Altered placental expression of kisspeptin and its receptor in pre-eclampsia

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Abstract

Kisspeptin, originally identified as metastatin, important in preventing cancer metastasis, has more recently been shown to be important in pregnancy. Roles indicated for kisspeptin in pregnancy include regulating trophoblast invasion and migration during placentation. The pregnancy-specific disorder pre-eclampsia (PE) is now accepted to begin with inadequate trophoblast invasion and the current study therefore sets out to characterise placental expression of both kisspeptin (KISS1) and its receptor (KISS1R) throughout pregnancy and in PE. Placental tissue was obtained from women undergoing elective surgical termination of early pregnancy ($n=10$) and from women following Caesarean section at term in normal pregnancy ($n=10$) and with PE ($n=10$). Immunohistochemistry of paraffin embedded sections and western immunoblotting were performed to

assess protein localisation and expression. Quantitative real-time PCR was carried out to evaluate mRNA expression of both *KISS1* and *KISS1R*. Protein and mRNA expression was found to mirror each other with *KISS1* expression found to be reduced in PE compared with that in normal term pregnancy. Interestingly, *KISS1R* expression at both the mRNA and protein levels was found to be increased in PE compared with that in normal term pregnancy. The current findings of increased *KISS1R* expression may represent a mechanism by which functional activity of *KISS1* is higher in PE than in normal pregnancy. Higher levels of activity of *KISS1R* may be involved in inhibition of trophoblast invasion and angiogenesis, which are associated with PE.

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Introduction

The primary translation product of *KISS1* is a 145 amino acid polypeptide known as Kp-145. Kisspeptins (KISS1), which are biologically active, containing 54 (Kp-54), 14 (Kp-14), 13 (Kp-13) and ten (Kp-10) amino acids, are then produced by post-translational cleavage of Kp-154. All kisspeptins share an Arg-Phe-NH₂ motif at the C-terminus, which is required for biological activity (Roa *et al.* 2008). The activity of *KISS1* is regulated via the expression of the KISS1 receptor (KISS1R), an orphan G-protein-coupled receptor GPR54 (Ohtaki *et al.* 2001).

The *KISS1* gene was originally identified as a human metastasis suppressor gene; however, additional roles have now been revealed including a fundamental role in reproductive function. Kisspeptins are known to be involved in the onset of puberty due to their role in stimulating hypothalamic GnRH release, being described as the gate-keeper of reproductive function (Seminara *et al.* 2003). Mutations in the *KISS1* gene have been shown to be associated with the onset of precocious puberty and hypogonadotropism (Luan *et al.* 2007), whereas inactivating

mutations in *KISS1R* result in failure to progress through puberty (de Roux *et al.* 2003). In addition to their central control of the gonadotrophic axis, *KISS1* has been found to be important in placentation, pregnancy and cardiovascular function (Bilban *et al.* 2004, Hiden *et al.* 2007, Mead *et al.* 2007b). The physiological importance of *KISS1* is further verified by the observations that KISS1R is expressed in a variety of human tissues including hypothalamus, placenta, aorta, coronary artery and umbilical vein (Janneau *et al.* 2002, Mead *et al.* 2007a).

Of particular importance to placentation is the involvement of KISS1 in cell migration, a process which is of crucial importance for trophoblast invasion. KISS1 has been shown to induce focal adhesion and stress fibre formation and also to phosphorylate focal adhesion kinase and paxillin, the intracellular signals needed for cell migration, which may associate with integrins to inhibit migration (Ohtaki *et al.* 2001). Furthermore, KISS1 has also been shown to diminish matrix metalloproteinase 9 (MMP9) expression by reduced NFκB (NFKB1) binding of the MMP9 promoter, thereby resulting in a reduction of cell migration (Yan *et al.* 2001). It is specifically Kp-10, produced by first trimester

trophoblast cells, that has been shown to inhibit migration (Bilban *et al.* 2004).

An important role for KISS1 in pregnancy has also been suggested due to the dramatic increase in circulating KISS1 levels in pregnancy (Horikoshi *et al.* 2003). Furthermore, a placental origin for the peptide has been suggested due to the observation that circulating KISS1 levels fall 5 days post-delivery compared with non-pregnant concentrations. However, the exact function for such an elevation within the gonadotrophin axis remains to be determined as LH levels are not elevated in pregnancy as would be predicted by the observed potent stimulation of LH following exogenous administration of KISS1 (Dhillon *et al.* 2006).

