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The potential role of the *ERRγ* pathway in placental dysfunction

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Abstract

Normal placental development and function is of key importance to fetal growth. Conversely aberrations of placental structure and function are evident in pregnancy complications including fetal growth restriction (FGR) and preeclampsia. Although trophoblast turnover and function is altered in these conditions, their underlying aetiologies and pathophysiology remains unclear, which hampers development of therapeutic interventions.

Here we review evidence that supports a role for Estrogen Related Receptor-gamma (*ERRγ*) in the development of placental dysfunction in FGR and preeclampsia. This relationship deserves particular consideration because *ERRγ* is highly expressed in normal placenta, is reduced in FGR and preeclampsia and its expression is altered by hypoxia, which is thought to result from deficient placentation seen in FGR and preeclampsia. Several studies have also found microRNA or other potential upstream regulators of *ERRγ* negatively influence trophoblast function which could contribute to placental dysfunction seen in FGR and preeclampsia. Interestingly, microRNAs regulate *ERRγ* expression in human trophoblast. Thus, if *ERRγ* is pivotally associated with the abnormal trophoblast turnover and function it may be targeted by microRNAs

or other possible upstream regulators in the placenta.

This review explores altered expression of *ERRγ* and upstream regulation of *ERRγ*-mediated pathways resulting in the trophoblast turnover, placental vascularisation, and placental metabolism underlying placental dysfunctions. This demonstrates that the *ERRγ* pathway merits further investigation as a potential therapeutic target in FGR and preeclampsia.

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1.0 Introduction

Placental dysfunction describes when the placenta fails to develop and/or function adequately to support the nutritional demands of the fetus, and is central to the development of both fetal growth restriction (FGR) and preeclampsia (Spinillo et al., 2019, Redman, 1991). FGR describes a fetus that does not reach its genetic growth potential. In clinical practice, this is often identified as a small for gestational age infant i.e. a baby whose estimated fetal weight (EFW) or birthweight is less than the 10th percentile for that stage of pregnancy (ACOG, 2019). However, being small for gestational age is not synonymous with FGR. True FGR affects 5%-10% of fetuses and

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is associated with both short-term and long-term complications including stillbirth, neonatal death, abnormal neurodevelopment, and cardiovascular and metabolic disorders in later life (Pels et al., 2019, Bernstein et al., 2000, Crispi et al., 2010, Gardosi et al., 2005, Ramirez-Velez et al., 2017). The majority of cases of FGR are mediated by abnormal placental structure and function (Spinillo et al., 2019). FGR may also co-exist with preeclampsia, which is defined as an elevation of maternal blood pressure with proteinuria occurring after 20 weeks' gestation (Brown et al., 2018). In addition to adverse effects on the fetus, preeclampsia is associated with maternal morbidity and mortality (Souza et al., 2013, Brown et al., 2018). Presently there are no effective therapies to treat FGR or preeclampsia, leaving a decision between expectant management or delivery indicated by deterioration in fetal or maternal condition. Therapeutic advances are in part impaired by an incomplete understanding of the mechanisms underlying the placental dysfunction evident in FGR and preeclampsia. Therefore, the identification of key causal pathways amenable to therapeutic manipulation is an important goal for research in this area. Here we review the evidence for involvement of one such pathway, that of estrogen-related receptor γ (*ERRγ*) in the human placenta (Kumar and Mendelson, 2011).

Estrogen-related receptor-γ is a member of the ERR family of orphan nuclear receptors, which is highly expressed in the human placenta (Takeda et al., 2009). Evidence suggests that *ERRγ* serves an important role in trophoblast differentiation, proliferation, and invasion, and may be involved in blood pressure homeostasis (Zhu et al., 2018a, Luo et al., 2014). In addition, deficient expression of *ERRγ* is linked to impaired placental mitochondrial function (Poidatz et al., 2012), which could lead to inadequate energy supply and thus reduced energy expenditure within the placenta. Due to its wide range of functions in relevant biological processes, it is plausible that *ERRγ* may also play a role in placental dysfunction underlying pregnancies complicated with FGR or preeclampsia.

To consider whether the *ERRγ* pathway has a causal role in placental dysfunction we have reviewed the literature to: (i) summarize knowledge regarding the role of *ERRγ* in

trophoblast, placental vascularisation and placental metabolism, (ii) discuss the evidence for aberrant expression of constituents of the *ERRγ* pathway in pregnancy complications, including FGR and preeclampsia, and (iii) consider the implications of altered *ERRγ* expression and how this may contribute to placental dysfunction.

2. Placental dysfunction underlying pregnancy complications

To assess whether a pathway may be involved in the pathophysiology of FGR and/or preeclampsia, its role in normal placental development requires consideration, followed by evaluation of whether the aberrant placental phenotype seen in these conditions is consistent with disruption of that pathway.

2.1 Normal placental development

In normal placental development, appropriate differentiation of cytotrophoblast cells, the trophoblast stem cell population of the placenta, is important; two different pathways arise within the developing placental villus: the extravillous and villous lineages. The extravillous trophoblasts (EVT) differentiate from cytotrophoblast cell columns and invade the uterus (interstitial invasion) and spiral arteries (endovascular invasion) to remodel the maternal blood vessels and produce dilated and compliant uterine arterioles (Figure 1A), thereby ensuring an adequate supply of oxygen and nutrients to support fetal growth (Pijnenborg et al., 1983). Proliferation, differentiation and fusion of villous cytotrophoblast maintains the syncytiotrophoblast, the multinucleated outer layer of the placenta responsible for placental transport, protective and endocrine functions (Jones and Fox, 1991) (Figure 1A and 1B). The villous cytotrophoblast and syncytiotrophoblast, together with a core of villous stromal cells containing fetoplacental blood vessels form the villous tree, which is the functional unit of the placenta (Jones and Fox, 1991) (Figure 1 A and 1B). There are five different types of villi, including mesenchymal villi, immature intermediate villi, stem villi, mature intermediate villi, and terminal villi. Terminal villi, which represent the final

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branches of the villous tree, exhibit a high degree of capillarization and fetoplacental vessels are separated from maternal blood by a thin layer of syncytiotrophoblast and endothelial cells termed the vasculo-syncytial membrane, which is optimised for gas and nutrient exchange in human placenta (Kingdom et al., 2000). Consequently, there is a close relationship between terminal villous structure and function.

2.2 Placental changes in FGR and preeclampsia

Compared to placentas from normal pregnancies, placentas from pregnancies complicated by FGR and/or pre-eclampsia may exhibit a number of structural and functional changes, including evidence of an unfolded protein response, increased trophoblast apoptosis and autophagy, and reduced trophoblast proliferation and metabolic function (Heazell et al., 2011, Heazell et al., 2008, Burton and Jauniaux, 2018, Curtis et al., 2013, Yung et al., 2019). In the syncytiotrophoblast, some nuclei are aggregated to form syncytial knots with features of apoptosis and a disordered proliferation, and the increased formation of syncytial knots is related to the conditions of placental dysfunction which have been found in the FGR placentas (Macara et al., 1996, Heazell et al., 2007). FGR placentas also show decreased volume and surface area of terminal villi, with elongated and less-branched capillary loops (Krebs et al., 1996, Jackson et al., 1995). It is hypothesized that some of these changes in villous tissue are secondary to reduced invasion of extravillous trophoblast earlier in pregnancy, leading to impaired perfusion of the intervillous space. There may also be abnormalities of the fetal-placental vasculature and a reduction in placental weight, all of which combine to result in insufficient delivery of nutrients to the developing fetus (Roberts and Post, 2008).

