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Short communication

SLC11A1 is expressed in the human placenta across multiple gestational ages

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A B S T R A C T

The human placenta functions as an innate immune barrier to prevent fetal infection. However, the molecular mechanisms accounting for placental resistance to pathogens are currently poorly understood. The solute carrier family 11 member 1 (SLC11A1) is a divalent cation transporter expressed primarily by macrophages and neutrophils that is essential for controlling infections by intracellular pathogens such as *Salmonella*, *Leishmania* and *Mycobacteria*. This report demonstrates that SLC11A1 is expressed in the syncytiotrophoblast of the human placenta at multiple gestational ages. These results suggest that SLC11A1 may play a role in blocking productive placental infections by certain intracellular pathogens.

1. Introduction

Pregnancy represents a significant window of susceptibility to pathogens, for infections cause severe complications that include miscarriage, preterm birth and congenital birth defects [1,2]. The human placenta has been proposed to serve as an innate immune barrier to infection in order to facilitate successful pregnancy. Indeed, previous studies demonstrated that the syncytiotrophoblast, the outer layer of the placenta in direct contact with maternal blood, is relatively resistant to infections by *Toxoplasma* gondii, *Listeria* monocytogenes and a wide variety of unrelated viruses [3–8]. Although the human placenta expresses a number of different antimicrobial molecules, including toll like receptors, nucleotide-binding oligomerization domain proteins, defensins, inflammatory cytokines and their corresponding receptors, the precise mechanisms by which it defends against infections remain poorly defined [1,2].

SLC11A1 (also known as natural resistance associated macrophage protein-1/NRAMP-1) is a divalent cation transporter that is critical for controlling and clearing infections by intracellular pathogens such as *Salmonella*, *Leishmania* and *Mycobacteria* [9–11]. Defects in murine SLC11A1 and human SLC11A1 allelic variants are associated with host susceptibility to these pathogens [9–13]. Expression of SLC11A1 has primarily been observed in professional phagocytes such as M1

macrophages and neutrophils, but has also been reported in neurons and dendritic cells [14–17]. Experimental evidence suggests that SLC11A1 functions to inhibit intracellular growth of select pathogens by restricting the availability of iron and magnesium ions, however, there is also experimental support for alternative modes of SLC11A1 action [9,10,18–20]. In this study, we investigated the expression of SLC11A1 in human placental villi from multiple gestational ages by RT-PCR and whole mount immunofluorescence (WMIF).

2. Methods

2.1. Human tissue collections

A total of 24 placentas was collected (9 first trimester, 5 s trimester and 10 term) for this study. First (6–12 week) and 2nd (14–22 week) trimester placentas were collected from elective terminations, while term (> 39 week) placentas were collected from healthy, uncomplicated pregnancies delivered by caesarean section. Placentas from mothers with known comorbid conditions such as chorioamnionitis, sexually transmitted diseases, chronic hypertension, diabetes, autoimmune disease, pre-eclampsia and renal disease, as well as reported smokers and illicit drug users were excluded from this study. Informed consent was obtained for collection of all tissues. This study and tissue

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collections were approved by the Institutional Human Subjects Review Board at the University of Rochester.

2.2. RNA isolation and RT-PCR

Detailed protocols for RNA isolation and RT-PCR are included in Supplemental methods.

2.3. Antibodies and whole mount immunofluorescence (WMIF)

Placental specimens were subjected to WMIF as previously described [21]. Details are included in Supplemental methods.

3. Results and discussion

3.1. The human syncytiotrophoblast expresses solute Carrier family 11 member 1 (SLC11A1)

To investigate SLC11A1 expression in the human placenta, RNA samples isolated from 1st trimester, 2nd trimester and term placental villi were subjected to standard RT-PCR using four distinct sets of SLC11A1-specific primers. The correct sized bands were detected in every placental RNA sample with each primer set tested (Fig. 1), indicating that the human placenta expresses SLC11A1 mRNA at multiple gestational ages.

