Expression of ABC transporters during syncytialization in preeclampsia

Visalakshi Sethuraman, MD[a], **Yong Pu, PhD**[b], **Jeremy Gingrich, PhD**[c], **Jiongjie Jing, PhD**[c], **Robert Long, MD**[d], **Isoken Nicholas Olomu, MD**[a], **Almudena Veiga-Lopez, DVM, PhD**[b,c]

[a]Division of Neonatology, Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan, USA.

[b]Department of Pathology, University of Illinois at Chicago

[c]Department of Animal Science, College of Agriculture and Natural Resources, Michigan State University, East Lansing, Michigan, USA.

[d]Department of Obstetrics and Gynecology, Sparrow Health System, East Lansing, Michigan, USA

Abstract

Preeclampsia complicates 2–8% of pregnancies and is associated with prematurity and intrauterine growth restriction. Cholesterol and sterol transport is a key function of the placenta and it is elicited through ATP binding cassette (ABC) transporters. ABCA1 expression changes during trophoblast cell fusion, a process required to form the placental syncytium that enables maternal-fetal nutrient transfer. ABCA1 expression is dysregulated in preeclamptic placentas. But whether ABC transporters expression during trophoblast fusion is disrupted in preeclampsia remains unknown. We investigated if cholesterol and sterol ABC transporters are altered in term and preterm preeclampsia placentas and during human cytotrophoblast syncytialization. Human placental biopsies were collected from healthy term (37 weeks; n=11) and term preeclamptic (36 6/7 weeks; n=8) and pre-term preeclamptic (28–35 weeks; n=8) pregnancies. Both, protein and mRNA expression for ABCA1, ABCG1, ABCG5, and ABCG8 were evaluated. Primary cytotrophoblasts isolated from a subset of placentas were induced to syncytialize for 96h and *ABCA1*, *ABCG1* and *ABCG8* mRNA expression evaluated at 0h and 96h. Protein and gene expression of ABC transporters were not altered in preeclamptic placentas. In the healthy Term group, *ABCA1* expression was similar before and after syncytialization. After 96 h of syncytialization, mRNA expression of *ABCA1* and *ABCG1* increased significantly, while *ABCG8* decreased significantly in term-preeclampsia, but not pre-term preeclampsia. While placental expression of ABCA1 and ABCG1 remained unaltered in term preeclampsia, the disruption in

Correspondence should be addressed to Almudena Veiga-Lopez, Department of Pathology, University of Illinois at Chicago, 909 S. Wolcott Ave, Rm 6093 Chicago, IL, 60612, USA. veiga@uic.edu.

their dynamic expression pattern during cytotrophoblast syncytialization suggests that cholesterol transport may contribute to the pathophysiologic role of the placenta in preeclampsia.

Keywords

placenta; trophoblast; ABC transporters; syncytialization

Introduction

Preeclampsia is a hypertensive disorder of pregnancy that develops after 20 weeks, and is characterized by edema, new onset hypertension, and proteinuria. This is typically accompanied by multiorgan dysfunction, particularly of the kidney and liver, and can additionally result in fetal growth restriction [1]. Preeclampsia and other hypertensive disorders of pregnancy complicate 2 – 8% of pregnancies and is one of the leading causes of maternal morbidity and mortality worldwide [2–4]. In preeclampsia with severe features, delivering the fetus and placenta remains the only definitive treatment; this often results in premature delivery.

It is well established that the placenta plays a central role in the pathogenesis of preeclampsia [5–7]. Development of abnormal placentation by mechanisms thought to include inadequate spiral artery remodeling resulting from suboptimal trophoblast invasion leads to narrow maternal vessels and placental ischemia [8, 9]. Compromised utero-placental perfusion is hypothesized to cause secretion of placental factors into maternal circulation that leads to aberrant maternal systemic inflammation, oxidative stress and angiogenic imbalance, all of which ultimately lead to the preeclampsia phenotype [10, 11] and have been associated intrauterine growth restriction (IUGR) [2–5, 7, 12]. However, the etiology of preeclampsia remains incompletely understood, and whether preeclampsia associated IUGR is due to impaired placental nutrient transport, including sterols, remains unknown.

Feto-maternal transfer of nutrients and hormones, and gas exchange occurs through the placental syncytium, a multinucleated layer of trophoblast cells. The syncytiotrophoblast forms the outer layer of the chorionic villous and is a polar epithelial layer that allows for transport of nutrients and gases between the mother and the fetus through active transport, passive transport, endocytosis, exocytosis, and/or pinocytosis [13, 14]. During embryogenesis, the syncytiotrophoblast layer originates from the trophectoderm layer of the embryo and forms by a multistep process of asymmetric division and fusion of mononuclear cytotrophoblast cells into a multinucleated syncytium [15]. The formation of the two-layered placental syncytiotrophoblast from the underlying layer of cytotrophoblast cells requires expression of the "captured" retroviral envelope syncytin genes [16, 17], fusion-related proteins like glial cell missing factor-1, and human chorionic gonadotropin genes [18], in addition to the loss of intercellular e-cadherin expression upon fusion into the multinucleated syncytia. However, the syncytialization process is not yet completely understood and considerable debate remains regarding key aspects of biochemical maturation and morphological differentiation of mononuclear cytotrophoblasts into the multinucleated syncytium. However, it has been shown that faulty syncytium

formation is associated with pregnancy complications such as preeclampsia and IUGR [5–7].

One of the key functions of the syncytiotrophoblast layer is active transport of molecules against a concentration gradient. This is mediated by transport proteins [19], including the ATP binding cassette (ABC) group of transporters [20–22]. ABC transporters are comprised of over 50 members in 7 subfamilies [23–25] and 15 of these transporters are highly expressed in the placenta (ABC-A1, A5, ABC-B1, B2, B3, ABC-C1, ABC-D3, ABC-E1, ABC-F1, ABC-G1, G2, G5, G8) [4, 20, 26]. ABC transporters are localized in both the apical and basolateral membrane of the syncytiotrophoblast [26, 27] where they efflux xenobiotics towards maternal circulation, minimizing fetal exposures [20, 26, 28] or efflux molecules into the fetal capillaries [20, 29]. Of note, ABC transporters expression in the placenta is dynamic and their expression has been reported to change with gestational age [30]. Among the ABC transporters expressed in the placenta, ABCA1, ABCG1, ABCG5 and ABCG8 participate in sterol and cholesterol transport [25]. ABCA1 is one of the major cholesterol transporters that effluxes cholesterol from endothelial cells, including those in placental blood vessels. ABCA1 also reduces inflammation and is hypothesized to prevent atherosclerosis [12, 31], which is supported in a mouse model with a specific point mutations in the ABCA1 binding sites [32].

