Corin, an enzyme with a putative role in spiral artery remodeling, is up-regulated in late secretory endometrium and first trimester decidua

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STUDY QUESTION: What is the nature of cellular Corin expression in human gestational tissues?

SUMMARY ANSWER: CORIN is expressed in non-pregnant late secretory phase endometrium, first trimester human implantation sites and is up-regulated with decidualization ex vivo.

WHAT IS KNOWN ALREADY: Adequate trophoblast invasion and spiral artery remodeling/transformation is critical for successful implantation. CORIN, best known for its role in activating atrial natriuretic peptide (ANP) to regulate blood pressure, has recently been proposed to be centrally involved in trophoblast invasion and spiral artery remodeling. It is postulated that ANP, activated by CORIN, promotes trophoblast invasion and that a deficiency causes pre-eclampsia. Mice deficient in either Corin or ANP displayed poor trophoblast invasion, impaired spiral artery remodeling and phenocopied human pre-eclampsia. However, the precise cellular localization of CORIN within human gestational tissues has not been well characterized.

STUDY DESIGN, SIZE, DURATION: We measured CORIN protein localization in a number of human gestational tissues relevant to early embryo/placental implantation: non-pregnant (NP) endometrial biopsies (n = 5 per phase of the menstrual cycle), first trimester placental bed biopsies (n = 12) and pre-term control (n = 10) and severe early onset preeclamptic placentas (n = 15). Endometrial stromal cells were isolated from human endometrial biopsies (n = 5) and induced to decidualize ex vivo. Finally, CORIN concentrations were measured in serum obtained from pregnant women during the first trimester of whom, 56 subsequently ended up with a healthy term delivery (controls), 18 developed fetal growth restriction (FGR) and 21 had a miscarriage.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We performed immunohistochemistry to assess CORIN localization. Changes in Corin mRNA expression in human endometrial stromal cells decidualized ex vivo were measured by quantitative RT–PCR, and levels of CORIN within human sera were measured by ELISA.

MAIN RESULTS AND THE ROLE OF CHANCE: CORIN was expressed in both NP late secretory phase endometrium and first trimester decidua within placental bed biopsies. Importantly, decidualization of primary human endometrial cells ex vivo significantly increased Corin expression (P < 0.05). CORIN was also detected within the villous cytotrophoblast, but there was no change in mRNA levels in placentas complicated by severe preterm pre-eclampsia when compared with pre-term controls. Although CORIN was detected in first trimester serum, levels did not change across gestation, nor could they predict miscarriage or FGR (other disorders of impaired placental invasion).
Introduction

Human placentation and trophoblast invasion is particularly aggressive, with trophoblasts invading the inner third of the uterine myometrium during early placentation (Pijnenborg et al., 1980). During this time, specialized endovascular trophoblasts plug the mouths of the maternal spiral arteries, preventing blood flow into the inter villous space. They also actively remodel the spiral arteries themselves, replacing both the medial muscularis and elastica and transiently the endothelial lining, creating low resistance, high volume vessels. The remodeled vessels allow a large volume of maternal blood to flow across the placental surface, maximizing the potential for oxygen, waste disposal and nutrient exchange. This provides optimal conditions for healthy fetal development across pregnancy (Redman and Sargent, 2005; Moffett, 2006). In the event of shallow trophoblast invasion or inadequate remodeling of spiral arteries, insufficient implantation occurs that results in poor blood and nutrient supply to the developing fetus and ultimately may manifest in diseases of pregnancy such as pre-eclampsia, fetal growth restriction (FGR) and miscarriage.