The placenta is a metabolically active organ that consumes a large volume of oxygen throughout gestation, with energy provision mainly dependent on mitochondrial activity by glucose utilization (Diamant et al., 1975, Malek et al., 1996). An imbalance of placental mitochondrial function with excessive generation of reactive oxygen and nitrogen species in placentas is observed in pregnancy complications such as FGR, and

pre-eclampsia (Biri et al., 2007, Atamer et al., 2005, Leduc et al., 2010). Taken together with the observation of altered perfusion of the intervillous space the critical relationship between hypoxia, reactive oxygen species (ROS), and how this leads to placental dysfunction needs to be considered.

2.3 A possible role of hypoxia / ROS in placental dysfunction in FGR and Preeclampsia

As stated above, hypoxia and hypoxia-reoxygenation can contribute to the elevation of reactive oxygen species (ROS), which can lead to increased oxidative DNA damage and depletion of local antioxidant defenses (Hung and Burton, 2006, Kimura et al., 2013). Placental hypoxia has been reported in both FGR and preeclampsia (Kimura et al., 2013). Furthermore, a hypoxic environment can reproduce elements of the trophoblast phenotype seen in these conditions. Culture in 2% or 9% O_2 reduces differentiation and induces apoptosis in third trimester primary cytotrophoblast (Levy et al., 2000, Alsat et al., 1996). Culture in 2% O_2 impaired differentiation and invasion in first-trimester primary cytotrophoblast (Genbacev et al., 1996), and term placental villous explants also exhibited reduced proliferation and induction of apoptosis when cultured at 1% compared to 6-8% O_2 (Heazell et al., 2008). Therefore, oxygen tension can modulate both the development of villous structure and trophoblast function. The molecular mechanisms responsible for these changes in trophoblast phenotype are still elusive, but recent reports suggest that it may, in part, be linked to activation of an unfolded protein response (UPR) by placental oxidative stress (Yung et al., 2019, Yung et al., 2008).

To understand the potential contribution of the *ERRγ* pathway in the pathogenesis of placental dysfunction underlying FGR and preeclampsia, the functions of *ERRγ* in pregnancy will be described, and the evidence that *ERRγ* signalling might be involved in the occurrence of placental dysfunction will be reviewed.

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3. The ERR family

Estrogen-related receptors (ERRs) are an NR3B (nuclear receptor 3B) group of the nuclear receptor subfamily, including *ERRα*, *ERRβ*, and *ERRγ*, which are encoded by *ESRRA*, *ESRRB*, and *ESRRG*, respectively. The NR3B group of nuclear receptors is one of the larger NR3 classes and includes the hormone receptors for estrogen, androgens, progesterone, aldosterone, and cortisol (Giguere et al., 1988, Giguere, 1999). Although ERRs share sequence homologies with the estrogen receptor (ER), the transcription of ERRs is not activated by estrogen, and information on the nature of endogenous ligands for ERRs remains to be established (Vanacker et al., 1999). ERRs can regulate transcription by binding to estrogen-related receptor elements (ERRE) in target genes, which include several molecules involved in the cellular energy metabolic pathway (Giguere, 2008).

3.1 Structure of ERRs

According to their sequence homology and function, the structural features of ERRs include an activation function (AF)-1 domain /N-terminal domain (NTD), a DNA -binding domain (DBD), a ligand-binding domain (LBD), and an AF-2 domain (Giguere, 1999). The NTD is a non-conserved domain and it includes an AF-1 domain and a variable amino acid domain. In *ERRγ* and *ERRα*, the NTD contains phosphorylation-dependent sumoylation sites that are embedded in a synergy control motif and may serve a role in regulating the transcriptional activity of ERRs (Tremblay et al., 2008). The synergy control motif may have a role in modulating higher-order interactions among transcriptional factors (Iniguez-Lluhi and Pearce, 2000). The ERRs' DBD exists the highest sequence homology in the three ERR isoforms: *ERRβ* and *ERRα* share 99% and 93% identical amino acid sequence with *ERRγ* respectively, which suggests that more than two ERRs might share some target genes (Heard et al., 2000). DBD contains two highly conserved zinc finger motifs, which recognize and bind a specific DNA sequence (TCAAGGTCA), denoted as an ERR response element (ERRE). The ERRE can be either a monomer, a homodimer, or a heterodimer, which

197 can modulate the translational activities of ERRs (Johnston et al., 1997, Dufour et al.,
198 2007). Moreover, ERRs and ERs have high homology in the DBD region (Giguere et
199 al., 1988); ERRs can recognize the ERRE embedded in estrogen response elements
200 (ERE), but only 21% of *ERRα* target promoters can be recognized by ERα in breast
201 cancer cell lines (Deblois et al., 2009). Despite this, several genes can be regulated by
202 both ERα and ERRs, including the human lactoferrin gene and monoamine oxidase B
203 (Yang et al., 1996, Zhang et al., 2006b).

204 The final structural part of ERRs is the LBD, a less conserved domain; there is a 77%
205 sequence homology between the LBDs of *ERRβ* and *ERRγ*, and 61% homology
206 between the *ERRα* and *ERRγ* (Heard et al., 2000). The homodimerization or
207 heterodimerization of LBD in *ERRγ* can also influence the translation of ERRs; the
208 homodimerization of *ERRγ* via LBD can enhance the activity of translation; conversely,
209 heterodimerization with ERRα inhibits the activity of both receptors (Huppunen and
210 Aarnisalo, 2004). The interaction between the LBD and its coactivator is ligand-
211 independent (Greschik et al., 2002). However, the crystal structure also showed that the
212 LBD can interact with ligands by a flexible ligand-binding pocket and importantly from
213 the perspective of understanding receptor signalling pathways, several synthetic
214 molecules can inhibit or stimulate the transcriptional function of ERRs by LBD,
215 including proliferator-activated receptor coactivator 1-alpha, diethylstilbestrol (DES),
216 and 4-hydroxytamoxifen (4-OH) (Kallen et al., 2004, Chao et al., 2006, Tremblay et
217 al., 2001a, Tremblay et al., 2001b). Bisphenol A (BPA) is a chemical and environment
218 contaminant used to produce plastics, which strongly binds to *ERRγ*-LBD (Takeda et
219 al., 2009). As the level of BPA in maternal blood and placental tissue is inversely
220 related to fetal weight in human pregnancy (Troisi et al., 2014), BPA-mediated
221 upregulation of placental *ERRγ* may provide a mechanistic link to explain the
222 association between elevated BPA levels and FGR (Takayanagi et al., 2006, Okada et
223 al., 2008).

