DI, Vince G. Oncogene and tumour
822 suppressor gene products during trophoblast differentiation in the first trimester. *Mol Hum*
823 *Reprod*. 1998;4:477-81.

824 [84] Roncalli M, Bulfamante G, Viale G, Springall DR, Alfano R, Comi A, et al. C-myc
825 and tumour suppressor gene product expression in developing and term human trophoblast.
826 *Placenta*. 1994;15:399-409.

827 [85] Matsuoka S, Edwards MC, Bai C, Parker S, Zhang P, Baldini A, et al. p57KIP2, a
828 structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor
829 suppressor gene. *Genes Dev*. 1995;9:650-62.

830 [86] Chilosi M, Piazzola E, Lestani M, Benedetti A, Guasparri I, Granchelli G, et al.
831 Differential expression of p57kip2, a maternally imprinted cdk inhibitor, in normal human
832 placenta and gestational trophoblastic disease. *Lab Invest*. 1998;78:269-76.

833 [87] Paumelle R, Tulasne D, Kherrouche Z, Plaza S, Leroy C, Reveneau S, et al.
834 Hepatocyte growth factor/scatter factor activates the ETS1 transcription factor by a RAS-
835 RAF-MEK-ERK signaling pathway. *Oncogene*. 2002;21:2309-19.

836 [88] Kauma S, Hayes N, Weatherford S. The differential expression of hepatocyte growth
837 factor and met in human placenta. *J Clin Endocrinol Metab*. 1997;82:949-54.

838 [89] Takai N, Ueda T, Narahara H, Miyakawa I. Expression of c-Ets1 protein in normal
839 human placenta. *Gynecol Obstet Invest.* 2006;61:15-20.

840 [90] Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility
841 and more. *Nat Rev Mol Cell Biol.* 2003;4:915-25.

842 [91] Genbacev O, McMaster MT, Fisher SJ. A repertoire of cell cycle regulators whose
843 expression is coordinated with human cytotrophoblast differentiation. *Am J Pathol.*
844 2000;157:1337-51.

845 [92] Korgun ET, Celik-Ozenci C, Acar N, Cayli S, Desoye G, Demir R. Location of cell
846 cycle regulators cyclin B1, cyclin A, PCNA, Ki67 and cell cycle inhibitors p21, p27 and p57
847 in human first trimester placenta and deciduas. *Histochem Cell Biol.* 2006;125:615-24.

848 [93] Bamberger AM, Bamberger CM, Aupers S, Milde-Langosch K, Loning T,
849 Makrigiannakis A. Expression pattern of the activating protein-1 family of transcription
850 factors in the human placenta. *Mol Hum Reprod.* 2004;10:223-8.

851 [94] Kudo Y, Yamada K, Fujiwara A, Kawasaki T. Evidence for modulation by ATP of
852 amino acid transport in human placenta. *Biochem Int.* 1988;16:485-93.

853 [95] Knofler M, Saleh L, Bauer S, Galos B, Rotheneder H, Husslein P, et al.
854 Transcriptional regulation of the human chorionic gonadotropin beta gene during villous
855 trophoblast differentiation. *Endocrinology.* 2004;145:1685-94.

856 [96] Potgens AJ, Drewlo S, Kokozidou M, Kaufmann P. Syncytin: the major regulator of
857 trophoblast fusion? Recent developments and hypotheses on its action. *Hum Reprod Update.*
858 2004;10:487-96.

859 [97] Holdsworth-Carson SJ, Permezel M, Riley C, Rice GE, Lappas M. Peroxisome
860 proliferator-activated receptors and retinoid X receptor-alpha in term human gestational
861 tissues: tissue specific and labour-associated changes. *Placenta.* 2009;30:176-86.

862 [98] Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control
863 of metabolism. *Endocr Rev.* 1999;20:649-88.

864 [99] Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D. Evolution of the nuclear receptor
865 gene superfamily. *EMBO J.* 1992;11:1003-13.

866 [100] Chinetti-Gbaguidi G, Fruchart JC, Staels B. Pleiotropic effects of fibrates. *Curr*
867 *Atheroscler Rep.* 2005;7:396-401.

868 [101] Issemann I, Green S. Activation of a member of the steroid hormone receptor
869 superfamily by peroxisome proliferators. *Nature.* 1990;347:645-50.