Corin is best known for its role in activating atrial natriuretic peptide (ANP), a signaling peptide well known for its role in regulating blood pressure (Yan et al., 2000; Wu and Wu, 2003; Wu et al., 2009). Highly expressed in heart muscle, CORIN cleaves pro ANP to produce its active form ANP. CORIN has recently been proposed to have a completely unexpected role in trophoblast invasion and spiral artery remodeling. Furthermore, it has been postulated that it contributes to the early pathogenesis of pre-eclampsia (Cui et al., 2012). In an elegant set of studies, Cui et al. (2012) utilized pregnant mice with genetic deletions to demonstrate that Corin or ANP knockouts developed hypertension and proteinuria, clinical characteristics of pre-eclampsia. In an intriguing finding, histological and immunohistochemical analysis of implantation sites indicated that trophoblast invasion in Corin- and ANP-deficient animals were markedly impaired when compared with the wild type. Moreover, spiral arteries within knock-out animals were smaller and less abundant than wild-type animals suggesting a direct impact upon spiral artery remodeling. Importantly, they were also able to demonstrate the importance of local CORIN produced ANP, by utilizing Corin knockout transgenic animals that only produced cardiac CORIN. In this set of studies, they showed that animals that produced CORIN in cardiac tissue (but not elsewhere, notably the uterus) still exhibited poor trophoblast invasion and spiral artery remodeling. Finally, they demonstrated that active ANP stimulated both BeWo cell (trophoblast/choriocarcinoma cell line) and primary trophoblast invasion in vitro.

Although Cui et al. (2012) demonstrated decreased Corin expression in term preeclamptic uterine biopsies when compared with normal term uterine biopsies, Corin mRNA and protein expression in first trimester human implantation sites and first trimester human gestational tissues were generally not well characterized in that study. Specifically, the exact cell type expressing CORIN within the uterine biopsies in humans was not well defined and no examination was made of first trimester implantation sites. Given that it appeared to be up-regulated in pregnancy and had a role in spiral arteriole remodeling, we speculated that CORIN was likely to be up-regulated in the endometrium during the period when it may receive a potential conceptus and during the first trimester when the pregnancy is being established.

To confirm the potential role of CORIN in trophoblast invasion and spiral artery remodeling during human implantation, we set out to characterize CORIN protein expression in relevant human gestational tissues. Specifically, we measured CORIN in NP endometrium across the menstrual cycle and in first trimester human implantation. To further confirm a decidual source, we undertook a functional analysis, examining whether Corin was up-regulated with experimental decidualization of human endometrial stromal cell ex vivo. Finally, we measured Corin in placentas obtained from cases of severe pre-eclampsia and levels in the serum across the mid first trimester and early second trimester, during the time of active spiral artery remodeling.

Materials and Methods

Tissue collection—placental tissue

Women presenting to two tertiary women’s hospitals in Melbourne, Australia, between 2008 and 2009 gave informed written consent for placental tissue collection. Placenta was obtained from pre-term pregnancies not complicated by pre-eclampsia (n = 10) and those complicated by severe early-onset pre-eclampsia (n = 15) as previously described (Kaitu’u-Lino et al., 2012a, 2012b, 2012c; Kaitu’u-Lino et al., 2012a, 2012b, 2012c). Briefly, severe preeclamptics were diagnosed in accordance with ACOG guidelines and included the presence of hypertension >160 out of 110 on two occasions greater than 6 h apart, proteinuria >5 g/day, oliguria <500 mL/day, visual disturbance, pulmonary edema, right upper quadrant pain, abnormal liver function, thrombocytopenia or FGR (ACOG, 2002).
Early-onset pre-eclampsia was defined as requiring delivery <34 weeks based on maternal or fetal indications. Pre-term control placentas were selected from women presenting with pre-term rupture of membranes or in spontaneous pre-term labor without evidence of infection, hypertensive disease or maternal co-morbidities. All pre-term controls delivered babies with a birth weight >20th centile corrected for gestation. Patient characteristics are shown in Table 1. Human Ethics approval was obtained for this study from both the Southern Health Human Research Ethics Committee and the Mercy Health Human Research Ethics Committee.

Tissue collection—placental bed biopsies

Uterine tissues (placental bed with trophoblast) for this study were obtained from women (n = 12) undergoing elective termination of pregnancy at 6–18 weeks gestational age as previously described (Robson et al., 2002). Written informed consent was obtained from all patients at the time of tissue collection, and ethics approval was provided by the Newcastle and North Tyneside Joint Ethics committee.

Tissue collection—endometrial tissue

Endometrial biopsies were collected by dilatation and curettage from fertile women who were scheduled for tubal ligation or were undergoing testing for tubal patency. Tissues were assessed by a pathologist and had no obvious endometrial pathology. The women had no steroid treatment or other relevant medication for at least 2 months before the collection of tissue. Samples were collected from five women for each phase of the menstrual cycle. Written and informed consent was obtained from all women participating in the study, and the protocols were approved by the Southern Health Human Research Ethics Committee.