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Thus, the structure of the ERRs, specifically that of the LBD and DBD, is vital to the regulation of ERR signalling, including that of *ERRγ*. Furthermore, abnormal placental expression of *ERRγ* in FGR and preeclampsia suggests a potential role for *ERRγ* in the development or potentiation of these pregnancy complications (Zhu et al., 2018a, Luo et al., 2014). This review will consider how *ERRγ* is regulated, its effects in trophoblast and how this may contribute to the phenotypes of placental dysfunction observed in FGR and preeclampsia.

3.2 *ERRγ*

Both fetal and adult organs abundantly express *ERRγ* (Heard et al., 2000), including the placenta, heart, and brain (Takeda et al., 2009, Heard et al., 2000, Misra et al., 2017). *ERRγ* can regulate blood pressure homeostasis, due to the high expression of *ERRγ* in kidneys which mediate aldosterone-stimulated sodium and water reuptake (Alaynick et al., 2010). In *ERRγ* null mice, the genes that regulate serum potassium and blood pressure were decreased in the kidney; RNA expression of the potassium channels, *Kcnj1*, *Kcne1*, and *Kcne2*, and kallikrein-kinin system genes kallikrein 1 (*Klk1*) and kallikrein 6 (*Klk6*), were significantly reduced in the kidneys of *ERRγ* null mice, (Alaynick et al., 2010). Other potential mechanisms by which *ERRγ* can regulate maternal blood pressure homeostasis during pregnancy are related to steroid 11β-hydroxylase (*Cyp11b1*) and aldosterone synthase (*Cyp11b2*) (Luo et al., 2014). In *ERRγ* heterozygous (*ERRγ*+/-) pregnant mice, expression of *CYP11b1* and *CYP11b2* is decreased in the mouse adrenal cortex, resulting in reduced production of aldosterone and a reduction in blood pressure; conversely, expression of *CYP11b1* and *CYP11b2* in WT pregnant mice is increased after exposure to the *ERRγ* agonist DY131, which increased maternal blood pressure (Luo et al., 2014). Given that development of preeclampsia involves abnormal elevation of maternal blood pressure, dysregulation of *ERRγ* signaling in the kidney and adrenal cortex may contribute to this phenomenon.

Placental *ERRγ* expression also plays an important role in the maintenance of pregnancy. Placenta has the highest expression of *ERRγ* in the human reproductive system (Figure

1B) (Takeda et al., 2009); expression of *ERRγ* increases over gestation and is higher in villous compared to extravillous trophoblast (Poidatz et al., 2012). *ERRγ* expression is dramatically increased during human cytotrophoblast cell differentiation, indicating a potential regulatory role (Kumar and Mendelson, 2011). Moreover, *ERRγ* also regulates genes involved in cellular energy homeostasis and metabolism; expression of key regulator genes involved in mitochondrial biogenesis (*PGC-1α* and *NRF-1*) and energy metabolism (*PDK4* and *MCAD*) decreased after silencing *ERRγ* in human first trimester placental primary cytotrophoblast (Poidatz et al., 2012). As these studies indicate that *ERRγ* signaling may influence multiple aspects of normal placental function, we will review the evidence for the involvement of *ERRγ* in regulating trophoblast function, hypoxic responses, placental vascularisation, placental metabolism, and other regulators in the human placenta (Table 1 and Figure 2).

3.3 The effect of *ERRγ* on trophoblast function

3.3.1 Proliferation

ERRγ knockdown has been shown to reduce proliferation of the extravillous-like trophoblast cell line HTR-8/SVneo, via decreasing the expression of its downstream gene, 17β-hydroxysteroid dehydrogenase type 1 (*HSD17B1*) (Zhu et al., 2018a). *HSD17B1* is an enzyme capable of converting estrone to 17β-estradiol in the metabolism of estrogen. Abnormal expression of *HSD17B1* has been reported in both FGR and preeclampsia (Zhu et al., 2018a); previous studies have revealed that a reduced plasma *HSD17B1* expression level could be considered a potential prognostic factor for preeclampsia (Ohkuchi et al., 2012, Ishibashi et al., 2012). Ohkuchi *et al*. (Ohkuchi et al., 2012) examined 128 normal pregnant women and 30 pregnancies complicated with preeclampsia and found that reducing maternal plasma levels of *HSD17B1* correlated with the occurrence of preeclampsia, implicating *HSD17B1* in the pathogenesis of the disease, possibly by influencing the process of estrogen metabolism. Since estrogen can reduce the proliferation of HTR-8/SVneo cell line (Patel et al., 2015), this might suggest a relationship between low level of *HSD17B1* in maternal serum,

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placental estrogen metabolism, and trophoblast proliferation. Moreover, the mRNA and protein level of *HSD17B1* was decreased in placentas complicated with FGR (Zhu et al., 2018a). Therefore, aberrant regulation of *HSD17B1* by *ERRγ* may contribute to placental dysfunction, by its ability to regulate the proliferation of cytotrophoblast cells which is disrupted in FGR and preeclampsia.

3.3.2 Differentiation

There is also evidence that *ERRγ* may influence cytotrophoblast differentiation via its role as a regulator of the aromatase *CYP19A1*, the voltage-gated potassium (K_V7) channel family, or via interactions with two other downstream genes, placenta specific-1 (*PLAC1*), and 11β-hydroxysteroid dehydrogenase 2 (*HSD11B2*).

The cytochrome P-450 (CYP) family members include *CYP11A1* and *CYP19A1*, and hydroxysteroid dehydrogenases (HSDs), such as 3β-HSD and 17β-HSD; these enzymes play a vital role in placental hormone synthesis and metabolism (Payne and Hales, 2004). C19 steroid precursors can be converted into estrogen via activating aromatase P450, which is encoded by the *CYP19A1/ hCYP19* gene and only expressed in the syncytiotrophoblast, not in trophoblast stem cells or cytotrophoblast (Fournet-Dulguerov et al., 1987, Kamat et al., 1998). Notably, *ERRγ* has been shown to stimulate the expression of *hCYP19 in vitro*, via binding to its promotor to increase estrogen levels in a 20% O_2 culture environment, which promotes trophoblast differentiation. When human second-trimester primary cytotrophoblasts were cultured in a hypoxic environment (2% O_2), both *ERRγ* and *hCYP19* expression decreased; however, elevating *ERRγ* expression restored *hCYP19* expression (Kumar and Mendelson, 2011).

ERRγ also induces mRNA and protein expression of the K_V7 family of potassium channels to regulate the differentiation of cytotrophoblast in second-trimester placentas. Voltage-gated K_V7 channels are encoded by the *KCNQ1-5* (α-subunit) and *KCNE1-5* (β-subunit) genes. The human placenta expresses many potassium channel genes, including the *KCNQ* and *KCNE* families, and the expression of *KCNQ3* and *KCNE5* is

markedly increased in placentas from pregnancies complicated with preeclampsia, particularly in the syncytiotrophoblast (Mistry et al., 2011). *ERRγ* induces mRNA and protein expression of the potassium channels *KLK1*, *KCNQ1*, *KCNE1*, *KCNE3* and *KCNE5* during primary cytotrophoblast differentiation, the effect of which was blocked by hypoxia (Luo et al., 2013). After examining the promoter, an ERRE located in the upstream region of the *KCNE1* and *KLK1* genes was identified, to which *ERRγ* can bind (Luo et al., 2013). In addition, expression of the oxygen-sensitive K^+ channel gene $K_V9.3$ was increased in FGR placentas, and expression of $K_V2.1$ was increased in chorionic plate veins from the same placentas (Corcoran et al., 2008). However, the relationship between *ERRγ* and expression of K^+ channels in functionally deficient placentas remains unclear.