KISS1 is present at the feto-maternal interface with abundant expression in the syncytiotrophoblast cells (Bilban *et al.* 2004). KISS1R expression has been localised to both the villous and invasive extravillous cytotrophoblast cells (EVT; Bilban *et al.* 2004). Interestingly mRNA and protein expressions of both KISS1 and KISS1R have been shown to be higher in first trimester placental trophoblast cells than in term gestation, contrasting with the increasing circulating KISS1 levels during pregnancy (Horikoshi *et al.* 2003, Bilban *et al.* 2004). This finding of the highest levels of expression of KISS1 and KISS1R in early pregnancy coincides with the time of maximal extravillous cytotrophoblast invasion and has therefore been suggested to represent a crucial control mechanism regulating placental development (Bilban *et al.* 2004). It is therefore suggested that altered placental expression of KISS1 and/or KISS1R may be associated with poor placentation and the associated disorder of pre-eclampsia (PE). Further support for this comes from the observation of lower circulating KISS1 concentrations in early second trimester serum samples from women who subsequently develop PE and intra-uterine growth restriction (Armstrong *et al.* 2009).

The objective of the current study was to investigate the expression of KISS1 and KISS1R in PE. The hypothesis to be tested was that the increased expression of KISS1 and/or KISS1R will be associated with PE.

Materials and Methods

After local ethical committee approval and with appropriate informed consent, placental tissue was obtained from women undergoing elective surgical termination of pregnancy (TOP) during early pregnancy (*n*=10; gestational age (obtained from last menstrual period) 8.8±0.9 weeks (mean±s.d.)) and at delivery in the third trimester from ten women with normal term pregnancy (gestational age 39.4±1.1 weeks) and ten women with PE (gestational age 37.6±2.6 weeks). Term samples from both with normal pregnancy and PE were obtained from women undergoing elective Caesarean section prior to labour. Additional early pregnancy placental samples were collected at St George's University of London (London, UK) with ethical approval from Wandsworth

Local Ethics Committee. Doppler ultrasound screening can characterise pregnancies into distinct groups, reflecting a proxy measure of the extent to which remodelling of the spiral arteries has occurred (Prefumo *et al.* 2004, Whitley *et al.* 2007). Maternal uterine artery Doppler velocimetry scans were taken in the Fetal Medicine Unit, St George's Hospital from women attending the clinic for elective TOP as previously described (Prefumo *et al.* 2004). High-resistance cases were defined as those with bilateral uterine diastolic notches and a mean resistance index (RI) >95th percentile. Normal resistance cases had no diastolic notches and a mean RI <95th percentile. These resistance groups represent cases most (high RI; *n*=6; gestational age 11.2±0.4 weeks) and least (normal RI; *n*=6; gestational age 11.1±0.3 weeks) likely to have developed PE had the pregnancy progressed (Melchiorre *et al.* 2008, Poon *et al.* 2009).

All women with PE met the International Society for the Study of Hypertension in Pregnancy definition (Brown *et al.* 2001). Clinical data for normal term pregnancy and PE subjects are shown in Table 1. Biopsies for immunohistochemistry were fixed in formalin, embedded in wax, and 5 µm serial sections were prepared. Biopsies of placental tissue, collected for RNA extraction, were snap frozen and stored at -80 °C.

Immunohistochemistry

Single immunohistochemical labelling was carried out using the Dako REAL EnVision detection system (Dako UK Ltd, Cambridgeshire, UK). The sections were first deparaffinised by immersion in xylene followed by rehydration by passing through descending concentrations of alcohol (100–70%) and finally immersion in running tap water. Antigen retrieval was performed by microwave heating these sections for 10 min in trisodium citrate buffer (10 mM sodium citrate, pH 6.0). Peroxidase block solution (Dako) was applied to sections for 5 min to block endogenous peroxidase activity. To reduce non-specific background staining, Ultra V Block

Table 1 Clinical details of term pregnancy subject groups. Data are expressed as mean (s.d.)