Endometrial stromal cell isolation and decidualization

Endometrial tissue biopsies taken at menstrual cycle days 8–21 were used for all in vitro studies. Human endometrial stromal cells (HESCs) were isolated from tissue by enzymatic digestion and filtration as described previously (Dimitriadis et al., 2002). This resulted in a 97% pure stromal cell culture. Cells were then induced to undergo decidualization by addition of 10^{-8} mol/l estradiol 17 (Sigma) plus 10^{-7} mol/l progesterone (P; P-0130, Sigma). Decidualization was confirmed by prolactin ELISA as previously described (Menkhorst et al., 2010).

Serum collection and assessment of CORIN by ELISA

Serum samples utilized for assessment of CORIN levels were obtained from a biobank of prospectively collected samples (Tong et al., 2008). All samples in every group were taken from women between 7 and 11 weeks of pregnancy. In the control group, 10 were taken at 7 weeks, 13 at 8 weeks, 14 at 9 weeks, 11 at 10 weeks and 8 at 11 weeks. The FGR cohort (n = 18) were taken on to deliver a baby at term that was less than the third centile at birth. The miscarriage cohort (n = 21) had a viable fetus (as indicated by fetal cardiac activity at ultrasound) at 7–11 weeks gestation, but subsequently suffered a miscarriage as previously described (Tong et al., 2008; Kaitu’u-Lino et al., 2011, 2012a, 2012b, 2012c; Kaitu’u-Lino et al., 2011). Patient characteristics are shown in Table 2. This study was approved by the Mercy Hospital for Women Ethics committee.

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**Table I** Patient characteristics of pre-term control and preeclamptic cohort.

<table>
<thead>
<tr>
<th></th>
<th>Pre-term control (n = 15)</th>
<th>Preeclamptic (n = 10)</th>
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<tbody>
<tr>
<td>Maternal age (years)</td>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.9 (21–43)</td>
<td>29.6 (19–39)</td>
</tr>
<tr>
<td>BMI* (kg/m²)</td>
<td>Mean (SEM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.2 (2.8)</td>
<td>27.2 (2.0)</td>
</tr>
<tr>
<td>Parity [% (n)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27 (4)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>1</td>
<td>53 (8)</td>
<td>40 (4)</td>
</tr>
<tr>
<td>≥2</td>
<td>20 (3)</td>
<td>30 (3)</td>
</tr>
<tr>
<td>Gestation at delivery (weeks)</td>
<td>Mean (SEM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.4 (0.96)</td>
<td>31.8 (1.02)</td>
</tr>
<tr>
<td>SBP at delivery (mmHg)</td>
<td>Mean (SEM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>118 (4.5)</td>
<td>146 (11.2)*</td>
</tr>
<tr>
<td>DBP at delivery (mmHg)</td>
<td>Mean (SEM)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 (3.9)</td>
<td>108 (3.3)**</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>Mean (SEM)</td>
<td></td>
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<tr>
<td></td>
<td>1770 (207)</td>
<td>1560 (221)</td>
</tr>
</tbody>
</table>

Clinical details of the two cohorts from whom placenta were obtained for analysis. The preeclampsia cohort all had severe pre-eclampsia necessitating pre-term delivery. Pre-term controls were delivered prematurely for indications other than pre-eclampsia.

SBP, systolic blood pressure; DBP, diastolic blood pressure.
*BMI data available for 7 out of 10 preeclampsics and 13 out of 15 preterm controls. **P < 0.05.