There is also evidence that *PLAC1* and *HSD11B2* are downstream effectors of *ERRγ*, and these two genes can regulate cytotrophoblast differentiation (Luo et al., 2013). The expression of *PLAC1* is elevated during trophoblast differentiation and conversely, reduced expression of *PLAC1* attenuates fusion of term primary human cytotrophoblast *in vitro* (Chang et al., 2016). Contrary to expectations, Sifakis *et al*. found high *PLAC1* expression in FGR placentas at term (Sifakis et al., 2018), although this may be linked to the aberrant differentiation and trophoblast turnover reported in FGR(Heazell et al., 2011, Huppertz, 2011). Combined with the observations of Luo et al., these data suggest that the effect of *PLAC1* on trophoblast differentiation might be mediated via *ERRγ*.

Another downstream gene of *ERRγ* is *HSD11B2*, an enzyme that converts active cortisol to inactive cortisone, which is expressed in villous syncytiotrophoblast (Pepe et al., 1999). Both mRNA and protein expression of *HSD11B2* is induced during term primary cytotrophoblast differentiation, and it is considered a marker for trophoblast differentiation (Hardy and Yang, 2002, Homan et al., 2006). During pregnancy, *HSD11B2* acts as a critical placental glucocorticoid barrier that protects the fetus from the harmful effects of excessive maternal glucocorticoids (Zhu et al., 2018b, Benediktsson et al., 1993). Placental *HSD11B2* expression correlates with fetal weight and postnatal growth velocity (Benediktsson et al., 1993). McTernan *et al.* (McTernan

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et al., 2001) showed that placental *HSD11B2* expression is decreased in FGR and demonstrated the importance of placental *HSD11B2* in regulating fetal growth. Studies of SGA placentas also reported low *HSD11B2* expression, which further revealed the relationship between *HSD11B2* and fetal weight (Struwe et al., 2007). Placental *HSD11B2* expression at birth is positively associated with fetal length at birth, whereas its expression is inversely related to growth velocity in the first year of life, and might therefore be a predictor of postnatal growth of fetuses with FGR (Tzschoppe et al., 2009). These studies support the relationship between abnormal differentiation of cytotrophoblast seen in FGR and preeclampsia and expression of *HSD11B2*, although the roles of *HSD11B2* in trophoblast function are still unclear. Since *ERRγ* regulates *HSD11B2* (Luo et al., 2013), the reduced effect of *HSD11B2* on trophoblast differentiation might be due to reduced levels of *ERRγ* in the presence of placental dysfunction.

3.3.3 Invasion

Invasion of the extravillous trophoblast into the uterine wall and subsequent remodeling of the uterine arterioles is critical for normal placental development and optimal uteroplacental perfusion. Knockdown of *ERRγ* resulted in the deficient invasion of the extravillous-like HTR-8/SVneo cell line (Liu et al., 2018, Zhu et al., 2018a). Liu et al. showed that overexpression of microRNA (miR)-320a inhibited HTR-8/SVneo invasion by regulating *ERRγ* signalling (Liu et al., 2018). Furthermore, Zhu et al. demonstrated that reduced expression of *ERRγ* in HTR-8/SVneo cells significantly impaired invasion via regulation of *HSD17B1* (Zhu et al., 2018a). Although a potentially significant finding, the relationship between *ERRγ* and the invasive capacity of extravillous trophoblast needs to be explored further using primary tissues.

3.4 The effect of *ERRγ* on response to hypoxia

A hypoxic environment alters the expression of many genes which are associated with trophoblast differentiation. The most well-studied oxygen sensor in trophoblast is

hypoxia-inducible factor 1α (HIF1α), which is reported to be elevated in FGR and pre-eclampsia (Rajakumar et al., 2004, Robb et al., 2017). HIF-1α regulates *ERRγ* expression in human trophoblast: culture in a 2% O_2 environment activates HIF-1α and decreases the expression of *ERRγ* and *hCYP19* (Kumar and Mendelson, 2011). Conversely, knockdown of *HIF-1α* in trophoblast prevents *ERRγ* suppression under hypoxic conditions (Kumar and Mendelson, 2011). Collectively, these findings demonstrate that *ERRγ* serves as an oxygen-dependent transcriptional factor regulated by *HIF-1α* to control the expression of downstream *hCYP19*. This relationship appears to be maintained *in vivo*, as low *ERRγ* expression has been reported in placentas from FGR pregnancies, which often show evidence of hypoxia and/or oxidative stress(Takagi et al., 2004). A preliminary study in a south Chinese population examined the mRNA and protein level of *ERRγ* in 28 FGR placentas and 30 matched appropriate for gestational age (AGA) placentas, and reported lower expression of *ERRγ* in FGR placentas (Zhu et al., 2018a). Poidatz et al. (Poidatz et al., 2015) also reported lower mRNA expression of *ERRγ* in 39 FGR placentas compared with a 30 controls in a European population. These studies support the hypothesis that *ERRγ* might play a role in placental dysfunction originating from placental hypoxia.

3.5 The effect of *ERRγ* on placental vascularisation

Although the trophoblast is critically important to the placental function, it is also widely acknowledged that impaired placental blood vessel development may be important in the aetiology of FGR (Hitschold et al., 1993). Several genes have been implicated in regulating placental vascularisation, including vascular endothelial growth factor A (*VEGFA*) (Ylikorkala et al., 2001, Burton et al., 2009). Maternal serum levels of *VEGFA,* an angiogenic factor that is crucial for placental angiogenesis during early gestation, are decreased in the 2nd and 3rd-trimester in pregnancies complicated with FGR (Bersinger and Odegard, 2005). Expression of *VEGFA* in primary vascular endothelial cells is also reduced in FGR placentas (Chui et al., 2014). The altered vascularisation seen in the placentas of women with FGR can potentially be attributed to dysregulated *ERRγ* expression. In mice, placentas from *ERRγ*-/- fetuses have

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393 significantly increased mRNA levels of *VEGFA,* compared with placentas from wild
394 type fetuses (Luo et al., 2014), and in *ERRγ* +/- pregnant mice, circulating levels of the
395 angiogenic, soluble receptor for *VEGF*, soluble fms-like tyrosine kinase-1(sFlt-1), were
396 significantly reduced (Luo et al., 2014). In the mouse myoblast cell line C2C12,
397 suppression of *ERRγ* can block the transcriptional expression of *VEGFA*, whilst in
398 HEK-293T cells, *ERRγ* has been shown to activate the *VEGFA* promoter (Matsakas et
399 al., 2012). This indicates that *ERRγ* may affect placental vascularisation via its
400 regulation of *VEGFA,* however further studies are required in humans to confirm this.