To localize SLC11A1 expression in the human placenta, villous tissues from 1st trimester, 2nd trimester and term specimens were stained with an anti-human SLC11A1 antibody and subjected to WMIF. SLC11A1 staining was observed in the outer layer of placentas from all gestational ages examined (Fig. 2A; Supplemental Figs. 1A and 2A). In 1st trimester tissues, SLC11A1 staining colocalized with hCG, which is expressed by the syncytiotrophoblast (Fig. 2A). At later gestational ages when hCG staining decreases dramatically [21], SLC11A1 staining colocalized with cytokeratin 7, which is expressed by the syncytiotropholast and villous cytotrophoblast (Supplemental Figures 1A and 2A). A similar staining pattern was observed with two different rabbit antibodies that were generated against peptides corresponding to different regions of human SLC11A1 (data not shown). No signal above background was detected in villous tissues stained with either mouse or rabbit isotype-control Abs and the corresponding secondary antibodies (Fig. 2B, data not shown) or secondary antibodies alone (Supplemental Figures 1B and 2B), supporting the conclusion that the SLC11A1 staining pattern is specific. These results demonstrate that SLC11A1, which is critical for clearing infections by certain intracellular pathogens, is expressed by the trophoblast layer of the human placenta at multiple gestational ages.
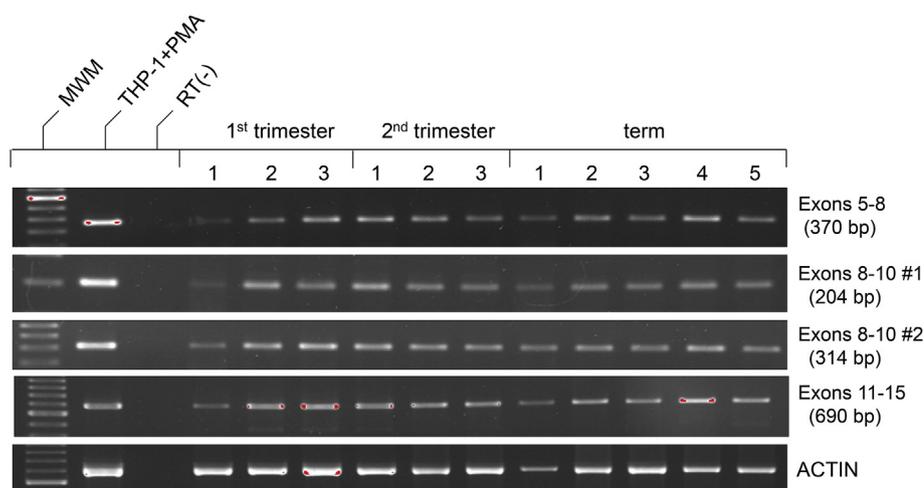
SLC11A1 is localized to late endosomal and lysosomal membranes in macrophages, and upon infection these vesicles fuse with pathogen-containing phagosomes [9,10,17]. Conflicting mechanisms have been proposed to explain the roles of SLC11A1 in clearing these infections. Based on studies in macrophages, SLC11A1 was proposed to transport iron out of pathogen-containing phagosomes, thereby depriving intracellular pathogens of essential cations required for viability [10]. A contrasting mechanism was proposed based in part on studies in Xenopus oocytes in which SLC11A1 transports iron into pathogen-containing vacuoles, which ultimately promotes hydroxy radical-mediated killing of the pathogens [9].

Although the precise antimicrobial mechanisms of SLC11A1 remain unclear, future studies are necessary to determine if it has similar functions in the placenta. SLC11A1 may also function in a broader context by regulating iron homeostasis in the placenta across gestation, and thus transport to the fetus. Interestingly, SLC11A1 gene polymorphisms have been linked with an increased risk for autoimmune diseases and infections by *Mycobacteria, Salmonella* and *Leishmania* species [9,12,13,22,23]. A subset of these SLC11A1 polymorphisms occur in the promoter region and regulate SLC11A1 transcription [24]. Thus, it would be intriguing to investigate whether there are associations between human SLC11A1 gene polymorphisms and the relative risk of congenital infections by SLC11A1-sensitive pathogens.

Declaration of interest

None.

Author contributions

IDP contributed to designing and performing the experiments, interpreting the results and writing the manuscript. LK contributed to interpreting the results and writing the manuscript. SPM conceptualized the study, and contributed to designing and performing the experiments, interpreting the results and writing the manuscript.