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**Table II** Patient characteristics of the cohorts from which serum was obtained in the first trimester of pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 56)</th>
<th>FGR (n = 18)</th>
<th>Miscarriage (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>30.6 (22–38)</td>
<td>31 (21–39)</td>
<td>30.9 (20–43)</td>
</tr>
<tr>
<td>Gravidity [% (n)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37.5 (21)</td>
<td>50 (9)</td>
<td>23.8 (5)</td>
</tr>
<tr>
<td>2</td>
<td>28.6 (15)</td>
<td>27.8 (5)</td>
<td>28.6 (6)</td>
</tr>
<tr>
<td>≥3</td>
<td>37.7 (20)</td>
<td>22.2 (4)</td>
<td>47.6 (10)</td>
</tr>
<tr>
<td>Parity [% (n)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60.7 (34)</td>
<td>77.8 (14)</td>
<td>57.1 (12)</td>
</tr>
<tr>
<td>1</td>
<td>32.1 (18)</td>
<td>11.1 (2)</td>
<td>23.8 (5)</td>
</tr>
<tr>
<td>≥2</td>
<td>7.2 (4)</td>
<td>11.1 (2)</td>
<td>19 (4)</td>
</tr>
<tr>
<td>Gestation at delivery (weeks)</td>
<td>Mean (SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.8 (0.11)</td>
<td>39.2 (0.33)</td>
<td>N/A</td>
</tr>
<tr>
<td>Birthweight (grams)</td>
<td>Mean (SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3510 (20)</td>
<td>2550 (68)*</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Control patients delivered a healthy baby at term, FGR patients delivered a baby at term whose birth weight was ≤ third centile. Miscarriage patients had a baby with fetal heartbeat at the time of sampling, but miscarried at less than 20 weeks gestation. **P < 0.0001.
Corin is expressed in first trimester decidua

Results

Patient characteristics

The clinical characteristics of the pre-eclampsia cohort and their controls are shown in Table 1, and the characteristics of the FGR patients, miscarriage patients and the controls from whom serum was collected are shown in Table 2. No significant differences between groups were identified besides those parameters related to diagnosis.

CORIN protein is expressed across the cycle in NP endometrium and present in late-secretory decidualized stromal cells and CD45 positive leukocytes

We first assessed CORIN protein production in NP endometrium across the menstrual cycle (Fig. 1). CORIN was absent in proliferative phase endometrium (Fig. 1A), with weak glandular staining observed in early secretory phase endometrium (Fig. 1B). In contrast, strong glandular epithelial staining was observed in both the mid- (Fig. 1C) and late-secretory (Fig. 1D) phase.

In the late secretory phase, CORIN expression was also noted in decidualized stromal cells (Fig. 1E), with stromal cell staining not observed during earlier phases of the menstrual cycle when decidualization had not yet occurred. Given the vast influx of inflammatory leukocytes during the late secretory phase, we also carried out CD45 immunostaining on adjacent serial sections that had been stained with CORIN (Fig. 1F). CD45 staining was abundant within late secretory phase sections (Fig. 1F). A subpopulation of CD45 positive leukocytes was also CORIN positive; however, the majority of CORIN positive cells were CD45 negative. Together, this finding indicates that both decidual cells and some CD45-positive leukocytes are CORIN positive in late secretory phase endometrium (Fig. 1E and F arrows).

CORIN is expressed in first trimester human implantation sites

Consistent with our findings of CORIN expression during the late secretory phase, CORIN expression was observed in glandular epithelial (cytokeratin positive—Fig. 2B) cells of first trimester implantation sites (Fig. 2A).

Interestingly, we found areas within the first trimester implantation sites, where decidual cells positive for CORIN surrounded spiral arteries (Fig. 2C, representative slide of a number of spiral arteries found). The presence of spiral arteries was confirmed by α smooth muscle actin (αSMA) staining (Fig. 2E). This finding would be consistent with a role of CORIN in facilitating spiral arteriole remodeling as suggested by Cui et al. (2012). However, we also observed strong CORIN positive decidua throughout the placental bed tissue (Fig. 2H). Interstitial extra villous cytotrophoblasts (confirmed by cytokeratin staining) within the vicinity of spiral arteries were CORIN negative (Fig. 2D). In contrast, EVTs distant from spiral arteries were variably positive for CORIN (Fig. 2F and G).

We also assessed CD45 staining in relation to CORIN positive cells in first trimester implantation sites. In these tissues, CD45 positive leukocytes were CORIN negative (Fig. 2H and I arrows).