401 **3.6 *ERRγ* and placental metabolism**

402 There is accumulating evidence that *ERRγ* plays a role in the regulation of several
403 mitochondrial functions, including mitochondrial biogenesis, oxidative
404 phosphorylation, and fatty acid oxidation, in the heart, kidney, skeletal muscle, and
405 placenta (Huss et al., 2002, Alaynick et al., 2007, Dufour et al., 2007, Kubo et al., 2009,
406 Alaynick et al., 2010, Fan et al., 2018). In the human placenta, *ERRγ* regulates
407 mitochondrial function by controlling gene networks involved in mitochondrial
408 biogenesis and fat and glucose metabolism in the villous trophoblast, including
409 pyruvate dehydrogenase kinase 4 (*PDK4*), medium chain acyl-CoA dehydrogenase
410 (*MCAD*), sirtuin 1 (*SIRT1*) and peroxisome proliferator-activated receptor γ (*PPARγ*)
411 coactivator 1 α (*PGC-1α*) (Poidatz et al., 2012).

412 In human term primary villous cytotrophoblast, expression of *PDK4* and *MCAD* was
413 decreased after knockdown of *ERRγ* expression (Poidatz et al., 2012), which implicates
414 *ERRγ* as a potential regulator of placental fatty acid oxidation and glucose metabolism
415 mediated via these genes. *PDK4* can phosphorylate the pyruvate dehydrogenase
416 complex (PDC), which facilitates the conversion of pyruvate to acetyl-CoA in
417 mitochondria, to inhibit the activity of PDC (Sugden and Holness, 2003); *MCAD* is an
418 enzyme which catalyzes the initial step of mitochondrial fatty acid oxidation (FAO)
419 (Schulz, 1991). *ERRγ* can stimulate the expression of *PDK4* in human liver cell lines
420 (HepG2 cells) and rat hepatoma cells (Zhang et al., 2006a, Lee et al., 2012); regulation

of the promoter of *PDK4* by *ERRγ* has been observed in mammary epithelial cells by using both ChIP and luciferase reporter assays, and the activation of the *ERRγ-PDK4* pathway attenuates glucose oxidation and decrease cell death and apoptosis (Kamarajugadda et al., 2012). Thus, a reduction in *ERRγ* would be expected to be associated with increased apoptosis and cell death, as is observed in FGR. This suggests that the *ERRγ-PDK4* signalling pathway in human placentas might contribute to the placental dysfunction. *MCAD* is one of the targets of *ERRα* and the increased expression of the *ERRα-MCAD* pathway serves an important role in the decidualization of human primary endometrial stromal cells (Bombail et al., 2010). Since *ERRγ* is similar to *ERRα*, it is possible that *ERRγ* also contributes to the regulation of *MCAD*, however further studies are needed to investigate this and to determine the role of the pathway in placental dysfunction.

SIRT1, *PGC-1α,* and *PGC-1β* are also coactivators of *ERRγ* that have known roles in regulating placental metabolism. *SIRT1*, a NAD(+)-dependent protein deacetylase, is expressed ubiquitously in different organs and is required for many cellular processes related to differentiation and metabolism (Leibiger and Berggren, 2006). *SIRT1* is expressed in both the syncytiotrophoblast and cytotrophoblast (Lappas et al., 2011). Findings from two recent studies in mice indicate that *SIRT1* plays a key role in trophoblast differentiation and placental development (Arul Nambi Rajan et al., 2018, McBurney et al., 2003). The differentiation of mouse trophoblast stem cells obtained from *SIRT1*-null mice was blunted *in vitro* (Arul Nambi Rajan et al., 2018), resulting in fetuses with FGR, and smaller placentas with deficient morphology including a thickened chorion and a more hypercellular labyrinth were observed (Arul Nambi Rajan et al., 2018, McBurney et al., 2003). Wilson et al. found *SIRT1* can deacetylate and increases *ERRα* DNA-binding activity by interacting with *ERRα in vivo* and *in vitro*, which also suggest a potential interaction between *ERRγ* and *SIRT1* (Wilson et al., 2010), since *ERRα* and *ERRγ* have structural and functional similarities. In HepG2 cells, small heterodimer partner interacting leucine zipper protein (*SMILE*) expression and its ability to repress *ERRγ* transactivation and downstream signaling, is dependent on the

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450 expression of *SIRT1* (Xie et al., 2009). *SIRT1* can also positively regulate the expression
451 of another *ERRγ* coactivator, *PGC-1α* (Amat et al., 2009, Gerhart-Hines et al., 2007).
452 *PGC-1α* and its family member, *PGC-1β,* act as transcriptional co-regulators of *ERRα*
453 and *ERRγ* to influence metabolism in many diseases, such as cardiovascular disease and
454 cancer (Liu et al., 2005, Huss et al., 2002, Torrano et al., 2016, Luo et al., 2017). In
455 human placental tissue, the mRNA expression level of *PGC-1α* and *SIRT1* correlated
456 with that of *ERRγ* in pregnancies complicated with preeclampsia and FGR (Poidatz et
457 al., 2015); low mRNA levels of *ERRγ*, *PGC-1α* and *SIRT1* have all been reported in
458 FGR placentas (Poidatz et al., 2015). Together, these studies suggest that methods to
459 modulate both *ERRγ* and its transcriptional co-regulators, may provide a potential
460 therapeutic strategy to improve placental metabolism and fetal growth. Therefore, we
461 will conclude by reviewing the microRNAs (miRNAs) that have been identified as
462 upstream regulators of *ERRγ,* and which may also contribute to the etiology of placental
463 dysfunction.

464 **3.7 Regulation of *ERRγ* in the human placenta by miRNAs**

465 miRNAs are short non-coding RNAs with 19-23 nucleotides which post-translationally
466 reduce gene expression in both animals and plants by mediating argonaute (AGO)
467 binding to the 3'-untranslated-region (3'-UTR) of mRNA (Baek et al., 2008). The
468 miRNA-induced silencing complex (miRISC), which includes the miRNAs and AGO,
469 degrades target mRNA and represses protein translation. Different miRNAs are
470 expressed in specific tissues, and by regulating different sets of target genes, specific
471 miRNAs can mediate many cellular processes, such as differentiation, proliferation,
472 and invasion (Anton et al., 2013, Li et al., 2014). In humans, more than 60% of protein-
473 coding genes are thought to be regulated by miRNAs, many of which are specifically
474 expressed in the placenta (Friedman et al., 2009). Expression of numerous miRNAs is
475 altered in pregnancy complications such as FGR and preeclampsia, which are
476 associated with placental dysfunction (Friedman et al., 2009, Zhang et al., 2010,
477 Hromadnikova et al., 2015). The following miRNAs are associated with placental
478 dysfunction and have been identified as potential upstream regulators of *ERRγ*.