Fig. 1. RT-PCR analysis of SLC11A1 mRNA expression in the human placenta. RNA isolated from 1st trimester (N = 3), 2nd trimester (N = 3) and term (N = 5) human villous tissues was subjected to standard RT-PCR using four distinct primer sets specific to human SLC11A1 exons 5–8, exons 8–10 and exons 11–15. RNA from human THP-1 monocytic cells activated with phorbol 12-myristate 13-acetate (PMA) served as a positive control for SLC11A1 mRNA expression (THP-1 + PMA) [25]. Actin mRNA expression was examined as a control for RNA integrity and quantification. Sequencing of the placental PCR products confirmed that they were SLC11A1 (data not shown). MWM, molecular weight markers; RT(−), RNA from term placental tissue was subjected to cDNA reactions without reverse transcriptase and the "products" used in the polymerase chain reactions for SLC11A1 as a negative control. PCR product sizes for each SLC11A1 primer set are indicated.
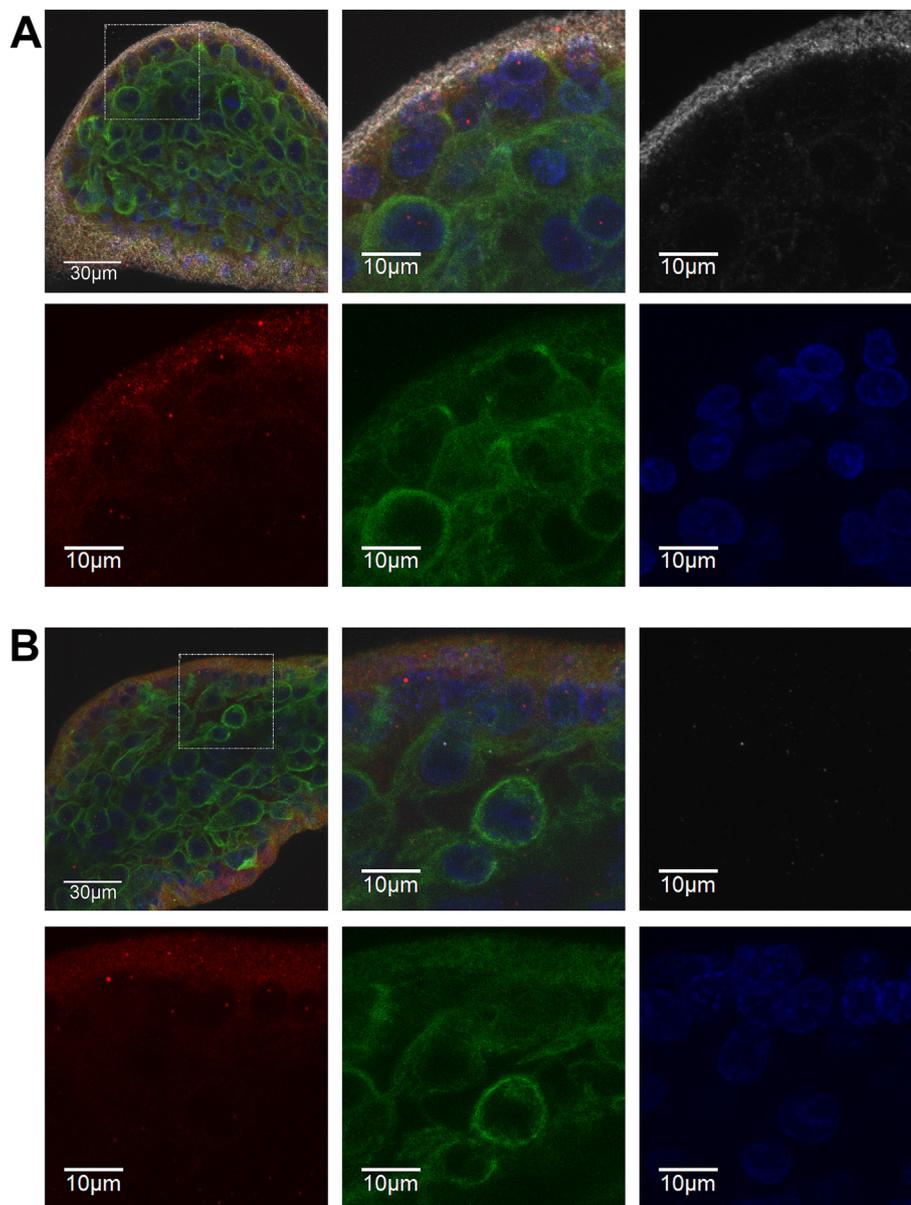
Fig. 2. WMIF analysis of SLC11A1 expression in 1st trimester placental villi. Villi from 1st trimester placentas (N = 6) were subjected to WMIF using: **A**) a mouse anti-human SLC11A1 Ab (grey; upper right panel), or **B**) mouse isotype control antibody (upper right panel), anti-CK7 Ab (green, lower left panels), anti-hCG Ab (red, middle right panels) and DAPI (blue, lower right panels) to stain for the DNA. Placental specimens from A and B were subsequently stained with the APC-conjugated goat anti-mouse IgG secondary antibody to visualize the SLC11A1 antibody and isotype control antibody staining, respectively. The upper left panels represent lower magnification views of the merged images, and the white boxes shown are magnified in the remaining images. The upper middle panels are the higher magnification, merged images. Images are representative Z-stacks of approximately 10 μm thickness. Scale bars represent 30 μM at the lower magnification and 10 μM at high magnification, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2018.11.009.

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