	Normal term pregnancy	Pre-eclampsia	P value
Number of subjects	10	10	NA
Maternal age (years)	28 (7.6)	32 (9.6)	NS
Maternal BMI (kg/m ²)	23.2 (3.3)	24.3 (4.4)	NS
Gestational age (weeks)	38.3 (1.8)	36.2 (2.1)	<0.05
Baby weight (kg)	3.6 (0.9)	3.5 (1.08)	NS
Systolic blood pressure (mmHg)	111 (18.6)	152 (10.5)	<0.000
Diastolic blood pressure (mmHg)	81 (15.7)	110 (15.5)	<0.000
Proteinuria (g/l)	NA	1.9 (1.9)	NA
Platelets (×10 ⁹ /l)	NA	185 (35.9)	NA
Creatinine (µmol/l)	NA	66 (7.0)	NA

(Thermo Scientific, Cheshire, UK) was applied to the sections for 5 min. Slides were then incubated for 1 h with appropriate mouse monoclonal antibody for either KISS1 (AbD Serotec, Oxford, UK; MCA3219Z; 1:50) or KISS1R (Novus Biologicals, Cambridge, UK; NLS1926; mouse monoclonal; 1:100). Specificity of staining was confirmed using positive control tissue. Negative controls were also performed in each staining run using no primary antibody.

Analysis of immunohistochemical labelling

All slides were analysed by the same observer (P J W) who was blinded to pregnancy stage or outcome. For analysis of placental sections, digital images of five randomly selected medium-power ($\times 200$) fields were captured on NIS-Elements F2.20 (Nikon UK Ltd, Surrey, UK). As described previously, the total percentage of positive labelled cells per $\times 200$ magnification field was determined using the 'positive pixel count' function with ImageScope software (Aperio Technologies Ltd, Bristol, UK; Williams *et al.* 2010). Results are expressed as 'positivity' that takes into account both the number of positive pixels and the intensity of staining. A visual check was performed to ensure accurate discrimination of immunolabelled regions.

RNA extraction

Total RNA from placental biopsies (50–100 mg) was obtained using 1 ml of Tri-reagent (Sigma) according to the manufacturer's instructions. RNA integrity was determined using an Agilent Bioanalyzer (Santa Clara, CA, USA) and all samples had an RNA integrity number > 8.0 . RNA purity and concentration were verified using a Nanodrop spectrophotometer (Thermo Scientific); all RNA samples had an A_{260}/A_{280} ratio of 1.85 or greater.

Following RNA extraction, 1 μg of each sample was reverse transcribed to cDNA in a 20 μl reaction using QuantiTect Reverse transcription kit (Qiagen). This kit contains a mix of random and oligo-dT primers thereby ensuring that cDNA is generated from all regions of RNA transcripts. The protocol also includes a genomic DNA elimination step prior to reverse transcription. Reverse transcription was performed using a Veriti 96-well thermal cycler (Applied Biosystems, Warrington, UK). The conditions used to generate first strand cDNA were 42 °C (15 min) and 95 °C (3 min). cDNA was diluted with

nuclease-free water (Applied Biosystems) to a standard concentration of 1 $\mu\text{g}/\mu\text{l}$ and stored at -80 °C until use.

Preparation of standards for quantitative real-time PCR

Standards for *KISS1*, *KISS1R* and for the reference genes tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (*YWHAZ*), hypoxanthine phosphoribosyltransferase 1 (*HPRT*) and succinate dehydrogenase subunit A (*SDHA*) were made from cDNA obtained from a randomly selected control placenta using semi-quantitative PCR. QuantiTect Primer assays (Qiagen) were used to detect *KISS1* (Hs_KISS1_1_SG; QT00016044; melting temperature 60 °C) and *KISS1R* (Hs_KISS1R_1_SG; QT00043134; melting temperature 60 °C); sequence information for the primers in these kits is not available. The reference genes *YWHAZ*, *HPRT* and *SDHA* were assessed using oligonucleotide primers generating specific intron-spanning products (Table 2).

The PCR programme comprised an initial denaturation stage (95 °C, 15 min), amplification (stage I, 94 °C (30 s); stage II, melting temperature (30 s); stage III, 72 °C (1 min)) and final extension (72 °C, 7 min; 8 °C 'hold'). Each PCR (final volume 50 μl) contained 23.5 μl nuclease-free water (Ambion, Grand Island, NY, USA); 25 μl 2 $\times$ ReddyMix PCR master mix containing 2.0 mM MgCl_2 and 0.2 mM of each dATP, dCTP, dGTP and dTTP (ThermoScientific, Abgene, Surrey, UK); 0.5 μM forward and reverse primers; and 1 μl cDNA. The annealing temperature and cycle number of both primers were optimised and used in their linear range. Agarose gel electrophoresis (2.0–2.5%) and ethidium bromide staining confirmed the presence of products at the expected sizes. The resulting PCR product was extracted (QIAquick gel extraction kit, Qiagen), sequenced, and results were cross-referenced with NCBI Nucleotide Blast to determine specificity of the target gene. After confirmation that the product was specific to the target gene, extracted PCR products were resuspended in nuclease-free water to allow preparation of a standard curve ranging from 10^7 to 10^1 copies/ μl . Standards were stored at -80 °C until its use in quantitative real-time PCR (qRT-PCR).