870 [102] Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the
871 peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell.*
872 1992;68:879-87.

873 [103] Sher T, Yi HF, McBride OW, Gonzalez FJ. cDNA cloning, chromosomal mapping,
874 and functional characterization of the human peroxisome proliferator activated receptor.
875 *Biochemistry.* 1993;32:5598-604.

876 [104] Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, et al. Isolation
877 of the human peroxisome proliferator activated receptor gamma cDNA: expression in
878 hematopoietic cells and chromosomal mapping. *Gene Expr.* 1995;4:281-99.

879 [105] Jones PS, Savory R, Barratt P, Bell AR, Gray TJ, Jenkins NA, et al. Chromosomal
880 localisation, inducibility, tissue-specific expression and strain differences in three murine
881 peroxisome-proliferator-activated-receptor genes. *Eur J Biochem.* 1995;233:219-26.

882 [106] Yoshikawa T, Brkanac Z, Dupont BR, Xing GQ, Leach RJ, Detera-Wadleigh SD.
883 Assignment of the human nuclear hormone receptor, NUC1 (PPARD), to chromosome
884 6p21.1-p21.2. *Genomics.* 1996;35:637-8.

885 [107] Chen Y, Jimenez AR, Medh JD. Identification and regulation of novel PPAR-gamma
886 splice variants in human THP-1 macrophages. *Biochim Biophys Acta.* 2006;1759:32-43.

887 [108] Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, et al. The
888 organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol*
889 *Chem.* 1997;272:18779-89.

890 [109] Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, et al. Structural organization
891 of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene:
892 alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc Natl*
893 *Acad Sci U S A.* 1995;92:7921-5.

894 [110] Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JP. The association
895 between the peroxisome proliferator-activated receptor-gamma2 (PPARG2) Pro12Ala gene
896 variant and type 2 diabetes mellitus: a HuGE review and meta-analysis. *Am J Epidemiol.*
897 2010;171:645-55.

898 [111] Zhang H, VBi Y, Hu C, Lu W, Zhu D. Association between the Pro12Ala
899 polymorphism of PPAR- γ gene and the polycystic ovary syndrome: A meta-analysis of case-
900 control studies. *Gene.* 2012;503:12-7.

901 [112] Krey G, Keller H, Mahfoudi A, Medin J, Ozato K, Dreyer C, et al. Xenopus
902 peroxisome proliferator activated receptors: genomic organization, response element
903 recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *J*
904 *Steroid Biochem Mol Biol.* 1993;47:65-73.

905 [113] Gearing KL, Crickmore A, Gustafsson JA. Structure of the mouse peroxisome
906 proliferator activated receptor alpha gene. *Biochem Biophys Res Commun.* 1994;199:255-63.

907 [114] Green S, Chambon P. Nuclear receptors enhance our understanding of transcription
908 regulation. *Trends Genet.* 1988;4:309-14.

909 [115] Evans RM. The steroid and thyroid hormone receptor superfamily. *Science.*
910 1988;240:889-95.

911 [116] Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM. Convergence of 9-cis
 912 retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation
 913 of their receptors. *Nature*. 1992;358:771-4.

914 [117] Gervois P, Torra IP, Chinetti G, Grotzinger T, Dubois G, Fruchart JC, et al. A
 915 truncated human peroxisome proliferator-activated receptor alpha splice variant with
 916 dominant negative activity. *Mol Endocrinol*. 1999;13:1535-49.

917 [118] Gray JP, Davis JW, 2nd, Gopinathan L, Leas TL, Nugent CA, Vanden Heuvel JP. The
 918 ribosomal protein rpL11 associates with and inhibits the transcriptional activity of peroxisome
 919 proliferator-activated receptor-alpha. *Toxicol Sci*. 2006;89:535-46.

920 [119] Gray JP, Burns KA, Leas TL, Perdew GH, Vanden Heuvel JP. Regulation of
 921 peroxisome proliferator-activated receptor alpha by protein kinase C. *Biochemistry*.
 922 2005;44:10313-21.

923 [120] Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell*.
 924 1995;83:841-50.

925 [121] Sumanasekera WK, Tien ES, Davis JW, 2nd, Turpey R, Perdew GH, Vanden Heuvel
 926 JP. Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated
 927 receptor-alpha (PPARalpha) and PPARbeta activity. *Biochemistry*. 2003;42:10726-35.