Statistical analyses

Continuous variables were compared using either an unpaired t-test to assess parametric data or a Mann–Whitney U-test for non-parametric data. P ≤ 0.05 was considered significant. A Chi-square test was used to assess differences in categorical data. All statistical analysis was undertaken using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Cytokeratin, αSMA and CD45 immunohistochemistry

Cytokeratin and αSMA immunohistochemistry was carried out as detailed for CORIN. Primary antibodies used were rabbit anti-human cytokeratin (Dako) at 0.18 μg/ml applied overnight at 4 °C, mouse anti-human αSMA (Dako) at 0.7 μg/ml applied overnight at 4 °C or mouse anti-human CD45 (Dako) at 7 μg/ml for 1 h at 37 °C. For isotype controls, primary antibody was substituted with either Rabbit IgG or Mouse IgG at matching concentrations.

RT–PCR

To assess Corin mRNA expression in decidualized stromal cells and pre-eclamptic and pre-term placenta, RNA was extracted using RNeasy mini kit (Qiagen, Valencia, CA, USA). Then, 0.2 – 1 μg of RNA was converted to cDNA using Vilo (Invitrogen) as per the manufacturer’s guidelines. Taqman gene expression assays for Corin and GAPDH were used (Applied Biosystems, Carlsbad, CA, USA). RT–PCR was performed on the CFX 384 (Biorad, Hercules, CA, USA) using FAM-labeled Taqman universal PCR mastermix (Applied Biosystems) with the following run conditions: 50 °C for 2 min; 95 °C for 10 min; 95 °C for 15 s and 60 °C for 1 min (40 cycles). PCR product was confirmed by gel electrophoresis. Relative quantification was determined using the comparative CT method.

CORIN immunohistochemistry

CORIN immunohistochemistry was conducted on paraffin sections (2-5 μm) of formalin-fixed tissues. Sections were dewaxed in xylene and rehydrated through descending concentrations of ethanol. Sections were then heated for 20 min on defrost in a 700-W microwave, followed by cooling to room temperature (RT) for 30 min. They were washed for 10 min in phosphate-buffered saline pH 7.6 (PBS) and immersed in 3% H2O2 in methanol for 10 min at RT. Sections were then washed with PBS, before immersion in Dako blocking buffer (DAKO) for 10 min and then incubated for 1 h at RT with rabbit anti-human CORIN (Abcam, Cambridge, UK) at 2 μg/ml in 1%BSA/PBS. For isotype controls, primary antibody was substituted with rabbit IgG. The SuperPicTure kit (Invitrogen, Carlsbad, CA, USA) was applied according to the manufacturer’s instructions to reveal the CORIN staining. Sections were lightly counterstained with Harris hematoxylin (Accustain; Sigma Diagnostics, Castle Hill, New South Wales, Australia), dehydrated and mounted using DPX mounting medium (BDH Laboratory Supplies, Poole, England). For co-localization studies, serial sections (2 μm) were stained in the same manner as described above.

Samples were assayed for CORIN using commercially available duoset ELISAs from R&D systems (MN, USA). Proteins were measured in serum samples according to the manufacturer’s instructions. Optical density was determined using a BioRad X-Mark microplate spectrophotometer (BioRad), and CORIN levels were determined using BioRad Microplate manager 6 software. The minimum detection limit for the assay was 62.5 pg/ml.

We also assessed CD45 staining in relation to CORIN positive cells in first trimester implantation sites. In these tissues, CD45 positive leukocytes were CORIN negative (Fig. 2H and I arrows).
Corin is up-regulated with endometrial decidualization

To assess the relationship between Corin expression and decidualization, we undertook a functional model of decidualization whereby human endometrial stromal cells were induced to decidualize by the addition of estrogen and progesterone. Cell pellets and conditioned media were collected at day 2 (no decidualization), day 7 (mild decidualization) and day 14 (full decidualization). We confirmed the occurrence of decidualization by measuring prolactin in the media (Fig. 3B). We found Corin mRNA expression increased with decidualization ex vivo of human endometrial stromal cells (Fig. 3A). This functionally confirms that Corin increases in expression with decidualization.

CORIN is expressed by villous cytотrophoblasts

Given that we observed some positive staining of CORIN in EVTs, we next examined whether CORIN was expressed in placental tissue. We found that CORIN protein was readily detectable in villous cytотrophoblasts in placentas collected from both severe...
early onset preeclamptic patients (Fig. 4A) and gestationally matched pre-term controls (Fig. 4B). Interestingly, CORIN staining was not present in the syncytiotrophoblast. Real-time RT–PCR indicated no significant difference in Corin mRNA expression in the placenta obtained from cases of severe preterm pre-eclampsia \((n = 10)\) and pre-term, normotensive, gestation matched controls \((n = 15; \text{Fig. 4C})\).

