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EC.1.
PREECLAMPSIA ASSOCIATES WITH INCREASED RECK EXPRESSION IN TROPHOBLAST AND REDUCED MIGRATION, INVASION, AND ENDOTHELIAL-LIKE DIFFERENTIATION OF FIRST TRIMESTER HUMAN TROPHOBLAST CELLS

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Objectives: In normal pregnancies, the trophoblast invades the maternal decidua reaching and modifying the spiral arteries. The trophoblast differentiates to an endothelial-like phenotype, which is required to increase the blood flow to the placenta. However, in preeclampsia the trophoblast invasion and differentiation capacity is affected, a phenomenon that emerges as a potential cause of this syndrome. Reversion-inducing-cysteine-rich-protein with kazal motifs (RECK) is a plasma membrane protein that inhibits different metalloproteinases, acting as a key regulator of cell migration, invasion, and angiogenesis. **Objectives:** To determine the role of RECK on migration, invasion, and endothelial-like differentiation of human trophoblast and its expression and localization in human placentas from normal and preeclampsia pregnancies.
Methods: Expression and localization of RECK in the human first trimester trophoblast cell line HTR8/SVneo and in placentas from normal pregnancy and early preeclampsia were evaluated by western blot and immunofluorescence. Cells were transfected whit the expression vectors for human RECK or shRNA against RECK. Migration/invasion was assayed by the Boyden chambers migration/invasion assays. The endothelial-like differentiation was evaluated by *in vitro* pre-stablished endothelial-vascular tubes formation assay.
Results: RECK protein was detected at the plasma membrane of HTR-8/SVneo cells. Knockdown cells for RECK showed increased ($P<0.05$, $n = 3$) migration (1.4 ± 0.1 fold), invasion (2.2 ± 0.2 fold), and tubes formation (1.4 ± 0.1 fold). These phenomena were reduced by overexpressing this protein. RECK was also detected in the syncytiotrophoblast in human placentas, and preeclampsia resulted in higher protein abundance (1.4 ± 0.2 fold, $P<0.05$, $n = 5$) compared with placentas from normal pregnancies.
Conclusion: RECK is a protein expressed from early in pregnancy in human trophoblast where it could play a role in the pathogenesis of preeclampsia.
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EC.2.
MATERNAL BIRTHWEIGHT, EARLY PREGNANCY BODY MASS INDEX AND RISK OF PREGNANCY COMPLICATIONS

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Objectives: The intrauterine environment plays a critical role in health after birth. Low birthweight is known to be associated with adult-onset diseases including hypertension, cardiovascular disease (CVD), stroke and type 2 diabetes. Also, emerging evidence demonstrates a strong link between pregnancy complications and subsequent CVD. We examined the influence of maternal birthweight on the risk of development of pregnancy complications including preeclampsia (PE), gestational hypertension (GHTN), small for gestational age (SGA) pregnancy, spontaneous preterm birth (sPTB) and gestational diabetes mellitus (GDM).
Methods: This study includes 5336 nulliparous women who were recruited during their first pregnancies to SCOPE (SCreening fOr Pregnancy Endpoints), a multicentre prospective cohort study. Detailed information was collected at 15 and 20 weeks' gestation and the women were followed up throughout pregnancy. Each woman's birthweight was self-reported and confirmed via medical records when possible. A maternal birthweight of 2500-3500g was considered the reference group.
Results: After adjusting for confounders, maternal birthweight <2500g was associated with increased risk of gestational hypertension (aOR = 1.8, 95% CI = 1.1-3.0) compared to the reference group. Women who were born with a birthweight <2500g and subsequently became overweight or obese were at increased risk of gestational hypertension (aOR = 4.2, 95% CI = 2.3-8.2) and preeclampsia (aOR = 4.0, 95% CI = 2.1-7.5) compared to women who were born with a birthweight ≥ 2500g and remained lean.
Conclusion: These Results confirm previous findings that women who are small at birth and become overweight or obese as adults are at increased risk of developing major pregnancy complications.

EC.3.
RETINOIC ACID-INDUCED PLACENTAL VASCULAR HYPOPLASIA WITH PATCHED-1 UP-REGULATION IN RATS

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Objectives: Retinoic acid (RA)-induced rats exhibit low birth weight; however, the underlying mechanisms are not known. RA has been reported to induce Patched-1 (PTCH1) expression, which decreases cell proliferation and tissue differentiation by inhibiting the Hedgehog pathway. Here, we investigated the potential role of RA-induced PTCH1 expression for fetal growth in the placenta.
Methods: RA (60 mg/kg) was orally administered to Sprague-Dawley rats at embryonic day 10 (E10). The placental tissue samples were obtained from three RA-induced rats and three normal rats at E20. The levels of Ki67 and Hedgehog-related proteins, including PTCH1, Gli1, and Hedgehog interfering proteins, in the RA-induced rat placenta (RA-pl) and normal rat placenta (normal-pl) were analyzed. The structure of tissue around the placental vasculature in the RA-pl was compared with that in the normal-pl.
Results: The weight of the RA-pl was lower than that of the normal-pl. Furthermore, the level of PTCH1 in the labyrinth zone was higher in the RA-pl than in the normal-pl. However, Gli1 expression did not decrease in the RA-pl at this stage. There was no difference in the expression of Hedgehog interfering factors, including Hedgehog interacting protein, β-catenin, and secreted frizzled protein, between the groups. The analysis of tissue structure showed that the sinusoidal space in the normal-pl was larger than that in the RA-pl. Furthermore, the fetal capillaries in the terminal villi of RA-pl were significantly narrower with more branching than those in the normal-pl. Finally, ki67-positive sinusoidal trophoblast giant cells without pyknosis increased in the RA-pl, indicating reactivity to placental hypoxia due to placental vascular hypoplasia.
Conclusion: PTCH1 up-regulation in the placenta results in decreased vascular formation and blood flow in the sinusoidal space, leading to placental hypoxia and increased fetal capillary formation due to maintenance of undifferentiated state in trophoblasts.