928 [122] Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, et al. Fatty
 929 acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-
 930 activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol*.
 931 1997;11:779-91.

932 [123] Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, et al. Fatty
 933 acids and eicosanoids regulate gene expression through direct interactions with peroxisome
 934 proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A*. 1997;94:4318-
 935 23.

936 [124] Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The
 937 PPAR α -leukotriene B₄ pathway to inflammation control. *Nature*. 1996;384:39-43.

938 [125] Gupta RA, Tan J, Krause WF, Geraci MW, Willson TM, Dey SK, et al. Prostacyclin-
 939 mediated activation of peroxisome proliferator-activated receptor δ in colorectal cancer.
 940 *Proc Natl Acad Sci U S A*. 2000;97:13275-80.

941 [126] Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-
 942 δ 12, 14-prostaglandin J₂ is a ligand for the adipocyte determination factor PPAR γ .
 943 *Cell*. 1995;83:803-12.

944 [127] Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates
 945 macrophage gene expression through ligand activation of PPAR γ . *Cell*. 1998;93:229-
 946 40.

947 [128] Davies SS, Pontsler AV, Marathe GK, Harrison KA, Murphy RC, Hinshaw JC, et al.
 948 Oxidized alkyl phospholipids are specific, high affinity peroxisome proliferator-activated
 949 receptor γ ligands and agonists. *J Biol Chem*. 2001;276:16015-23.

950 [129] Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA.
 951 An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-
 952 activated receptor γ (PPAR γ). *J Biol Chem*. 1995;270:12953-6.

953 [130] Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, et al. Ligand
 954 binding and co-activator assembly of the peroxisome proliferator-activated receptor- γ .
 955 *Nature*. 1998;395:137-43.

956 [131] Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S. Ligand type-
 957 specific interactions of peroxisome proliferator-activated receptor γ with transcriptional
 958 coactivators. *J Biol Chem*. 2000;275:33201-4.

959 [132] Shalev A, Siegrist-Kaiser CA, Yen PM, Wahli W, Burger AG, Chin WW, et al. The
 960 peroxisome proliferator-activated receptor alpha is a phosphoprotein: regulation by insulin.
 961 *Endocrinology*. 1996;137:4499-502.

962 [133] Juge-Aubry CE, Hammar E, Siegrist-Kaiser C, Pernin A, Takeshita A, Chin WW, et
 963 al. Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor
 964 alpha by phosphorylation of a ligand-independent trans-activating domain. *J Biol Chem*.
 965 1999;274:10505-10.

966 [134] Hu E, Kim JB, Sarraf P, Spiegelman BM. Inhibition of adipogenesis through MAP
 967 kinase-mediated phosphorylation of PPARgamma. *Science*. 1996;274:2100-3.

968 [135] Camp HS, Tafuri SR. Regulation of peroxisome proliferator-activated receptor gamma
 969 activity by mitogen-activated protein kinase. *J Biol Chem*. 1997;272:10811-6.

970 [136] Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK. Transcriptional
 971 activation by peroxisome proliferator-activated receptor gamma is inhibited by
 972 phosphorylation at a consensus mitogen-activated protein kinase site. *J Biol Chem*.
 973 1997;272:5128-32.

974 [137] Fournier T, Tsatsaris V, Handschuh K, Evain-Brion D. PPARs and the placenta.
 975 *Placenta*. 2007;28:65-76.

976 [138] Schaiff WT, Barak Y, Sadovsky Y. The pleiotropic function of PPAR gamma in the
 977 placenta. *Mol Cell Endocrinol*. 2006;249:10-5.

978 [139] Wendling O, Chambon P, Mark M. Retinoid X receptors are essential for early mouse
 979 development and placentogenesis. *Proc Natl Acad Sci U S A*. 1999;96:547-51.

980 [140] Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, et al. PPAR
 981 gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell*.
 982 1999;4:585-95.

983 [141] Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, et al. PPAR
984 gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Mol*
985 *Cell*. 1999;4:597-609.

986 [142] Nadra K, Quignodon L, Sardella C, Joye E, Mucciolo A, Chrast R, et al. PPARgamma
987 in placental angiogenesis. *Endocrinology*. 2010;151:4969-81.