qRT-PCR

qRT-PCR was used to quantify the expressions of *KISS1* and *KISS1R* relative to the reference genes *YWHAZ*, *HPRT* and *SDHA*. PCRs, set up in duplicate, were carried out in

Table 2 Primer sequences for reference genes used in quantitative RT-PCR

Gene name	Forward sequence 5'–3'	Reverse sequence 5'–3'	Melting temperature (°C)	Accession number
<i>YWHAZ</i>	CCTGCATGAAGTCTGTAAGTCTG	TTGAGACGACCCTCCAAGATG	60	NM_003406
<i>HPRT</i>	TGACACTGGCAAACAATGCA	GGTCCTTTTCACGACGAAGCT	55	NM_000194.2
<i>SDHA</i>	TGGGAACAAGAGGGCATCTG	CCACCACTGCATCAAATTCATG	55	NM_004168.2

25 µl volumes consisting of 1 µl of template cDNA, 12.5 µl of 2× QuantiFast SYBR Green PCR (Qiagen), 0.5 µM forward primer, 0.5 µM reverse primer and 9 µl nuclease-free water. Real-time PCR was performed on all samples using a Stratagene Mx3000P using a fast cycling programme consisting of an initial PCR activation step of 5 min at 95 °C followed by 40 cycles of denaturation at 95 °C for 10 s and combined annealing and extension step of 60 °C for 30 s. Melt curve analysis was performed at 95–55 °C. A no-template control and reverse transcription negative control sample were included in all assays.

PCR efficiencies were determined from the standard curves and were >97% for all genes. All standard curves were linear ($r^2 > 0.995$) from 10^7 to 10^1 copies. For each gene, the sample threshold cycle (C_t) and hence copy number/µl cDNA was determined from the standard curve using the MxPro software (Agilent). Normalisation of data was performed using geNorm software that calculated the normalisation factor of the reference genes for each sample (Vandesompele *et al.* 2002), data are expressed in arbitrary units.

Western immunoblotting

Normal term pregnancy ($n=10$), PE ($n=10$) placental tissues or high-RI ($n=6$) and normal-RI ($n=6$) early placental samples were homogenised in lysis buffer (CellLytic MT, Sigma–Aldrich) containing a protease inhibitor cocktail as per manufacturer instructions (Sigma–Aldrich). The protein concentration of the protein-containing supernatant was determined using a bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA). Each protein sample (30 µg) was used for western immunoblotting. Proteins were first separated by SDS–PAGE and then transferred to polyvinylidene fluoride membranes (Immobilon P, Millipore, Watford, UK). Membranes were blocked overnight in 10% Marvel in 0.2% Tween Tris-buffered saline at 4 °C. The following morning, membranes were washed and then incubated for 1 h in rabbit polyclonal anti-kisspeptin (Abcam, Cambridge, UK; ab80994; 10 µg/ml), anti-KISS1R (Abcam; ab100896; 1 µg/ml) or anti-β actin antibody (Abcam; ab8227; 1/2000), which was used as a loading control. Membranes were washed before 30 min incubation in HRP conjugated goat anti-rabbit IgG secondary antibody (Abcam; ab97080; 1:5000). Immunoreactivity was detected using AceGlow Ultra sensitive chemiluminescence substrate (PierceLab, Salisbury Green, UK) according to the manufacturer's instructions. Membranes were visualised using Quantity One 4.2.1 image analysis software (Bio–Rad). Data were corrected for background and then normalised to β-actin expression.

Statistical analysis

All analyses were performed using SPSS for Windows version 17.0 (Chicago, IL, USA). The Kolmogorov–Smirnov test was

used to assess the distribution of data. All data had a normal distribution and therefore between-group comparisons were made using a Student's *t*-test. The standard value of $P \leq 0.05$ was considered significant. Unless otherwise stated all data are expressed as mean $\pm$ S.E.M.