In studies of iatrogenic and spontaneous preterm delivery, static evaluations of ABCA1 expression show ABCA1 protein and mRNA expression are higher in preterm when compared with term placentas [33]. While this finding may be due to a gestational age-dependent effect, studies evaluating the placental expression of ABCA1 in preeclampsia have reported lower *ABCA1* mRNA and protein expression [34]. Similarly, within the syncytiotrophoblast layer of placentas from pregnancies complicated by preeclampsia, ABCA1 protein expression has been reported to be lower when compared with healthy term placentas [35]. During syncytialization, ABCA1 expression is dynamic, with an initial (12 h) increase in cytoplasmic and membrane expression followed by a decline in expression with the initiation of cell fusion (24 h). However, whether this dynamic expression change also occurs during syncytialization in the context of preeclampsia remains to be explored [36]. Notably, maternal circulating ABCA1 concentrations have been reported to be lower in preeclamptic patients and directly correlates with ABCA1 protein placental expression and circulating high-density lipoprotein, but is inversely related to low-density lipoprotein [37]. Some studies have suggested that low serum ABCA1 concentrations may be predictive of preeclampsia [38]. Studies have shown that ABCA1 overexpression results in enhanced cholesterol efflux from vascular endothelial cells and decreased atherosclerosis formation [37, 39–42]. Since atherosclerosis is involved in the pathogenesis of preeclampsia, downregulation of ABCA1 - the main cholesterol transporter - in preeclampsia may result in accumulation of cholesterol and atherosclerosis. This has been suggested to play a major role in pathogenesis of preeclampsia [37, 39–42].

However, to date, expression of other sterol transporters (ABCG5 and ABCG8) in pre-eclampsia remains unknown. Downregulation in the expression of the cholesterol transporter ABCG5 and sterol transporters ABCG1 and ABCG8 may also play a role, along with ABCA1, in the pathogenesis of preeclampsia. Syncytiotrophoblasts mediate active transport

of nutrients and sterols across the feto-maternal barrier, and abnormal cholesterol transport and atherosclerosis formation are thought to be involved in the development of preeclampsia [37, 39–41]. Therefore, a better understanding of dynamic changes in sterol and cholesterol ABC transporters during placental syncytialization in preeclampsia, which currently remains unexplored, may help determine if disruptions in these transporters contribute to the pathogenesis of this complex pregnancy disorder, and the goal of this study. To determine if ABC transporter expression is disrupted during cytotrophoblast fusion, we used placentas derived from healthy term and preeclamptic term and preterm pregnancies to evaluate the expression of sterol and cholesterol transporters in core biopsies and *in vitro* syncytialization of human primary cytotrophoblasts.

Materials and Methods

Ethics, exclusion criteria, and placenta collection

The institutional review boards at Michigan State University and Sparrow Hospital approved the project. All subjects gave written informed consent to participate in the study. Women admitted for C-section delivery at Sparrow Hospital (Labor & Delivery Service) were approached to participate in the study. Human placentas (n = 27) were obtained from three groups of patients: 1) healthy term pregnancies (Term; n = 11), 2) term preeclamptic pregnancies (Term-PE; n = 8) and 3) pre-term preeclamptic pregnancies (PT-PE; n = 8). We extracted maternal and neonatal anthropometric and clinical data from the mother's and infants' medical records. Healthy term pregnant mothers (at 37 weeks of pregnancy) with no complications and with none of the exclusion criteria (see below) were enrolled as healthy term controls. Mothers with a diagnosis of preeclampsia (at 36 6/7 weeks of pregnancy) were enrolled in the term preeclampsia group, while mothers with a diagnosis of preeclampsia (between 28 to 35 weeks of pregnancy) were enrolled in the preterm preeclampsia group.

Preeclampsia was defined by the following American Congress of Obstetricians and Gynecologists criteria [1]: 1) systolic blood pressure of 140 mmHg or more or diastolic blood pressure of 90 mmHg or more on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure and 2) proteinuria. Proteinuria was defined as a 24-h urine protein of >300 mg or protein to creatinine ratio of >0.3. Subjects with the following conditions were excluded from the study: multiple pregnancies, clinical chorioamnionitis, chromosomal anomalies, diabetes (gestational diabetes, type 1 diabetes, and type 2 diabetes), chronic hypertension (hypertension before pregnancy), gestational hypertension without the diagnosis of preeclampsia, major cardiovascular diseases, such as myocardial infarction, cholestasis, drug abuse, and smoking. All placentas were processed within 1 h of delivery. A core biopsy ~2 cm away from the periphery and the umbilical cord attachment was collected and kept frozen until further processing for mRNA and protein expression analyses. From a subset of placentas, additional placental tissue (~60 – 80 g) was collected for primary cell isolation and syncytialization assays (see below).

Human cytotrophoblast cell isolation, purification, and syncytialization

Human cytotrophoblast (hCTB) cells were isolated from a subset of placentas (n = 2 to 4 per clinical group) as previously described [43, 44]. In brief, placental tissue was rinsed with 0.9% NaCl, minced and dissociated with an enzyme digestion solution (Hank's Balanced Salt Solution, 25 mM HEPES, 2.5% trypsin, and ~300 IU/ml DNase I (Cat#: 15090–046, Gibco, Toronto, ON, Canada)). The cell suspension was collected, layered on fetal bovine serum (FBS, Cat#: 35–010-CV, Corning, Woodland, CA, USA), and centrifuged. The cell pellet was resuspended in Iscove's Modified Dulbecco's Medium (IMD, Cat#: I3390, MilliporeSigma, Saint Louis, MO, USA). Cell suspensions were filtered through a 100 μm nylon cell strainer, followed by a Percoll gradient centrifugation. The visible cell band between 30 – 50% Percoll was collected and resuspended in IMD medium supplemented with 10% FBS and 10% dimethyl sulfoxide (DMSO, Cat#: BP231–100, Thermo-Fisher, Rockford, IL, USA). The cells were then stored in liquid nitrogen until needed. Each of the hCTB primary cell cultures were seeded into a 24-well culture plate at a density of 1 $\times 10^6$ cells/well. hCTBs were cultured overnight to allow cell attachment and washed the following day with IMD medium to remove unattached cells. The cells were then treated with or without EGF (5 ng/ml, Cat#: E9644, Sigma Aldrich, St. Louis, MO, USA) for 96 h. After that, cells were washed with Dulbecco's phosphate-buffered saline and then lysed with cell lysis buffer and collected for RNA extraction (Qiagen, Hilden, Germany). *ABCA1*, *ABCG1* and *ABCG8* mRNA expression was evaluated in cultured cytotrophoblasts before fusion induction (at 0 h) and at 96 h. Each primary cell culture served as a biological replicate.

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted using a RNeasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol (n = 27). RNA quality and concentration were measured by Nanodrop (Thermo Fisher Scientific, Wilmington, NC, USA). A total of 1 μg RNA (A260/A280: 2.0 ± 0.05, RNA concentration: 150 ± 50 ng/μl) was reverse transcribed into complementary DNA (cDNA) using a High-Capacity cDNA Reverse Transcription Kit (Promega, Madison, WI) and 20 μl reaction volume. Quantitative real-time polymerase chain reaction (qRT-PCR; QuantStudio 5, Thermo Fisher Scientific, Carlsbad, CA, USA) was performed to quantify expression of the genes *ABCA1*, *ABCG1*, and *ABCG8*. Primer sequences are shown in Supplemental Table 1. Expression levels for indicated genes were calculated using the CT method, normalized against 60S ribosomal protein L27 (*RPL27*), and presented as relative fold-change to that of the control. All experiments and qRT-PCR assays were run in triplicate. The cDNA amplification reaction (50 ng) consisted of template denaturation and polymerase activation at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. Melt curve analyses were performed for all genes, and the specificity and integrity of the PCR products were confirmed by the presence of a single peak melt curve. Three technical replicates were run per gene and condition.