988 [143] Tarrade A, Lai Kuen R, Malassine A, Tricottet V, Blain P, Vidaud M, et al.
989 Characterization of human villous and extravillous trophoblasts isolated from first trimester
990 placenta. *Lab Invest*. 2001;81:1199-211.

991 [144] Waite LL, Person EC, Zhou Y, Lim KH, Scanlan TS, Taylor RN. Placental
992 peroxisome proliferator-activated receptor-gamma is up-regulated by pregnancy serum. *J Clin*
993 *Endocrinol Metab*. 2000;85:3808-14.

994 [145] Wang Q, Fujii H, Knipp GT. Expression of PPAR and RXR isoforms in the
995 developing rat and human term placentas. *Placenta*. 2002;23:661-71.

996 [146] Tarrade A, Schoonjans K, Pavan L, Auwerx J, Rochette-Egly C, Evain-Brion D, et al.
997 PPARgamma/RXRalpha heterodimers control human trophoblast invasion. *J Clin Endocrinol*
998 *Metab*. 2001;86:5017-24.

999 [147] Tarrade A, Rochette-Egly C, Guibourdenche J, Evain-Brion D. The expression of
1000 nuclear retinoid receptors in human implantation. *Placenta*. 2000;21:703-10.

1001 [148] Schaiff WT, Carlson MG, Smith SD, Levy R, Nelson DM, Sadovsky Y. Peroxisome
1002 proliferator-activated receptor-gamma modulates differentiation of human trophoblast in a
1003 ligand-specific manner. *J Clin Endocrinol Metab*. 2000;85:3874-81.

1004 [149] Tarrade A, Schoonjans K, Guibourdenche J, Bidart JM, Vidaud M, Auwerx J, et al.
1005 PPAR gamma/RXR alpha heterodimers are involved in human CG beta synthesis and human
1006 trophoblast differentiation. *Endocrinology*. 2001;142:4504-14.

1007 [150] Pavan L, Tarrade A, Hermouet A, Delouis C, Titeux M, Vidaud M, et al. Human
 1008 invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of
 1009 PPARgamma in cell invasion process. *Carcinogenesis*. 2003;24:1325-36.

1010 [151] Rauwel B, Mariame B, Martin H, Nielsen R, Allart S, Pipy B, et al. Activation of
 1011 peroxisome proliferator-activated receptor gamma by human cytomegalovirus for de novo
 1012 replication impairs migration and invasiveness of cytotrophoblasts from early placentas. *J*
 1013 *Virol*. 2010;84:2946-54.

1014 [152] Schaiff WT, Bildirici I, Cheong M, Chern PL, Nelson DM, Sadovsky Y. Peroxisome
 1015 proliferator-activated receptor-gamma and retinoid X receptor signaling regulate fatty acid
 1016 uptake by primary human placental trophoblasts. *J Clin Endocrinol Metab*. 2005;90:4267-75.

1017 [153] Fournier T, Handschuh K, Tsatsaris V, Evain-Brion D. Involvement of PPARgamma
 1018 in human trophoblast invasion. *Placenta*. 2007;28 Suppl A:S76-81.

1019 [154] Fournier T, Handschuh K, Tsatsaris V, Guibourdenche J, Evain-Brion D. Role of
 1020 nuclear receptors and their ligands in human trophoblast invasion. *J Reprod Immunol*.
 1021 2008;77:161-70.

1022 [155] Handschuh K, Guibourdenche J, Guesnon M, Laurendeau I, Evain-Brion D, Fournier
 1023 T. Modulation of PAPP-A expression by PPARgamma in human first trimester trophoblast.
 1024 *Placenta*. 2006;27 Suppl A:S127-34.

1025 [156] Handschuh K, Guibourdenche J, Cocquebert M, Tsatsaris V, Vidaud M, Evain-Brion
 1026 D, et al. Expression and regulation by PPARgamma of hCG alpha- and beta-subunits:
 1027 comparison between villous and invasive extravillous trophoblastic cells. *Placenta*.
 1028 2009;30:1016-22.

1029 [157] Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion
 1030 D, et al. Human chorionic gonadotropin expression in human trophoblasts from early

1031 placenta: comparative study between villous and extravillous trophoblastic cells. *Placenta*.
1032 2007;28:175-84.