3.7.1 miR-320a

miR-320a levels are increased in the placentas of women with late-onset preeclampsia, and overexpression of miR-320a in HTR-8/SVneo cells inhibits mRNA and protein expression of *ERRγ* (Gao et al., 2018). Key functional roles for *ERRγ* in the placenta appear to be modulated by miR-320a: direct regulation of *ERRγ* by miR-320a inhibits migration, invasion, and proliferation and indirectly modulates levels of *VEGFA* in both HTR-8/SVneo cells and human umbilical vein endothelial cells (HUVECs) (Gao et al., 2018, Liu et al., 2018). However, to our knowledge, expression levels of miR-320a in FGR placentas has yet to be assessed.

3.7.2 Other ERRγ regulatory miRNAs

Several other miRNAs have been implicated in placental dysfunction by regulating proliferation, invasion, or invasion of trophoblastic-like cell lines, and by reducing *ERRγ* expression in other cell lines, these include miR-378a-5p, miR-424, miR-377, and miR-204-5p (Eichner et al., 2010, Cheng et al., 2018, Zou et al., 2019). miR-378a-5p inhibits both mRNA and protein levels of *ERRγ* in the breast cancer cell line, BT-474 (Eichner et al., 2010); it also enhances the invasion and migration of HTR8/SVneo cells and reduces BeWo cell differentiation (Luo et al., 2012, Nadeem et al., 2014). miR-424 expression was increased in FGR placentas (Huang et al., 2013) and miR-424 overexpression inhibited protein expression of *ERRγ* in HTR/8SVneo cells. However, this study did not identify a regulatory relationship between miR-424 and the 3'-UTR of *ERRγ,* thus more in-depth studies of miR-424 are required in the future (Zou et al., 2019).

miR-377 is more highly expressed in human term placentas compared with first-trimester placentas, and overexpression of miR-377 in the first-trimester placental explants reduced cytotrophoblast proliferation (Farrokhnia et al., 2014). Furthermore, miR-377 inhibits the expression levels of *SIRT-1* in human retinal endothelial cells; taking into account the reported interaction between *ERRγ* and *SIRT-1* in the human

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placenta, miR-377 may also regulate *ERRγ* expression (Cui et al., 2019). As overexpression of miR-204-5p reduced the invasion of BeWo cells and JEG3 cells (Yu et al., 2015), and miR-204-5p overexpression reduced the differentiation of C1C12 myoblast cells by directly targeting 3'-UTR of *ERRγ*, this data suggest that a direct regulatory relationship may also exist between miR-204-5p and *ERRγ* in the placenta (Cheng et al., 2018). Since the above studies only used cell lines to assess trophoblast function, more data derived from primary placental models are needed; specifically those that focus on the relationship between individual miRNAs, *ERRγ* and its downstream effectors, and their roles in the etiology of FGR; these relationships have been summarized in Figure 3.

Manipulation of the expression of miRNAs upstream of *ERRγ* may represent an additional approach to correct placental dysfunction; accumulating studies *in vivo* and *in vitro* indicate the possibility of developing an inverse agonist of *ERRγ* as a promising treatment for *ERRγ*-related anaplastic thyroid cancer, breast cancer, and type 2 diabetes (Kim et al., 2019, Kim et al., 2012, Vernier et al., 2020). Our group has demonstrated that targeted miRNA inhibitors can be used to selectively manipulate placental function: targeted inhibition of trophoblast miR-145 and miR-675 expression promoted cytotrophoblast proliferation in human first-trimester villous placental explants and increased fetal and placental weight when administered intravenously to pregnant mice (Beards et al., 2017). Therefore, exploring the regulatory pathway of *ERRγ* in the human placenta could inform the development of potential new therapeutic approaches for pregnancy complications involving placental dysfunction, like FGR or preeclampsia.

4.0 Summary

Even though many studies have focused on the pathogenesis of placental dysfunction underlying FGR and preeclampsia, the precise pathophysiological mechanisms and biochemical pathways in the placenta are still unclear, which limits options for therapeutic discovery, making a better understanding of the underpinning placental pathways a priority. The most obvious changes in the placenta in FGR and preeclampsia

include abnormal trophoblast function, increased cell death, altered metabolism and nutrient transport, hypoxia and oxidative stress, and aberrant villous structure. Since *ERRγ* is highly expressed in the human normal term placenta, and it holds key roles in the regulation of cell invasion, differentiation, cellular energy homeostasis, hypoxic responses and metabolism, we argue that involvement of the *ERRγ* pathway in the placental dysfunction underlying FGR and preeclampsia is plausible, and thorough exploration may offer new therapeutic options. In support of this hypothesis, several studies have revealed significantly lower levels of *ERRγ* mRNA and protein in the human placenta in FGR, and *ERRγ* can regulate the invasion and proliferation of human trophoblast cell lines. Furthermore, additional evidence of disruption of both upstream regulators and downstream effectors of *ERRγ* provides evidence that the pathway is intact, and functions as expected in the human placenta. These data highlight that *ERRγ* may be involved in the development and pathogenesis of placental dysfunction by influencing trophoblast function and further studies of the regulation of this pathway are needed. By better understanding the intrinsic role of *ERRγ* as a regulator of trophoblast function, metabolism, and cell turnover, this in turn might provide new ideas for the treatment of placental dysfunction underpinning FGR and preeclampsia in the future.

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Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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Author contribution statement

ZZ, LKH, KF, and AEPH conceived and designed the research. ZZ drafted the manuscript. All authors read and approved the final manuscript.

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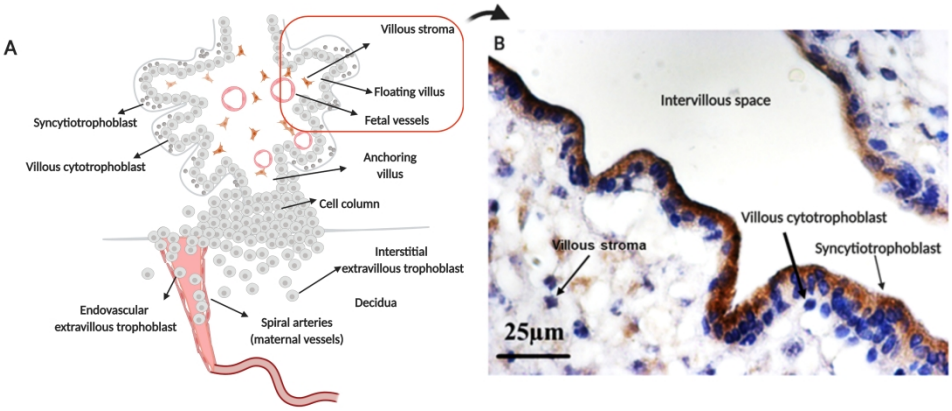

Figure 1. Schematic showing villous structure, trophoblast lineages and ERRγ localization in the human placenta.