EC.4.
EXPRESSION AND LOCALIZATION OF SPECIFIC MIRNAS IN HUMAN TERM PLACENTA BY *IN SITU* HYBRIDIZATION

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Objectives: MicroRNAs (miRNAs) are small non-coding RNAs (≈22 nucleotides) that regulate gene expression at the post-transcriptional level. They modulate cellular processes including cell proliferation, differentiation, and apoptosis. Numerous miRNAs are expressed by the placenta and can be altered in pathological conditions. However, the cellular localization of most placental miRNAs is unknown, precluding a deeper understanding of their functions. In this study, in situ hybridization (ISH) was used to investigate the expression and localization of miR-21-5p, miR-141-3p and miR-519d-3p in human term placentas.
Methods: Human placental villous fragments were collected immediately after cesarean section, fixed in 5% buffered formalin for 24 - 48 h, and routinely processed for paraffin embedding. Following, six μm-thick sections were prepared. ISH for miR-21-5p, miR-141-3p, and miR-519d-3p was performed using double digoxygenin-labeled miRCURY LNA™ microRNA Detection Probes, according to manufacturer's instructions. Tissue sections were counterstained with nuclear fast red.
Results: In syncytiotrophoblast cells, miR-141-3p and miR-519d-3p were strongly expressed by most of the cells, whilst miR-21-5p was in general weakly expressed. Only traces of miR-141-3p staining were observed in some stromal villous cells and endothelial cells. Likewise, a faint and conspicuous expression of miR-519d-3p was detected in stromal villous cells and endothelial cells. While stromal villus cells presented a faint expression some cells showed a strong staining for miR-21-5p. Some endothelial cells were intensely positive for miR-21-5p and the others showed a pale staining.
Conclusion: The investigated miRNAs showed particular patterns of expression in placental villous cells. This indicates a cell-specific role of placental miRNAs, which is lost when miRNA expression is analyzed in homogenized placental tissues. Therefore, for a better comprehension of miRNA roles in placental biology, we preconize the combination of morphological in situ techniques with molecular approaches.

MC.1.
THE HYPERTENSIVE PREGNANCY DISEASE PREECLAMPSIA CAUSES LINGERING VASCULAR STIFFENING AND BRAIN ABNORMALITIES INDICATIVE OF NEURONAL DAMAGE

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Objectives: Having had preeclampsia (PE) is more predictive of cardiovascular disease than obesity. Neither the underlying mechanisms, nor the kinetics are understood. We used state-of-the-art imaging and tonometry to quantify vascular and brain abnormalities in post-PE patients.
Methods: Magnetic Resonance Imaging (MRI) assessed overt neuronal damage while ^{1}H MR Spectroscopy quantified 10 compounds relevant to neuronal health. Tonometry combined with standard blood pressure (SphygmoCor) was used to calculate central (CPP) and peripheral (PPP) with an FDA-approved algorithm.
Results: In our patient group, both the CPP and PPP were trending higher in post-PE patients (mean PPP in controls 34.75, standard deviation (SD) 8.46; mean PPP in PE 46.33, SD 8.38; mean CPP in healthy 26.5, SD 5, mean CPP in PE 38.33, SD 8.74). Brain volumetrics were similar between the groups and no prior strokes were identified. N-acetylaspartate (NAA) and NAA+N-Acetylaspartylglutamate (NAAG) measurements normalized to creatine (Cr) indicated neuronal damage in the post-PE patients' posterior grey matter (Mean NAA/CR in the posterior grey matter in healthy 1.24 SD 0.032 in PE 1.19 SD 0.07; (NAA+NAAG)/Cr mean in healthy 1.37 SD 0.14 in PE 1.23 SD 0.12).
Conclusion: While these still relatively young PE patients did not display any obvious brain changes, abnormalities suggestive of neuronal damage were clearly detectable with our more sensitive MRS methods. The systemic vascular stiffening observed here is potentially indicative of a more profound vascular pathology than previously appreciated in this patient group. Over time, our study seeks to address whether vascular stiffening precedes the neuronal damage and in how far either are continuous processes or were triggered during the index pregnancy only.

MC.2.
PLACENTAL ANTECEDENTS OF PREECLAMPSIA AND SMALL FOR GESTATIONAL AGE

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Objectives: Preeclampsia and reduced fetal growth are major causes of the ~7 million perinatal and infant deaths occurring globally each year and both are associated with placental dysfunction. The objective of this study is an extensive RNA-seq analysis of the placental transcriptome at term in order to identify placental mRNAs differentially expressed in pregnancies with preeclampsia (PE) and severe small for gestational age (SGA) fetuses. The ultimate aim is to identify previously unrecognized pathways which are important in the pathophysiology of these great obstetrical syndromes.