Western blotting

Western blotting was completed as previously reported [45] with the following modifications. In brief, protein was extracted from whole placental tissue lysates (n = 8/group) using a RIPA buffer (VWR Life Sciences, Radnor, PA, USA), and protein concentration determined via the Pierce BCA protein assay kit (Thermo Fisher, Rockford, IL, USA). All samples were run on a 10% SDS-polyacrylamide gel. Protein was then transferred from the gel to a nitrocellulose membrane, blocked with 5% non-fat milk in tris-buffered saline with 0.25% tween-20 (TBS-T) for 1 h at room temperature, and incubated with primary antibodies ABCA1 (ab18180, Abcam, Cambridge, UK), ABCG5 (ab175421, Abcam, Cambridge, UK), or β-actin (A1978, Sigma, St. Louis, MO, USA) overnight at 4 °C. Membranes were then washed three times in TBS-T and incubated with HRP-conjugated secondary antibodies (115–035-003, anti-mouse-HRP and 111035–003, anti-rabbit-HRP, Jackson ImmunoResearch, West Grove, PA) diluted in blocking solution for 1 h at room temperature in the dark. Membranes were subjected to enhanced chemiluminescence (Western Bright ECL K-12045-D50, Advansta, Menlo Park, CA, USA) and developed using a Thermo MyECL imager. Quantification of band intensities was performed using ImageJ software. Protein loading was accounted for by normalizing the target protein band to control β-actin band for each sample. Three technical replicates were run per gene and condition.

Statistics

All data are presented as mean ± SEM. Appropriate transformations were applied, as needed, to account for the normality of data. Comparisons among the treatment groups were analyzed by ANOVA with Tukey posthoc tests using PASW Statistics. Differences were considered significant at *P < 0.05*.

Results

Maternal and neonatal clinical data are shown in Table 1. There were no significant differences in maternal age, height, weight, 1 min apgar score and neonatal birth weight percentiles among groups. Maternal BMI was lower in Term-PE group when compared with the PT-PE group. Neonates' birth weight was lower in the PT-PE group compared to the term and Term-PE groups.

ABC transporters placental expression

ABC transporters A1 (ABCA1) and G5 (ABCG5) were detectable in placental core biopsies of all three groups of placentas. Protein expression of ABCA1 was lower but not significantly different (P > 0.05) in term and preterm preeclampsia groups when compared with the term control group (Figure 1). Expression of ABCG5 protein was not different among groups (Figure 1). Although numerically higher in preterm preeclampsia placentas, *ABCA1* mRNA expression was not significantly different when compared with the term control group (Figure 2). *ABCG1* and *ABCG8* mRNA expression was not different among groups (Figure 2).

ABC transporter expression during syncytialization

Dynamic changes in mRNA expression of the ABC transporters *ABCA1*, *ABCG1* and *ABCG8* were evaluated before (0 h) and after (96 h) human cytotrophoblast syncytialization (with or without epidermal growth factor (EGF) supplementation. *ABCA1* mRNA expression in human cytotrophoblasts was detectable before syncytialization (0 h) in all groups (Figure 3A). In healthy term and PT-PE hCTBs, *ABCA1* mRNA expression was not significantly different after 96 h of syncytialization compared to 0 h regardless of EGF supplementation. In term-PE placentas, *ABCA1* mRNA expression was higher after 96 h of syncytialization compared to 0 h and this increase was significantly higher upon exposure to EGF. At 96 h, the expression of *ABCA1* was significantly higher in the term-PE group compared to both, the healthy term and PT-PE groups.

ABCG1 mRNA expression in human cytotrophoblasts was detectable before syncytialization (0 h) in all groups (Figure 3B). In healthy term hCTBs and PT-PE, *ABCG1* mRNA expression was not significantly different after 96 h of syncytialization compared to 0 h regardless of EGF supplementation. A significant increase in mRNA *ABCG1* during syncytialization was observed in hCTBs derived from term preeclamptic placentas and this effect was further enhanced with EGF supplementation. At 96 h, the expression of *ABCG1* was significantly higher in the term-PE group compared to the healthy term and PT-PE groups.

ABCG8 mRNA expression in human cytotrophoblasts was detectable before syncytialization (0 h) in all groups (Figure 3C). In term-PE, hCTBs *ABCG8* mRNA expression was significantly lower after 96 h of syncytialization with EGF supplementation compared to 0 h. *ABCG8* mRNA expression remained unaltered in healthy term and PT-PE hCTBs during syncytialization regardless of EGF supplementation. At 0 h, the expression of *ABCG8* was significantly higher in the term-PE group compared to both, the healthy term and PT-PE groups.

Discussion

Localized to both the apical and basolateral membrane of the syncytiotrophoblast layer [26, 27], cholesterol and sterol ABC transporters play essential functions in the influx and efflux of molecules across the feto-maternal interface [20, 26, 28, 29]. In this study, we have investigated 1) the dynamic changes in ABC transporters expression in cytotrophoblasts derived from healthy placentas and 2) if their expression was affected in preeclampsia, a common pregnancy complication. We first observed that the expression of cholesterol and sterol transporters ABCA1, ABCG1, ABCG5 and ABCG8 in core biopsies of term preeclamptic placentas was not altered. We then investigated the dynamic changes in ABC transporters during syncytialization *in vitro*. We have demonstrated that in human cytotrophoblasts derived from term preeclamptic placentas, the expression of ABCA1 and ABCG1 was significantly higher at the end of the cell fusion process compared to the cytotrophoblasts derived from healthy term placentas. Notably, these changes in ABC transporters were not evident in cytotrophoblasts derived from preterm preeclamptic placentas. Our findings suggest a previously unrecognized dynamic expression of these transporters during syncytialization in the context of term preeclampsia.

ABC transporter expression in placental core biopsies

Expressed throughout pregnancy in the placenta [46], ABCA1 has been demonstrated to play a vital role in cholesterol transport across the syncytiotrophoblast [47, 48]. In this study we evaluated the expression of ABCA1 in preeclampsia, a pregnancy disorder associated with maternal hyperlipidemia [49, 50]. We observed no significant difference in *ABCA1* mRNA or protein in the placental tissue of term or preterm preeclamptic pregnancies when compared to healthy term controls. Similarly, we did not observe any difference in *ABCG1* expression, a transporter that originates from the same family and exhibits similar and complementary functions to ABCA1 [51, 52]. These findings are like those reported for placentas derived from late-onset preeclampsia [53]. Interestingly, in the same study, *ABCA1* expression was lower in placentas derived from early-onset preeclampsia when compared to age-matched controls. While differences in placental ABCA1 expression across pregnancy are not always apparent [46], differences in early- but not late-onset suggests that onset time of preeclampsia or gestational age are key factors to be considered when studying ABC transporters in the context of preeclampsia. Other studies have reported lower ABCA1 expression in villous placental biopsies from preeclamptic placentas [34]. The inconsistencies among studies likely relates to the small sample size per group (n = 8–15), demographics and clinical factors such as age, race, ethnicity, parity, and concomitant chronic diseases, and the multifactorial etiology of preeclampsia [54] that adds to the heterogeneity of the clinical cases [55].

The sterol transporter ABCG8 is also highly expressed in the placenta [25], but has not yet been studied in the context of placental cell fusion or in association with preeclampsia. While ABCG5 had not yet been reported to be expressed in the placenta, both ABCG8 and ABCG5 are responsible for the efflux of cholesterol and sterols [56] and can prevent atherogenesis [12]. In our study, and like that observed for ABCA1 and ABCG1, we did not see a difference in the expression of ABCG8 or ABCG5 in core biopsies derived from preeclamptic placentas compared to the healthy term group. Despite their key role in facilitating absorption of dietary cholesterol [47, 57–61] and the association between preeclampsia and hyperlipidemia [49, 50], baseline expression of all the ABC transporters investigated was not altered in preeclampsia.