386x160mm (300 x 300 DPI)

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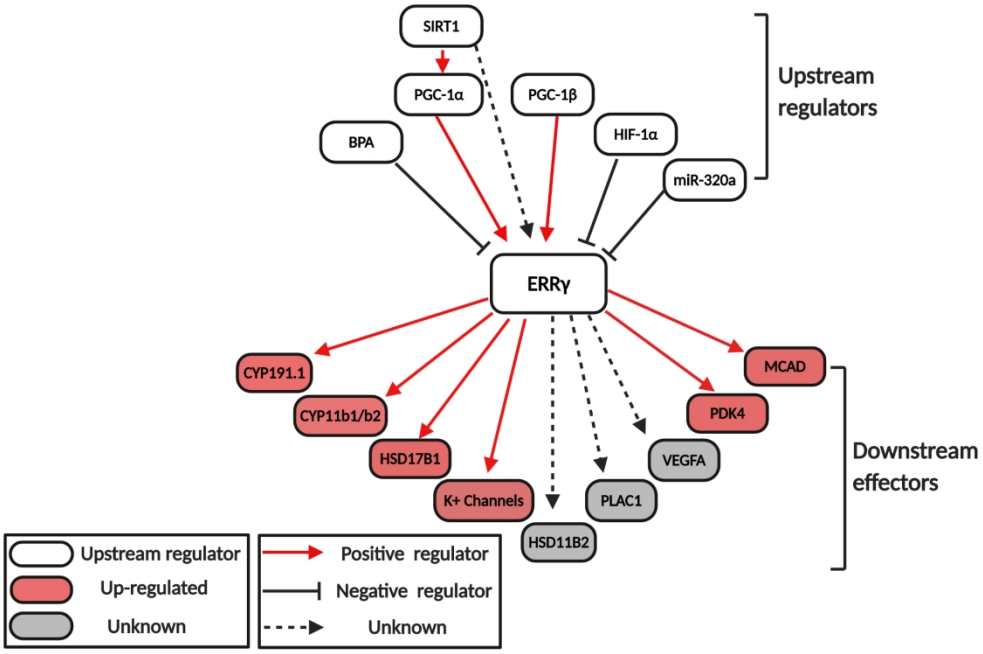

Figure 2. Diagrammatic representation of known upstream regulators and downstream effectors of ERRγ in the placenta.

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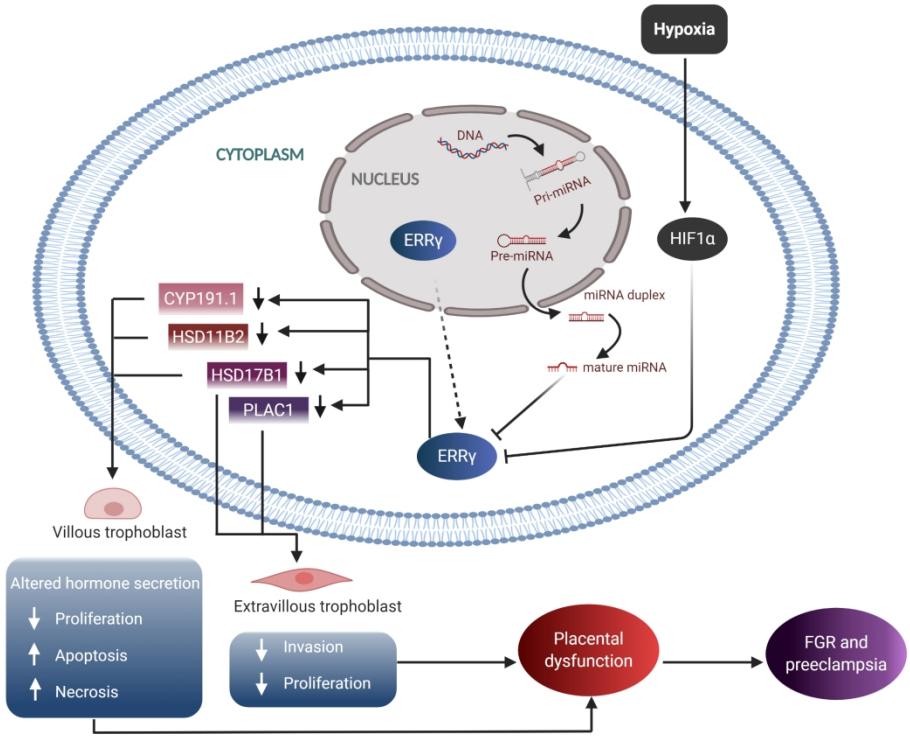

Figure 3. Proposed mechanism by which placental ERRγ expression and function is altered in pregnancies complicated by FGR and preeclampsia.

218x169mm (300 x 300 DPI)

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Figure 1. **Schematic showing villous structure, trophoblast lineages and *ERRγ* localization in the human placenta.** Figure 1A: Extravillous trophoblast that are situated at the end of the cell column invade the decidua and remodel the maternal spiral arterioles to produce dilated and compliant uterine vessels. Villous cytotrophoblast differentiates and fuses to form the outer multinucleated syncytiotrophoblast which transports nutrients and gases from the maternal to fetal circulation. Figure 1B light micrograph showing immunostaining of *ERRγ* in first trimester placental explants. *ERRγ* is mainly observed in the cytoplasm of the syncytiotrophoblast and cytotrophoblast. Arrows indicate villous cytotrophoblast, syncytiotrophoblast and villous stroma. Scale bar represents 25μm.

Figure 2. **Diagrammatic representation of known upstream regulators and downstream effectors of *ERRγ* in the placenta.** Expression of the depicted genes is known to be altered in the FGR or preeclampsia placenta, where a red arrow indicates a positive effect and a black line indicates a negative effect. Meanwhile, a black dot arrow suggests an unclear relationship between *ERRγ* and downstream effectors. *CYP11b1*: cytochrome P450 family 11 subfamily B member 1; *HSD11B2*: hydroxysteroid 11-beta dehydrogenase 2; *HSD17B1*: hydroxysteroid 17-beta dehydrogenase 1; *PLAC1*: placenta-specific 1; *VEGFA*: vascular endothelial growth factor A; *PDK4*: pyruvate dehydrogenase kinase 4; *MCAD*: medium-chain acyl-CoA dehydrogenase; BPA: bisphenol A; *PCG-1α/β*: peroxisome proliferator-activated receptor-γ coactivator-1 alpha/beta; *HIF-1α*: Hypoxia-inducible factor 1-alpha.

Figure 3. Proposed mechanism by which placental *ERRγ* expression and function is altered in pregnancies complicated by FGR and preeclampsia. The hypoxic environment of the maternofetal interface in FGR results in upregulation and activation of *HIF1α* in the placenta, resulting in inhibition of *ERRγ* expression. The expression of several miRNAs is upregulated in FGR and we propose that amongst these are key *ERRγ* regulatory miRNAs. Binding of these miRNAs to the 3'UTR of *ERRγ* results in mRNA degradation and inhibition of *ERRγ* protein translation. This leads to reduced

expression of genes downstream of *ERRγ*, including *CYP19*, *HSD17B1*, *HSD11B2* and *PLAC1*. These downstream genes play an important role in placental hormone production and regulating different aspects of cytotrophoblast function, including villous cytotrophoblast proliferation and extravillous trophoblast invasion.

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Table 1. Relationship of *ERRγ* to upstream regulators and downstream effectors demonstrated in studies of placenta.