1033 [158] Malassine A, Frendo JL, Evain-Brion D. Trisomy 21- affected placentas highlight
1034 prerequisite factors for human trophoblast fusion and differentiation. *Int J Dev Biol*.
1035 2010;54:475-82.

1036 [159] Yang M, Lei ZM, Rao Ch V. The central role of human chorionic gonadotropin in the
1037 formation of human placental syncytium. *Endocrinology*. 2003;144:1108-20.

1038 [160] Schild RL, Schaiff WT, Carlson MG, Cronbach EJ, Nelson DM, Sadovsky Y. The
1039 activity of PPAR gamma in primary human trophoblasts is enhanced by oxidized lipids. *J*
1040 *Clin Endocrinol Metab*. 2002;87:1105-10.

1041 [161] Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion
1042 D, et al. Human chorionic gonadotropin produced by the invasive trophoblast but not the
1043 villous trophoblast promotes cell invasion and is down-regulated by peroxisome proliferator-
1044 activated receptor-gamma. *Endocrinology*. 2007;148:5011-9.

1045 [162] Guibourdenche J, Handschuh K, Tsatsaris V, Gerbaud P, Leguy MC, Muller F, et al.
1046 Hyperglycosylated hCG is a marker of early human trophoblast invasion. *J Clin Endocrinol*
1047 *Metab*. 2010;95:E240-4.

1048 [163] Arck P, Toth B, Pestka A, Jeschke U. Nuclear receptors of the peroxisome
1049 proliferator-activated receptor (PPAR) family in gestational diabetes: from animal models to
1050 clinical trials. *Biol Reprod*. 2010;83:168-76.

1051 [164] Giaginis C, Spanopoulou E, Theocharis S. PPAR-gamma signaling pathway in
1052 placental development and function: a potential therapeutic target in the treatment of
1053 gestational diseases. *Expert Opin Ther Targets*. 2008;12:1049-63.

1054 [165] Jawerbaum A, Capobianco E. Review: Effects of PPAR activation in the placenta and
1055 the fetus: implications in maternal diabetes. *Placenta*. 2011;32 Suppl 2:S212-7.

1056 [166] Capparuccia L, Marzioni D, Giordano A, Fazioli F, De Nictolis M, Busso N, et al.
1057 PPARgamma expression in normal human placenta, hydatidiform mole and choriocarcinoma.
1058 Mol Hum Reprod. 2002;8:574-9.

1059 [167] Toth B, Bastug M, Scholz C, Arck P, Schulze S, Kunze S, et al. Leptin and
1060 peroxisome proliferator-activated receptors: impact on normal and disturbed first trimester
1061 human pregnancy. Histol Histopathol. 2008;23:1465-75.

1062 [168] Toth B, Bastug M, Mylonas I, Scholz C, Makovitzky J, Kunze S, et al. Peroxisome
1063 proliferator-activated receptor-gamma in normal human pregnancy and miscarriage. Acta
1064 Histochem. 2009;111:372-8.

1065 [169] Rodie VA, Young A, Jordan F, Sattar N, Greer IA, Freeman DJ. Human placental
1066 peroxisome proliferator-activated receptor delta and gamma expression in healthy pregnancy
1067 and in preeclampsia and intrauterine growth restriction. J Soc Gynecol Investig. 2005;12:320-
1068 9.

1069 [170] Holdsworth-Carson SJ, Lim R, Mitton A, Whitehead C, Rice GE, Permezel M, et al.
1070 Peroxisome proliferator-activated receptors are altered in pathologies of the human placenta:
1071 gestational diabetes mellitus, intrauterine growth restriction and preeclampsia. Placenta.
1072 2010;31:222-9.

1073 [171] Jawerbaum A, Capobianco E, Pustovrh C, White V, Baier M, Salzberg S, et al.
1074 Influence of peroxisome proliferator-activated receptor gamma activation by its endogenous
1075 ligand 15-deoxy Delta12,14 prostaglandin J2 on nitric oxide production in term placental
1076 tissues from diabetic women. Mol Hum Reprod. 2004;10:671-6.

1077 [172] Castell AL, Hieronimus S, Lascols O, Fournier T, Fenichel P. Vascular placental
1078 abnormalities and newborn death in a pregnant diabetic woman with familial partial
1079 lipodystrophy type 3: A possible role for peroxisome proliferator-activated receptor gamma.
1080 Diabetes Metab. 2012.