ABC transporter expression during cytotrophoblast syncytialization

In vitro, human cytotrophoblasts syncytialize spontaneously at a low rate, but this process of cell fusion can be enhanced with the supplementation of EGF [62, 63]. Previous studies have shown changes in the expression of ABCA1 and ABCG1 during *in vitro* syncytialization of human cytotrophoblasts within the first 12 to 24 h in culture [36]. Our results showed that *ABCA1* and *ABCG1* mRNA expression did not change in healthy term cytotrophoblasts after syncytialization (96 h in culture). The discrepancy between our findings and previous studies [36] likely relates to the timing of cell fusion induction when ABCA1 expression was evaluated. Nikitina et al. [36] observed that at 24h, with progressive syncytium formation, ABCA1 staining intensity by immunohistochemistry was reduced as a result of ABCA1 being dispersed into the cytoplasm of the forming syncytial membrane; although no quantification was provided. To our knowledge, no previous study has investigated the expression of ABCA1 during the syncytialization process beyond 24

h. A change in expression during the initial fusion stages points to a transient change in ABCA1 expression that is likely associated with early events (commitment stage) of the cell fusion process when cell-to-cell adhesion and intercellular communication is required [64]. An upregulation in ABCA1 at this early stage of fusion suggests that cholesterol transfer may be a key event prior to the cell-to-cell fusion stage. Overall, the process of cytotrophoblast cell fusion, its regulation, and the role of this transient increase in ABCA1 during early cytotrophoblast fusion remains poorly understood. Notably, given that EGF (used in this study) accelerates and promotes cytotrophoblast fusion, the differences between these two studies should consider that the syncytialization process evaluated in Nikitina et al., [36] was only restricted to spontaneous syncytialization while this study included both, spontaneous and EGF-mediated syncytialization.

Both, ABCA1 and ABCG1 are highly expressed in the placenta [25, 29, 36] and their function as cholesterol and sterol transporters makes them relevant to fetal and placental development [65]. Despite this, studies of ABCA1 and ABCG1 in the context of placental dysfunction remain scarce [29, 36, 65, 66]. In this study we have demonstrated that both, ABCA1 and ABCG1 are upregulated at the end of *in vitro* syncytialization (96 h) in cytotrophoblasts derived from term preeclamptic placentas. Previous studies have demonstrated that ABCA1 expression transiently increases during the early syncytialization stages (12 h) [36]. This supports the hypothesis that the increase in Term-PE cytotrophoblasts at 96 h may relate to a defect in the ability of preeclamptic cytotrophoblasts to provide proper cholesterol trafficking signals during the fusion process. This, in turn, continues to be exacerbated until the end of the fusion process, even with the addition of EGF. A limitation of using primary human cytotrophoblasts is that they do not form monolayers in culture. Therefore, even though they retain their apical-basal aspect in culture, studies that evaluate the functional transport of cholesterol *in vitro* are challenging and were not tested here. Since we did not evaluate fusion simultaneously in these cultures, we cannot correlate changes in ABC transporter expression to fusion events and/or syncytia formation; an aspect that should be evaluated in future studies.

One of the few studies that has evaluated the expression of ABC transporters in primary cytotrophoblasts derived from preeclamptic placentas (without fusion induction) used a semi-quantitative analysis to measure ABCA1 protein expression [66]. Cytotrophoblasts derived from preeclamptic placentas accompanied with IUGR had lower ABCA1 expression, but not ABCG1 [66]. Notably, this effect was lost in preeclamptic samples without IUGR [66] suggesting that cholesterol transport regulation plays an important role in fetal outcomes in preeclampsia. These findings are similar to our results where ABCA1 and ABCG1 expression in Term-PE at 0 h is numerically lower, but not significantly different, from that of the healthy term controls. Unfortunately, the small sample size for the fusion experiment did not allow us to discriminate between preeclampsia with and without IUGR. Overall, our results suggest that alterations in the normal pattern of ABCA1 and ABCG1 expression during syncytialization in term preeclamptic cytotrophoblasts is likely a reflection of a disruption of the normal syncytialization process. Intriguingly, we did not observe the same upregulation in *ABCA1* and *ABCG1* after fusion in preterm preeclamptic cytotrophoblasts as observed in the term preeclamptic group. This was the case regardless of EGF stimulation. While prematurity in the context of preeclampsia is associated with

severity [67], it is possible that the disruption in ABCA1 and ABCG1 observed in term preeclamptic cytotrophoblasts is reflective of an alternative mechanistic pathway with a similar phenotype, in that preterm preeclamptic cytotrophoblasts have additional disruptions that prevent cytotrophoblasts from spontaneously fusing or responding to the EGF stimulus.

However, our results do not provide us with enough insights to demonstrate either scenario. Future studies should simultaneously evaluate cell fusion events and ABC transporter expression in the context of prematurity in preeclampsia. One of the limitations of the study was the absence of a preterm control group without preeclampsia. The use of a preterm group would have enabled a comparison with a matched gestational age. This is especially important as ABC transporter expression has been reported to change across pregnancy [33, 46]. However, the creation of an idiopathic preterm delivery control group would be challenging given the frequency of infection, inflammation, and malplacentation in this setting [68]. Even when no obvious histopathological changes are observed in a preterm placenta, other abnormalities may be associated with preterm delivery [69].

ABCG8 is highly expressed in the human placenta [25]. However, to date, no study is available regarding expression of ABCG8 in human trophoblasts or during syncytialization. Similar to the ABC transporters ABCA1 and ABCG1, *ABCG8* expression did not change during cytotrophoblast fusion in the healthy term group. However, ABCG8 expression was lower in cytotrophoblasts derived from preeclamptic placentas when stimulated with EGF. Decrease in ABCG8 suggests that this ABC transporter plays a different role during trophoblast fusion than that oABCA1 and ABCG1, which have been reported to have redundant roles [51, 52].

Conclusion

While the static expression of ABCA1 and ABCG1 transporters remained unaltered in term preeclampsia, disruption of the dynamic expression pattern of ABCA1 and ABCG1 during cytotrophoblast syncytialization suggests that cholesterol transport may contribute to the pathophysiologic role of the placenta in preeclampsia. Future studies should investigate if the dynamic changes in ABC transporters during trophoblast syncytialization are associated with cell fusion events. Only the inclusion of a larger sample size from a diverse population will allow us to evaluate if there is a direct association between altered expression of cholesterol and sterol transporters with preeclampsia. Understanding if changes in these transporters occurs due to hypertensive conditions during pregnancy, ABC and sterol transporters should also be evaluated in other hypertensive disorders of pregnancy, such as chronic or gestational hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

Abbreviations

ABC	ATP binding cassette
ABCA1	ATP binding cassette transporter type A1
ABCG1	ATP binding cassette transporter type G1
ABCG5	ATP binding cassette transporter type G5
ABCG8	ATP binding cassette transporter type G8
BMI	Body Mass index
DMSO	dimethyl sulfoxide
EGF	epidermal growth factor
IUGR	intrauterine growth restriction
IQ	interquartile range
hCTB	human cytotrophoblast
IMD	Iscove's Modified Dulbecco's Medium
mRNA	messenger ribonucleic acid
Term-PE	Term preeclampsia
PT-PE	preterm preeclampsia
PTL	preterm labor
SROM	spontaneous rupture of membranes

References

[1]. ACOG, American College of Obstetricians and Gynecologists Practice Bulletin No. 202 Summary: Gestational Hypertension and Preeclampsia, Obstet Gynecol 133(1) (2019) 211–214.