**Figure 2** CORIN and CD45 expression in first trimester placental bed biopsies. In first trimester placental bed biopsies, (A) CORIN was expressed in glandular epithelium, as confirmed by (B) cytokeratin immunohistochemistry. (C) CORIN was also strongly localized to decidual cells surrounding (E) \(\alpha\)SMA positive spiral arteries. (D) Cytokeratin positive extravillous trophoblasts were also observed within the same vicinity. (F) Weak CORIN staining was also observed in trophoblasts removed from spiral arteries, as confirmed by (G) cytokeratin staining. To assess whether CD45 positive leukocytes (I) were CORIN positive (H), serial sections were stained. (H and I) Serial staining indicated that CD45 positive leukocytes were CORIN negative. Panels A–E shown at \(\times 100\) magnification, panels F and G at \(\times 400\) and panels H, I at \(\times 250\). Arrows indicate cells that are CD45 positive, CORIN negative. Images shown are representative areas taken from \(n = 12\) patient samples.

Serum CORIN levels do not change across the first trimester of pregnancy and are not altered in pregnancies destined to miscarry or develop FGR.

Finally, we measured serum CORIN concentrations in serum collected from 7 to 11 weeks gestation, the time of active spiral artery
remodeling. Although serum CORIN levels were elevated in pregnant sera when compared with NP sera, there was no change in levels across the first trimester (Fig. 4D).

In this set of serum samples, we also assessed whether there were differential levels of serum CORIN concentrations at 7–11 weeks gestation in pregnancies destined to be complicated by either FGR (lesser than fifth centile) or subsequent miscarriage (there was detectable fetal cardiac activity on the day of serum sampling) (Fig. 4E). We chose these complications because they are likely to also be disorders of abnormal placentation. In this sample set, there were insufficient numbers of cases of pre-eclampsia so we could not examine this complication. Mean serum CORIN levels in first trimester were not different in women destined to have either of these pregnancy complications, when compared with those who had healthy pregnancy outcomes.

**Discussion**

Placentation proceeds in a tightly controlled series of events, specifically designed to allow adequate blood flow to the feto-placental unit throughout pregnancy. Inadequate placentation can result in diseases of pregnancy such as FGR and pre-eclampsia, or in severe cases pregnancy loss (miscarriage). In this report, we examined a panel of relevant human gestational tissues and provide evidence demonstrating that Corin is up-regulated with experimental decidualization ex vivo and is localized to both decidual cells surrounding spiral arteries in first trimester implantation sites and in secretory phase NP endometrium. Our findings in humans, taken together with the recent report from Cui et al., suggest that CORIN may play an important role in early placentation and that its dysregulation may contribute to the pathogenesis of diseases such as FGR and pre-eclampsia.

Our finding that CORIN is also expressed in NP secretory phase endometrium is novel. Because CORIN is a transmembrane protein (Yan et al., 2000), it is possible that it plays an important local enzymatic role in normal uterine biology that warrants further investigation. Moreover, given that CORIN seems specific to the secretory phase, is present in endometrial glands and therefore might be secreted into the uterine lumen, it is tempting to speculate that it might have roles in endometrial receptivity and blastocyst-endometrial dialog as CORIN was produced by endometrial glands. Thus, besides a potential role in promoting trophoblast invasion, it could have a role in the very earliest stages of initial pregnancy implantation.

Our demonstration that Corin is up-regulated with stromal cell decidualization and strongly localized to the decidual cells of pregnancy provides compelling evidence to support a role of CORIN in activating ANP within these cells that may then promote trophoblast invasion. Certainly, a number of studies have indicated that soluble factors produced by decidual cells can enhance trophoblast migration and alter extravillous trophoblast protein expression (Hannan et al., 2006; Menkhorst et al., 2012). Thus, it is highly plausible that CORIN is produced by decidual cells and promotes trophoblast migration into spiral arteries through its activation of pro-ANP and that this mechanism may be altered in pre-eclampsia. Our in vitro data also suggest that CORIN expression in the decidua is regulated by progesterone.