Results

Immunohistochemistry

KISS1- and KISS1R-positive immunolabellings were present in all samples examined. KISS1 protein expression was significantly reduced in PE placental samples (1.21 ± 0.20) compared with normal term pregnancy (3.72 ± 0.276 ; $P < 0.001$). There was also a significant reduction in KISS1 expression between early pregnancy (6.18 ± 0.384) and term normal pregnancy placenta ($P < 0.001$; Fig. 1). Placental KISS1R expression was found to be higher in PE (3.01 ± 0.138) than in normal term pregnancy (1.73 ± 0.125 ; $P < 0.001$). Protein expression of KISS1R was also found to be lower in term pregnancy placental samples than in early pregnancy samples (4.04 ± 0.199 ; $P < 0.001$; Fig. 1).

Figure 1 (a) Photomicrographs to show placental expression of KISS1 and KISS1R in early pregnancy, normal term pregnancy and PE. Staining was predominantly localised to syncytiotrophoblast cells (indicated with open headed arrow) with a small amount of staining present in villous cytotrophoblast cells (indicated with diamond headed arrow). Positive labelled cells appear brown; magnification $\times 200$. (b) Graph of immunohistochemical labelling for KISS1 and KISS1R. Data are expressed as mean $\pm$ S.E.M. positivity.

Western immunoblotting

Protein expression as determined by western immunoblotting also identified an increased expression of KISS1 in term normal pregnancy placenta (20.3 ± 3.31) compared with PE placental samples (4.9 ± 0.94 ; $P < 0.001$; Fig. 2). Placental KISS1R expression was increased in PE (114 ± 3.7) and term normal pregnancy (24.3 ± 2.81 ; $P < 0.001$; Fig. 2). Additionally, early pregnancy samples identified that women with high uterine artery RI (less spiral artery remodelling and a higher risk of developing PE) expressed lower levels of KISS1 protein (39 ± 4.4) than did women with a normal uterine artery RI and a lower risk of developing PE (177.7 ± 6.8 ; $P < 0.001$; Fig. 3). KISS1R expression was also found to be higher in high-RI placental samples (180.7 ± 6.7) than in normal-RI placental samples (84 ± 5.1 ; $P < 0.001$; Fig. 3).

mRNA

mRNA expressions for both *KISS1* and *KISS1R* as assayed by qRT-PCR displayed similar expression patterns to that seen for protein expression (Fig. 4). mRNA expressions of the three reference genes *YWHAZ*, *HPRT* and *SDHA* did not vary between groups. The highest levels of expressions for both *KISS1* (2.49 ± 0.144) and *KISS1R* (2.33 ± 0.064) were found in early pregnancy placenta compared with normal term pregnancy ($P < 0.001$; Fig. 4). *KISS1* expression was reduced in PE placental samples (0.286 ± 0.08) compared

Figure 2 Graph to show protein expression levels determined by western immunoblotting in term normal pregnancy (NP) and PE. Photomicrographs show representative samples for each antibody. Data are shown as mean $\pm$ S.E.M. relative expression normalised to β -actin.

Figure 3 Graph and photomicrographs to show protein expression levels determined by western immunoblotting in early pregnancy placental samples from women with high (high-RI) and normal RI (normal-RI). RI was used as a proxy measure of the extent of uterine vessel remodelling, which is impaired in PE.

with term normal pregnancy (0.95 ± 0.17 ; $P = 0.003$; Fig. 3). However, expression of *KISS1R* mRNA was significantly higher in PE (1.57 ± 0.19) than in term normal pregnancy (0.80 ± 0.13 ; $P = 0.003$).

Discussion

This study demonstrates reduced expression of KISS1 and increased expression of KISS1R in PE compared with control term pregnancy at both the protein and mRNA levels. Additionally, this study has confirmed previous observations of highest levels of expression of both KISS1 and KISS1R in early pregnancy. The results of this study indicate that reduced expression of KISS1 and increased expression of KISS1R may be involved in the pathogenesis of PE due to alterations in expression at both mRNA and protein levels compared with normal pregnancy. It is interesting to note that in early pregnancies screened by uterine artery Doppler ultrasound as a proxy measure of the extent of uterine vessel remodelling (which is impaired in PE), there was also reduced KISS1 and increased KISS1R expressions in those with a high RI compared with those with normal RI, suggesting that these differences are apparent early on and at a stage at which the pathology of PE is becoming established.