[2]. Ghulmiyyah L, Sibai B, Maternal mortality from preeclampsia/eclampsia, Semin Perinatol 36(1) (2012) 56–9. [PubMed: 22280867]

[3]. Duley L, The global impact of pre-eclampsia and eclampsia, Semin Perinatol 33(3) (2009) 130–7. [PubMed: 19464502]

[4]. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM, The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP), Hypertens Pregnancy 20(1) (2001) IX–XIV. [PubMed: 12044323]

[5]. Himmelmann K, Himmelmann A, Niklasson A, Svensson A, Hypertension in pregnancy and size at birth, Blood Press 5(5) (1996) 278–84. [PubMed: 8879600]

[6]. Soma H, Yoshida K, Mukaida T, Tabuchi Y, Morphologic changes in the hypertensive placenta, Contrib Gynecol Obstet 9 (1982) 58–75. [PubMed: 6754249]

[7]. Brosens I, Dixon HG, Robertson WB, Fetal growth retardation and the arteries of the placental bed, Br J Obstet Gynaecol 84(9) (1977) 656–63. [PubMed: 911717]

[8]. Burton GJ, Woods AW, Jauniaux E, Kingdom JC, Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy, Placenta 30(6) (2009) 473–82. [PubMed: 19375795]

[9]. Zhou Y, Damsky CH, Fisher SJ, Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?, J Clin Invest 99(9) (1997) 2152–64. [PubMed: 9151787]

[10]. Redman CW, Sargent IL, Placental stress and pre-eclampsia: a revised view, Placenta 30 Suppl A (2009) S38–42. [PubMed: 19138798]

[11]. Maynard S, Epstein FH, Karumanchi SA, Preeclampsia and angiogenic imbalance, Annu Rev Med 59 (2008) 61–78. [PubMed: 17937587]

[12]. Schumacher T, Benndorf RA, ABC Transport Proteins in Cardiovascular Disease-A Brief Summary, Molecules 22(4) (2017).

[13]. Hill EP, Longo LD, Dynamics of maternal-fetal nutrient transfer, Fed Proc 39(2) (1980) 239–44. [PubMed: 7353680]

[14]. Tetro N, Moushaev S, Rubinchik-Stern M, Eyal S, The Placental Barrier: the Gate and the Fate in Drug Distribution, Pharm Res 35(4) (2018) 71. [PubMed: 29476301]

[15]. Bastida-Ruiz D, Yart L, Wuillemin C, Ribaux P, Morris N, Epiney M, Martinez de Tejada B, Cohen M, The fine-tuning of endoplasmic reticulum stress response and autophagy activation during trophoblast syncytialization, Cell Death Dis 10(9) (2019) 651. [PubMed: 31501418]

[16]. Dupressoir A, Vernochet C, Harper F, Guegan J, Dessen P, Pierron G, Heidmann T, A pair of co-opted retroviral envelope syncytin genes is required for formation of the two-layered murine placental syncytiotrophoblast, Proc Natl Acad Sci U S A 108(46) (2011) E1164–73. [PubMed: 22032925]

[17]. Handwerger S, New insights into the regulation of human cytotrophoblast cell differentiation, Mol Cell Endocrinol 323(1) (2010) 94–104. [PubMed: 20036312]

[18]. Milano-Foster J, Ray S, Home P, Ganguly A, Bhattacharya B, Bajpai S, Pal A, Mason CW, Paul S, Regulation of human trophoblast syncytialization by histone demethylase LSD1, J Biol Chem 294(46) (2019) 17301–17313. [PubMed: 31591264]

[19]. Hayward CEJ, Sibley RLPC, Mechanisms of Transfer Across the Human Placenta, in: Polin A, Rowitch Benitz and Fox (Ed.), Fetal and Neonatal Physiology, Elsevier, Philadelphia, 2017, pp. 121–133.

[20]. Bloise E, Ortiga-Carvalho TM, Reis FM, Lye SJ, Gibb W, Matthews SG, ATP-binding cassette transporters in reproduction: a new frontier, Hum Reprod Update 22(2) (2016) 164–81. [PubMed: 26545808]

[21]. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ, Schellens JH, Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues, Cancer Res 61(8) (2001) 3458–64. [PubMed: 11309308]

[22]. Higgins CF, ABC transporters: from microorganisms to man, Annu Rev Cell Biol 8 (1992) 67–113. [PubMed: 1282354]

[23]. Walker N, Filis P, Soffientini U, Bellingham M, O'Shaughnessy PJ, Fowler PA, Placental transporter localization and expression in the Human: the importance of species, sex, and gestational age differencesdagger, Biol Reprod 96(4) (2017) 733–742. [PubMed: 28339967]

[24]. Dean M, Hamon Y, Chimini G, The human ATP-binding cassette (ABC) transporter superfamily, J Lipid Res 42(7) (2001) 1007–17. [PubMed: 11441126]

[25]. HumanProteinAtlas, The Human Protein Atlas: ATP binding cassette transporters. https://www.proteinatlas.org/search/ATP+binding+cassette+transporters. (Accessed 6/21/2020 2020).

[26]. Joshi AA, Vaidya SS, St-Pierre MV, Mikheev AM, Desino KE, Nyandege AN, Audus KL, Unadkat JD, Gerk PM, Placental ABC Transporters: Biological Impact and Pharmaceutical Significance, Pharm Res 33(12) (2016) 2847–2878. [PubMed: 27644937]

[27]. Aye IL, Paxton JW, Evseenko DA, Keelan JA, Expression, localisation and activity of ATP binding cassette (ABC) family of drug transporters in human amnion membranes, Placenta 28(8–9) (2007) 868–77. [PubMed: 17482262]

[28]. Ceckova-Novotna M, Pavek P, Staud F, P-glycoprotein in the placenta: expression, localization, regulation and function, Reprod Toxicol 22(3) (2006) 400–10. [PubMed: 16563694]

[29]. Aye IL, Singh AT, Keelan JA, Transport of lipids by ABC proteins: interactions and implications for cellular toxicity, viability and function, Chem Biol Interact 180(3) (2009) 32739.

[30]. Mathias AA, Hitti J, Unadkat JD, P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages, Am J Physiol Regul Integr Comp Physiol 289(4) (2005) R963–9. [PubMed: 15961534]

[31]. Stamatikos A, Dronadula N, Ng P, Palmer D, Knight E, Wacker BK, Tang C, Kim F, Dichek DA, ABCA1 Overexpression in Endothelial Cells In Vitro Enhances ApoAI-Mediated Cholesterol Efflux and Decreases Inflammation, Hum Gene Ther 30(2) (2019) 236–248. [PubMed: 30079772]

[32]. Price NL, Rotllan N, Zhang X, Canfran-Duque A, Nottoli T, Suarez Y, Fernandez-Hernando C, Specific Disruption of Abca1 Targeting Largely Mimics the Effects of miR-33 Knockout on Macrophage Cholesterol Efflux and Atherosclerotic Plaque Development, Circ Res 124(6) (2019) 874–880. [PubMed: 30707082]

[33]. Cheng-Mao X, Yan L, Li L, Hua J, Xiao-Ju W, Jie-Wen Z, Placental ABCA1 Expression Is Increased in Spontaneous Preterm Deliveries Compared with Iatrogenic Preterm Deliveries and Term Deliveries, Biomed Res Int 2017 (2017) 8248094.