There is emerging evidence that decidual immune cells [dendritic cells, T-regulatory cells, macrophages and uterine natural killer cells (uNK)] are active participants facilitating placental implantation (Smith et al., 2009; Harris, 2010), and their dysregulation may have a role in the early pathogenesis of pre-eclampsia (Matthiesen et al., 2005). For example, uNK cells may have an important paracrine role (via release of cytokines) to help trophoblasts invade the decidua, and this interplay may be disrupted in pre-eclampsia [for review see (Redman and Sargent, 2005)]. Therefore, we examined whether leukocytes were positive for CORIN (CD 45 positivity). Interestingly, in this study, we identified CORIN positive leukocytes (CD45 positive) in NP late secretory phase endometrium, but did not identify any within first trimester implantation sites. This suggests that the leukocytes around the maternal–fetal interface have an altered phenotype when compared with those of the NP endometrium. However, the functional significance of leukocytes switching from a CORIN positive phenotype in the late secretory phase to CORIN negative in first trimester pregnancy (and possible cross...
talk with CORIN positive decidual cells) is unclear and merits further study.

We also detected CORIN in the villous cytotrophoblasts of both pre-term control and severe early onset preeclamptic placentas, but identified no change in the level of Corin mRNA expression between the two cohorts. Importantly, a previous report indicates that pro-ANP is also localized to the villous cytotrophoblast layer of placenta, and thus it is likely that CORIN plays a role in its activation within cytotrophoblast cells. However, the action of CORIN and/or ANP within placenta itself at this stage is unclear and is likely to be distinct from its role in decidua during early pregnancy.

Finally, we also measured serum CORIN across the first trimester of pregnancy and found that levels did not increase across gestation. Given that placentally derived proteins increase in the maternal serum across gestation (Tong et al., 2004; Tong et al., 2012), these data suggest that there is modest or no placentally derived CORIN represented in the maternal circulation. However, in our study, there was clearly a rise in serum CORIN with pregnancy when

Figure 4 Corin expression in placenta and serum CORIN levels across first trimester pregnancy and in pregnancies complicated by FGR or miscarriage. (A) CORIN was localized to villous cytotrophoblast cells of severe early onset preeclamptic placentas and (B) gestationally matched pre-term control placentas. Panel A and B are shown at × 250 magnification. (C) No significant difference in Corin mRNA expression was identified between pre-term control or severe early onset preeclamptic placentas. (D) Serum CORIN levels were measured in controls across the first trimester of pregnancy, the time of spiral artery remodeling. No significant change was identified between 7 and 11 weeks gestation, although a NP pool of serum had significantly lower CORIN levels, n = 10, at 7 weeks, 13 at 8 weeks, 14 at 9 weeks, 11 at 10 weeks and 8 at 11 weeks. (E) Serum CORIN levels were also compared between first trimester control pregnancies and those complicated by FGR or those that went on to miscarry. No significant change between groups was identified. For control samples n = 56, for FGR samples n = 18 and for spontaneous abortion samples n = 21. Data are expressed as mean ± SEM, *P < 0.05.
Hannan NJ, Jones RL, White CA, Salamonsen LA. The chemokines, Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, Huang X, et al. to demonstrate an increase in CORIN protein in human endometrium and gestational tissue and to suggest that serum CORIN is unable to predict these complications.

In summary, this is the first study to characterize corin mRNA and CORIN protein in human endometrium and gestational tissue and to demonstrate an increase in corin expression with decidualization. Our data support findings from Cui et al. to suggest that CORIN may indeed be a key protein involved in early trophoblast migration and spiral artery remodeling in humans. Our study suggests that measurement of serum CORIN in the first trimester of pregnancy is unlikely to predict pregnancy complications, possibly because cardiac CORIN production obscures measurement of CORIN production from gestational tissues. Nevertheless, further study of CORIN biology in early pregnancy may elucidate new therapeutic targets to improve implantation quality in early pregnancy and potentially reduce the rates of pregnancy complications resulting from inadequate implantation.

Authors’ roles

All authors fulfilled the roles required for authorship including involvement in conception, design, acquisition, analysis and interpretation of data and drafting and revising article and final approval.

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Conflict of interest

None declared.

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