	Tissue/cell type/subject	Reference	Study Methods	Main finding	Influence on trophoblast function	Definition of FGR / preeclampsia (where applicable)
Downstream						
hCYP19	Mid-trimester primary cytotrophoblasts	Kumar & Mendelson (2011)	RT-PCR; WB; ChIP;	*ERRγ* is an oxygen-dependent transcription factor and mediates *hCYP19* expression in trophoblast differentiation.	Differentiation	
Cyp11b1	Late-onset PE placenta; Mouse model	Luo *et al*. (2014)	RT-PCR; IHC; WB; ChIP; LRA	*ERRγ* is increased in placenta in PE and can influence the blood pressure in pregnant mice by targeting *Cyp11b1*.	Reduced production of aldosterone	PE defined as maternal blood pressure (≥140/90 mmHg) and proteinuria (proteinuria ≥300mg per 24 hours or ≥1+ protein by dipstick from 2 random urine specimens or ≥2+protein by 1 dipstick) after 20 weeks of gestation.
Potassium (K^+) channels	Mid-trimester primary trophoblast cells; Term FGR placenta	Luo *et al.* (2013); Corcoran *et al*. (2008)	RT-PCR; WB; ChIP; LRA; Whole-genome gene expression arrays	Hypoxia inhibits the expression of *ERRγ* and K^+ channels; *ERRγ* can regulate K^+ channels that may be	Differentiation	FGR defined as the individualized birth weight ratio (IBR) ≤ 5th centile for gestational age.

					associated with PE and identified three *ERRγ* potential effectors, including *HSD11B2*, *HSD17B1*, and *PLAC1*; Oxygen-sensitive K^+ channel gene $K_V9.3$ was increased in FGR placenta and $K_V2.1$ was increased in FGR placental vein		
	HSD11B2	Cytotrophoblast from mid-trimester placenta; Early-onset FGR placenta (n=15) and later-onset FGR placentas (n=4)	McTernan *et al*. (2001); Luo *et al*. (2013)	RT-PCR	*HSD11B2* mRNA is decreased in *ERRγ* knockdown mid-trimester cytotrophoblasts. *HSD11B2* is decreased in both early-onset and later-onset FGR placentas, when compared with gestational matched normal placentas.	Not reported	FGR are diagnosed with at least three of four following ultrasound features: 1) fetal abdominal circumference ≤ 3rd centile for weeks of gestation, 2) abnormal fetal growth velocity 3) severe oligohydramnios (amniotic fluid index ≤3rd percentile for gestational age), 4) absent or reversed velocities in umbilical artery Doppler waveforms.

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	PLAC1	Placental villi of the human first trimester and term placenta	Chang *et al*. (2016)	RT-PCR; IHC; WB	*PLAC1* is increased during the trophoblast differentiation and low expression inhibits the cell fusion.	Differentiation	FGR were diagnosed as a fetus with reduced growth velocity, which is less than 10th centile after 20 gestational weeks.
		Later-onset FGR placenta	Sifakis *et al*. (2018)	RT-PCR	*PLAC1* is increased in FGR placenta.	Not reported	
	HSD17B1	Late-onset FGR placenta; HTR-8 cell lines	Zhu *et al*. (2018)	RT-PCR; IHC; WB; LRA	*ERRγ* can regulate *HSD17B1* that is associated with FGR	Decrease invasion; proliferation	FGR defined as estimated fetal weight is less than 10th centile.
	VEGFA	Mouse model	Luo *et al*. (2014)	RT-PCR	The expression of *VEGFA* is decreased in *ERRγ* deficient mice placenta	No detection reported	
	PDK4	Primary trophoblast Bewo cell line	Poidatz *et al*. (2012)	RT-PCR, IHC	The expression of *ERRγ* is increased during trophoblast differentiation; *PDK4* is decreased after inhibiting *ERRγ* expression.	Differentiation	
	MCAD	First trimester human primary cytotrophoblast; Bewo cell line	Poidatz *et al*. (2012)	RT-PCR, IHC	*MCAD* is decreased after inhibiting *ERRγ* expression.	Differentiation	

Upstream							
	BPA	Placentas from low birth weight infant; 587 children	Troisi *et al*. (2014); Miao *et al*. (2011)	GC-MS analysis, Interview	Negative relationship between BPA and fetal weight; *ERRγ* is a receptor of BPA in the placenta.	Not reported	Low birth weight defined as the infant weight less than 2500g at birth;
	PGC-1α	FGR and PE placenta; Late-onset FGR placenta	Poidatz *et al*. (2015)	IHC, RT-PCR, Quantification Mitochondrial DNA	In FGR and PE placenta, the expression of *ERRγ*, *PGC-1α* and *SIRT1* is decreased.	No detection reported	FGR defined as a birth weight less than 10th centile. PE was diagnosed as an elevated maternal blood pressure (systolic and diastolic blood pressure ≥140/90mmHg) and proteinuria (≥ 300mg per 24 hours) after 20 weeks of gestation.
	SIRT1	FGR and PE placenta; Late-onset FGR placenta	Poidatz *et al*. (2015)	IHC, RT-PCR, Quantification Mitochondrial DNA	In FGR and PE placenta, the expression of *ERRγ*, *PGC-1α* and *SIRT1* is decreased.	No detection	FGR defined as a birth weight less than 10th centile. The definition of PE has been mentioned in the part of *PCG-1α*.
	MiR-320a	Late-onset PE placenta; HTR-	Gao *et al*. (2018)	RT-PCR; IHC; WB; LRA	MiR-320a regulates *ERRγ* in PE	Decreased invasion;	PE defined as increased diastolic and systolic maternal

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		8/SVneo				No change in proliferation and migration	blood pressure with proteinuria.
		HTR-8/SVneo; HUVECs	Liu *et al*. (2018)	RT-PCR; WB; LRA	MiR-320a directly target *ERRγ* and may indirectly control the expression of *VEGFA* to influence the function of both trophoblast and vein endothelial cells.	Decreased invasion, proliferation and migration; Increase in apoptosis	
	HIF-1α	Mid-trimester primary trophoblast cells	Kumar & Mendelson (2011)	RT-PCR; WB; ChIP assay	*HIF-1α* can mediate *ERRγ* expression in trophoblast differentiation	Differentiation	

ERRγ, estrogen related receptor γ; *hCYP19*, cytochrome P-450; RT-PCR, reverse transcription polymerase chain reaction; WB, western blot; CHIP, chromatin immunoprecipitation; FGR, fetal growth restriction; *CYP11b1*, cytochrome P450 family 11 subfamily B member 1; IHC, immunochemistry; LRA, luciferase reporter assay; PE, preeclampsia; *HSD11B2*, hydroxysteroid 11-beta dehydrogenase 2; *HSD17B1*, hydroxysteroid 17-beta dehydrogenase 1; *PLAC1*, placenta-specific 1; *VEGFA*, vascular endothelial growth factor A; *PDK4*, pyruvate dehydrogenase kinase 4; SGA, small for gestational age; *MCAD*, medium-chain acyl-CoA dehydrogenase; BPA, bisphenol A; GC-MS, gas chromatography-mass spectrometry; *PCG-1α*, peroxisome proliferator-activated receptor-γ coactivator-1 alpha; HUVECs, human umbilical vein endothelial cells; *HIF-1α*, Hypoxia-inducible factor 1-alpha.