[34]. Chigusa Y, Kondoh E, Mogami H, Nishimura F, Ujita M, Kawasaki K, Fujita K, Tatsumi K, Konishi I, ATP-binding cassette transporter A1 expression is decreased in preeclamptic placentas, Reprod Sci 20(8) (2013) 891–8. [PubMed: 23275468]

[35]. Korner M, Wenger F, Nikitina L, Baumann M, Surbek D, Albrecht C, PP141. The lipid transporters ABCA1 and ABCG1 are differentially expressed in preeclamptic and IUGR placentas, Pregnancy Hypertens 2(3) (2012) 315–6.

[36]. Nikitina L, Wenger F, Baumann M, Surbek D, Korner M, Albrecht C, Expression and localization pattern of ABCA1 in diverse human placental primary cells and tissues, Placenta 32(6) (2011) 420–30. [PubMed: 21501868]

[37]. Liu L, Zhang M, Li L, Li C, Min X, Zheng M, Expression and clinical significance of ATP-binding cassette transporter 1 in serum and placental tissue in Chinese patients with preeclampsia, Gynecol Obstet Invest 78(3) (2014) 194–200. [PubMed: 25139615]

[38]. Liu L, Zhang M, Min X, Cai L, Low Serum Levels of ABCA1, an ATP-Binding Cassette Transporter, Are Predictive of Preeclampsia, Tohoku J Exp Med 236(2) (2015) 89–95. [PubMed: 26004638]

[39]. Hentschke MR, Poli-de-Figueiredo CE, da Costa BE, Kurlak LO, Williams PJ, Mistry HD, Is the atherosclerotic phenotype of preeclamptic placentas due to altered lipoprotein concentrations and placental lipoprotein receptors? Role of a small-for-gestational-age phenotype, J Lipid Res 54(10) (2013) 2658–64. [PubMed: 23898049]

[40]. Staff AC, Dechend R, Pijnenborg R, Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health, Hypertension 56(6) (2010) 1026–34. [PubMed: 20956732]

[41]. Kim SH, Kim GJ, Umemura T, Lee SG, Cho KJ, Aberrant expression of plasma microRNA-33a in an atherosclerosis-risk group, Mol Biol Rep 44(1) (2017) 79–88. [PubMed: 27664032]

[42]. Sandhofer A, Iglseder B, Kaser S, More E, Paulweber B, Patsch JR, The influence of two variants in the adenosine triphosphate-binding cassette transporter 1 gene on plasma lipids and carotid atherosclerosis, Metabolism 57(10) (2008) 1398–404. [PubMed: 18803945]

[43]. Petroff MG, Phillips TA, Ka H, Pace JL, Hunt JS, Isolation and culture of term human trophoblast cells, Methods Mol Med 121 (2006) 203–17. [PubMed: 16251745]

[44]. Ticiani E, Gingrich J, Pu Y, Vettathu M, Davis J, Martin D, Petroff MG, VeigaLopez A, Bisphenol S and Epidermal Growth Factor Receptor Signaling in Human Placental Cytotrophoblasts, Environ Health Perspect 129(2) (2021) 27005. [PubMed: 33605785]

[45]. Pu Y, Pearl S, Gingrich J, Jing J, Martin D, Murga-Zamalloa CA, Veiga-Lopez A, Multispecies study: low-dose tributyltin impairs ovarian theca cell cholesterol homeostasis through the RXR pathway in five mammalian species including humans, Arch Toxicol 93(6) (2019) 1665–1677. [PubMed: 31006824]

[46]. Bhattacharjee J, Ietta F, Giacomello E, Bechi N, Romagnoli R, Fava A, Paulesu L, Expression and localization of ATP binding cassette transporter A1 (ABCA1) in first trimester and term human placenta, Placenta 31(5) (2010) 423–30. [PubMed: 20338636]

[47]. Lindegaard ML, Wassif CA, Vaisman B, Amar M, Wasmuth EV, Shamburek R, Nielsen LB, Remaley AT, Porter FD, Characterization of placental cholesterol transport: ABCA1 is a potential target for in utero therapy of Smith-Lemli-Opitz syndrome, Hum Mol Genet 17(23) (2008) 3806–13. [PubMed: 18775956]

[48]. Venkateswaran A, Repa JJ, Lobaccaro JM, Bronson A, Mangelsdorf DJ, Edwards PA, Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols, J Biol Chem 275(19) (2000) 14700–7. [PubMed: 10799558]

[49]. Jin WY, Lin SL, Hou RL, Chen XY, Han T, Jin Y, Tang L, Zhu ZW, Zhao ZY, Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China, BMC Pregnancy Childbirth 16 (2016) 60. [PubMed: 27000102]

[50]. Spracklen CN, Smith CJ, Saftlas AF, Robinson JG, Ryckman KK, Maternal hyperlipidemia and the risk of preeclampsia: a meta-analysis, Am J Epidemiol 180(4) (2014) 346–58. [PubMed: 24989239]

[51]. Yvan-Charvet L, Wang N, Tall AR, Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses, Arterioscler Thromb Vasc Biol 30(2) (2010) 139–43. [PubMed: 19797709]

[52]. Frambach S, de Haas R, Smeitink JAM, Rongen GA, Russel FGM, Schirris TJJ, Brothers in Arms: ABCA1- and ABCG1-Mediated Cholesterol Efflux as Promising Targets in Cardiovascular Disease Treatment, Pharmacol Rev 72(1) (2020) 152–190. [PubMed: 31831519]

[53]. Plosch T, Gellhaus A, van Straten EM, Wolf N, Huijkman NC, Schmidt M, Dunk CE, Kuipers F, Winterhager E, The liver X receptor (LXR) and its target gene ABCA1 are regulated upon low oxygen in human trophoblast cells: a reason for alterations in preeclampsia?, Placenta 31(10) (2010) 910–8. [PubMed: 20709391]

[54]. Rana S, Lemoine E, Granger JP, Karumanchi SA, Preeclampsia: Pathophysiology, Challenges, and Perspectives, Circ Res 124(7) (2019) 1094–1112. [PubMed: 30920918]

[55]. Benton SJ, Leavey K, Grynspan D, Cox BJ, Bainbridge SA, The clinical heterogeneity of preeclampsia is related to both placental gene expression and placental histopathology, Am J Obstet Gynecol 219(6) (2018) 604 e1–604 e25. [PubMed: 30278173]

[56]. Graf GA, Yu L, Li WP, Gerard R, Tuma PL, Cohen JC, Hobbs HH, ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion, J Biol Chem 278(48) (2003) 48275–82. [PubMed: 14504269]

[57]. Brown JM, Yu L, Protein mediators of sterol transport across intestinal brush border membrane, Subcell Biochem 51 (2010) 337–80. [PubMed: 20213550]

[58]. Duan LP, Wang HH, Wang DQ, Cholesterol absorption is mainly regulated by the jejunal and ileal ATP-binding cassette sterol efflux transporters Abcg5 and Abcg8 in mice, J Lipid Res 45(7) (2004) 1312–23. [PubMed: 15102882]