The present finding of a reduction in placental KISS1 expression is in agreement with the reports of lower circulating levels in the first and second trimester of pregnancy in women who deliver small for gestational age neonates and who subsequently developed PE and/or intra-uterine growth restriction and with the suggestion that KISS1 represents a suitable biomarker for PE (Smets *et al.* 2008, Armstrong *et al.* 2009, Logie *et al.* 2011). KISS1 was originally

Figure 4 Graph to show mRNA expression of *KISS1* and *KISS1R* as determined by qRT-PCR. Data are expressed as mean $\pm$ S.E.M. arbitrary units calculated following normalisation of copy number using normalisation factor obtained from the three housekeeping genes *YWHAZ*, *HPRT* and *SDHA*.

identified due to its anti-metastatic properties and within full term placenta and in the trophoblast derived cell line HTR8/Svneo Kp-10 has been shown to inhibit cell migration (Bilban *et al.* 2004, Roseweir *et al.* 2012). However, this finding has yet to be reproduced in early pregnancy placental samples. Furthermore, the current finding is in agreement with the recent report that has shown decreased expression of *KISS1* in women with recurrent early pregnancy loss (Park *et al.* 2012), again indicating an important role for *KISS1* in establishment of the placenta. The finding of reduced levels of *KISS1* in PE may appear to contradict our understanding of the pathophysiology of PE, which is accepted to begin with inadequate trophoblast invasion. However, it has been suggested that lower levels of *KISS1* within the placenta in pathological pregnancies may signal low invasive capacity, where reduced invasive capacity would be expected (Smets *et al.* 2008). Smets *et al.* describe how expression of kisspeptin is highest during the period of maximal trophoblast invasion and suggest that high invasive capacity is counteracted by the inhibitory effect of high levels of kisspeptin, and a low expression of kisspeptin signals low invasive capacity. Alternatively, smaller less invasive placentae may develop in PE, which have a reduced capacity to produce *KISS1*.

A further possible explanation for the decreased expression of *KISS1* in the pathophysiology of PE may relate to the simultaneous increase in *KISS1R*, which may serve to maximise the activity of *KISS1* and thereby inhibit trophoblast migration, which is crucial to the successful development of the placenta. In this manner the reduced

expressions of both mRNA and protein of *KISS1*, which in itself would lead to a reduction in inhibition of cell migration, are negated via the higher levels of expression of *KISS1R*, thereby achieving higher activity levels of *KISS1*, resulting in increased inhibition of cell migration. *KISS1* has also been shown to be angiostatic (Ramaesh *et al.* 2010) and therefore increased overall *KISS1* activity due to increased expression of *KISS1R* may result in inhibition of angiogenesis within human placental vessels leading to the development of PE.

A second explanation for the observed reduction in *KISS1* expression in placenta of women with PE may be that *KISS1* expression is reduced as a result of the reduced trophoblast invasion during the early stages of pregnancy that leads to reduced expression of *KISS1* as a mechanism to try to increase trophoblast invasion and placental development. A further possible explanation may be because the current study has examined *KISS1*, which will identify the smaller peptide products Kp-54, Kp-14, Kp-13 and Kp-10. To the best of the authors' knowledge, with the exception of Kp-10, which has been studied extensively (Bilban *et al.* 2004), the function of the other kisspeptin fragments in human placentation has not been described. Further functional studies are required to determine the exact functional effects of each of these biological active kisspeptins to examine if they all have the same function within the placenta.

Possible limitations of the present study are that the reduction in *KISS1* and *KISS1R* expressions in PE may represent a secondary change rather than being responsible for the primary pathogenesis of PE; however, the confirmation of higher levels of *KISS1R* and lower levels of *KISS1* in early placenta pregnancy samples indicates that these findings are likely to be important in the early placental changes associated with PE. Furthermore, the present sample size is relatively small ($n=10$) in each group and these findings need to be replicated in a much larger cohort to validate the present findings. Although mRNA and protein expressions mirrored each other, it is important to consider that altered expression does not necessarily correlate with altered function and therefore further functional studies are warranted. A further possible limitation of the study is the difference in gestational age at delivery between the term normal pregnancy and PE sample groups. The obstetric management for women with PE is delivery, which is frequently preterm, making it difficult to collect gestational age-matched samples of women who meet the ISSHP guidelines for diagnosis of PE at full term (Turner 2010).

This study shows altered expression of *KISS1* and its receptor *KISS1R* in PE suggesting a role in the pathophysiological changes that occur in this disorder.

Declaration of interest

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

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