[59]. Aye IL, Waddell BJ, Mark PJ, Keelan JA, Placental ABCA1 and ABCG1 transporters efflux cholesterol and protect trophoblasts from oxysterol induced toxicity, Biochim Biophys Acta 1801(9) (2010) 1013–24. [PubMed: 20570635]

[60]. Stefulj J, Panzenboeck U, Becker T, Hirschmugl B, Schweinzer C, Lang I, Marsche G, Sadjak A, Lang U, Desoye G, Wadsack C, Human endothelial cells of the placental barrier efficiently deliver cholesterol to the fetal circulation via ABCA1 and ABCG1, Circ Res 104(5) (2009) 600–8. [PubMed: 19168441]

[61]. Chatuphonprasert W, Jarukamjorn K, Ellinger I, Physiology and Pathophysiology of Steroid Biosynthesis, Transport and Metabolism in the Human Placenta, Front Pharmacol 9 (2018) 1027. [PubMed: 30258364]

[62]. Strauss JF 3rd, Kido S, Sayegh R, Sakuragi N, Gafvels ME, The cAMP signalling system and human trophoblast function, Placenta 13(5) (1992) 389–403. [PubMed: 1335147]

[63]. Morrish DW, Bhardwaj D, Dabbagh LK, Marusyk H, Siy O, Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta, J Clin Endocrinol Metab 65(6) (1987) 1282–90. [PubMed: 3500181]

[64]. Gerbaud P, Pidoux G, Review: An overview of molecular events occurring in human trophoblast fusion, Placenta 36 Suppl 1 (2015) S35–42. [PubMed: 25564303]

[65]. Palinski W, Maternal-fetal cholesterol transport in the placenta: good, bad, and target for modulation, Circ Res 104(5) (2009) 569–71. [PubMed: 19286612]

[66]. Baumann M, Korner M, Huang X, Wenger F, Surbek D, Albrecht C, Placental ABCA1 and ABCG1 expression in gestational disease: Pre-eclampsia affects ABCA1 levels in syncytiotrophoblasts, Placenta 34(11) (2013) 1079–86. [PubMed: 23880356]

[67]. Amaral LM, Wallace K, Owens M, LaMarca B, Pathophysiology and Current Clinical Management of Preeclampsia, Curr Hypertens Rep 19(8) (2017) 61. [PubMed: 28689331]

[68]. Goldenberg RL, Culhane JF, Iams JD, Romero R, Epidemiology and causes of preterm birth, Lancet 371(9606) (2008) 75–84. [PubMed: 18177778]

[69]. Burke KT, Colvin PL, Myatt L, Graf GA, Schroeder F, Woollett LA, Transport of maternal cholesterol to the fetus is affected by maternal plasma cholesterol concentrations in the golden Syrian hamster, J Lipid Res 50(6) (2009) 1146–55. [PubMed: 19122238]

Highlights

- Placental expression of ABC transporters is unaltered in preeclampsia

- ABC transporters have a dynamic expression during trophoblast syncytialization

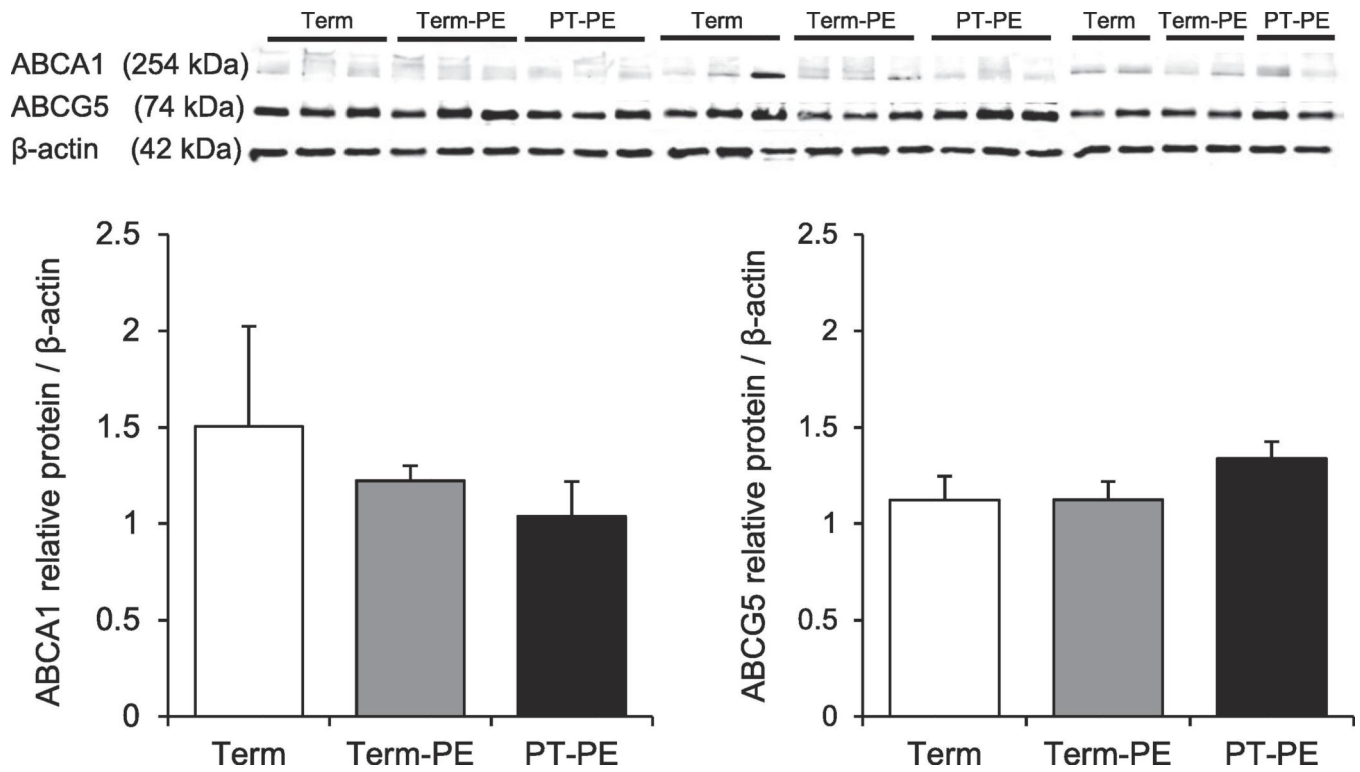- ABC transporters expression during cell fusion is disrupted in term preeclampsia

Figure 1.
ABCA1 and ABCG5 protein expression (mean ± SEM) in human placental core biopsies in term (Term, *open bars*), term-preeclamptic (Term-PE, *gray bars*) and preterm-preeclamptic (PT-PE, *closed bars*) placentas (n = 24 (8/group)). Significance was set at P < 0.05.
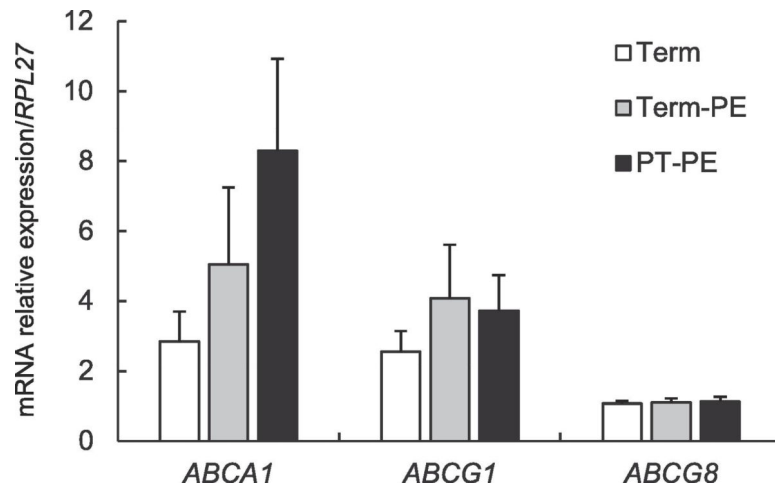
Figure 2.
Relative mRNA expression (mean ± SEM) of *ABCA1*, *ABCG1*, *ABCG8* in human placental core biopsies of term (Term, *open bars*, n = 11), term preeclampsia (Term-PE, *gray bars*, n = 8) and preterm preeclampsia (PT-PE, *closed bars*, n = 8) pregnancies. Significance was set at P < 0.05.
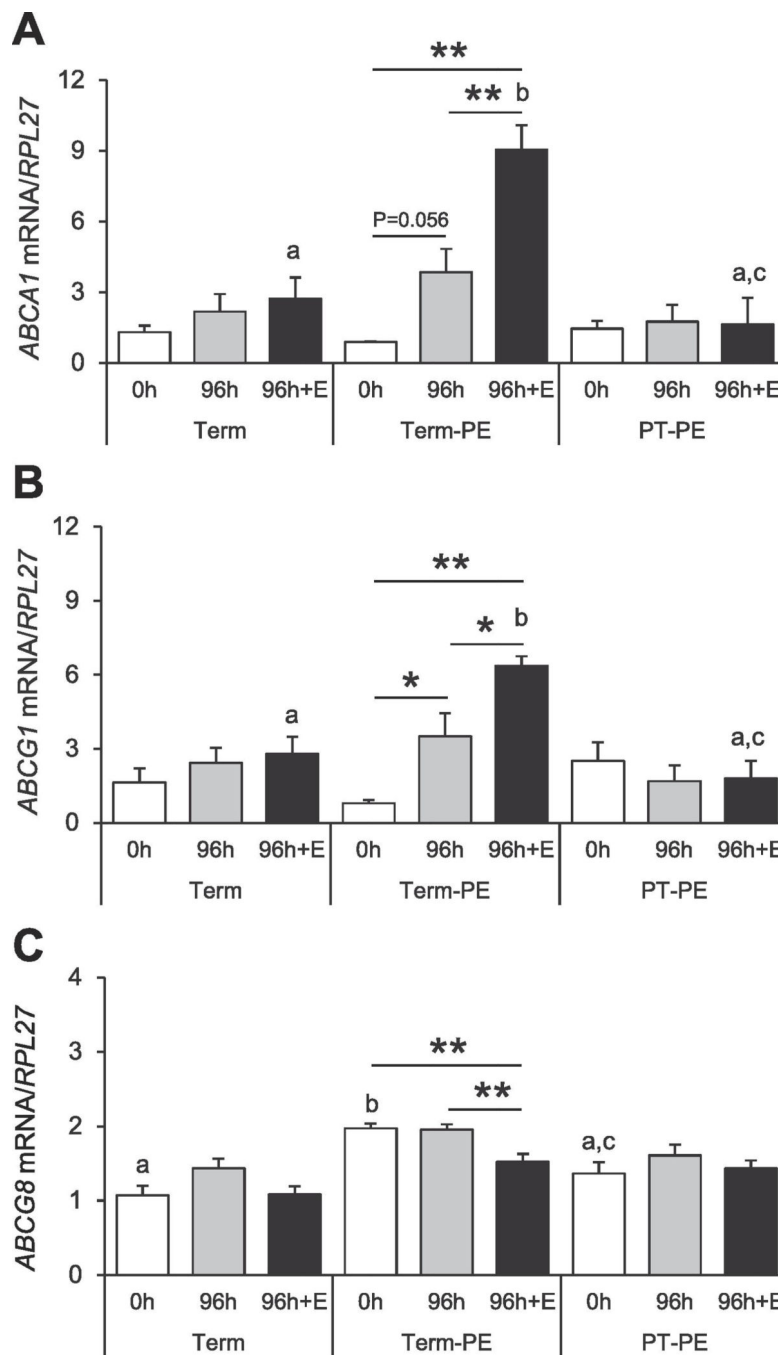
Figure 3.
Relative mRNA expression (mean ± SEM) of *ABCA1* (*top*), *ABCG1* (*middle*), and *ABCG8* (*bottom*) in cytotrophoblasts before (0 h, *open bars*) and after 96 h of syncytialization with (*closed bars*) and without (*gray bars*) epidermal growth factor (EGF) derived from healthy term (Term, n = 4), term preeclampsia (Term-PE, n = 4) and preterm preeclampsia (PT-PE, n = 2) cytotrophoblasts. Asterisks denote differences between groups (* P < 0.05 and ** P < 0.01).

Table 1.

Clinical characteristics

	Term (n = 11) 37 weeks		Term - PE (n = 9) 36 6/7 weeks		PT - PE (n = 8) 28–35 weeks		
	Mean ± SEM, percent, or N	Range	Mean ± SEM, percent or N	Range	Mean ± SEM, percent, or N	Range	*P value*
Age (years)	31.9 ± 1.0	(27 – 39)	28.7 ± 2.4	(19 – 41)	26.8 ± 1.4	(21 – 34)	*0.098*
Height (m)	1.7 ± 0.02	(1.6 – 1.8)	1.6 ± 0.02	(1.5 – 1.7)	1.6 ± 0.02	(1.5 – 1.7)	*0.060*
Maternal weight (kg)	89.0 ± 6.0	(69.9 – 120.2)	76.1 ± 8.6	(47.6 – 137.0)	102.2 ± 8.5	(74.8 – 148.8)	*0.085*
BMI (kg/m^2)	31.6 ± 1.9 ^{a,b}	(25.8 – 46.8)	28.9 ± 2.9^a	(20.5 – 50.2)	39.0 ± 3.2^b	(29 – 56.3)	***0.041***
Birth weight (g)	3,459 ± 155.5^a	(2,250 – 4,140)	3,488.3 ± 111.1^a	(2,995 – 4,240)	2,210 ± 211.3^b	(1,460 – 3,045)	***0.000***
Birth weight percentile	58.7 ± 5.7	(30.8 – 94.0)	66.1 ± 9.0	(18.8 – 99.6)	50.7 ± 11.0	(2.6 – 95.2)	*0.472*
Apgar Score – 1min (Median (IQR))	8 (0)	(8 – 9)	8 (1)	(4 – 9)	7 (1)	(6 – 8)	*0.053*
Newborn sex (Female/Male)	4/7		3/6		3/5		
Delivery (C-section/vaginal)	11/0		6/4		5/3		
SROM (%)	0 (0/11)		55.6 (4/5)		12.5 (1/7)		
History of PE (%)	9.1 (1/11)		11.1 (1/9)		12.5 (1/8)		
History of PTL/ miscarriage (%)	9.1 (1/11)		11.1 (1/9)		25 (2/6)		

Data expressed in mean ± SEM or percent unless otherwise specified. IQR: interquartile range, PE: preeclampsia, PT-PE: preterm preeclampsia, Term-PE: term preeclampsia, PTL: preterm labor, SROM: spontaneous rupture of membranes, and T: term control.

^{a} ^{b}denotes significant differences between groups.