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Original Research Article

Slc20a2 deficiency results in fetal growth restriction and placental calcification associated with thickened basement membranes and novel CD13 and lamininα1 expressing cells

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ABSTRACT

The essential nutrient phosphorus must be taken up by the mammalian embryo during gestation. The mechanism(s) and key proteins responsible for maternal to fetal phosphate transport have not been identified. Established parameters for placental phosphate transport match those of the type III phosphate transporters, Slc20a1 and Slc20a2. Both members are expressed in human placenta, and their altered expression is linked to preeclampsia. In this study, we tested the hypothesis that Slc20a2 is required for placental function. Indeed, complete deficiency of Slc20a2 in either the maternal or embryonic placental compartment results in fetal growth restriction. We found that Slc20a2 null mice can reproduce, but are subviable; ~50% are lost prior to weaning age. We also observed that 23% of Slc20a2 deficient females develop pregnancy complications at full term, with tremors and placental abnormalities including abnormal vascular structure, increased basement membrane deposition, abundant calcification, and accumulation of novel CD13 and lamininα1 positive cells. Together these data support that Slc20a2 deficiency impacts both maternal and neonatal health, and Slc20a2 is required for normal placental function. In humans, decreased levels of placental Slc20a1 and Slc20a2 have been correlated with early onset preeclampsia, a disorder that can manifest from placental dysfunction. In addition, preterm placental calcification has been associated with poor pregnancy outcomes. We surveyed placental calcification in human preeclamptic placenta samples, and detected basement membrane-associated placental calcification as well as a comparable lamininα1 positive cell type, indicating that similar mechanisms may underlie both human and mouse placental calcification.

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1. Introduction

All living organisms require phosphorus. It is a key component of cell membranes and DNA, and plays important roles in energetics and protein signaling. Phosphorus is found circulating through blood vessels and traveling between extracellular and intracellular environments in the form of phosphoric acid, $H_2PO_4^-$. The majority of phosphorus is found in bone in the form of hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$.

Intracellular concentrations of phosphorus greatly exceed extracellular concentrations observed in serum, and phosphorus must move against a concentration gradient to produce high intracellular concentrations [1]. Transmembrane transporters move phosphorus across the cell membrane in the form of phosphoric acid. In medical literature, the term phosphate (technically referring to PO_4^{-3}) is often used when referring to either elemental phosphorus or phosphoric acid. Thus, proteins that transport phosphoric acid are referred to as phosphate transporters, including the type III sodium-dependent phosphate transporters Slc20a1/PiT-1 and Slc20a2/PiT-2.

Phosphorus is consumed through diet in adults and is transferred from the mother to the fetus during pregnancy. The human fetus contains ~16–30 g of phosphorus at birth, with the majority obtained at the time of bone growth during late gestation [2,3]. Despite the rapid cell division, extensive protein signaling, bone development, and other phosphate-dependent processes that occur during this time in development, maternal to fetal phosphate transport mechanisms remain unknown. Several characteristics have been established, however. Fetal serum has been shown to have a higher concentration of phosphate than maternal serum; because transport flux is against a concentration gradient, transfer is believed to be active [4,5]. In addition, placental phosphate transport is regulated by parathyroid hormone, pH, and sodium availability, characteristics that match those of Slc20a1 and Slc20a2 [6–8].

The majority of maternal to fetal nutrient transfer occurs across the placenta. Recent studies support that Slc20a1 and Slc20a2 are poised to control placental phosphate transport. Both Slc20a1 and Slc20a2 are expressed in human placentas, and their altered expression is associated with placental dysfunction and preeclampsia [9,10]. Slc20a1 global KO mice are early embryonic lethal, precluding analysis of Slc20a1 roles in the late term placenta [11–13]. In this study, we sought to test the hypothesis that Slc20a2 plays an important role in placental function.

2. Materials and methods

2.1. Specimens

2.1.1. Mouse specimens

All mouse work was performed with IACUC approval (protocol #2224-08) from the University of Washington, Seattle. C57BL/6NTac-Slc20a2<tm1a(EUCOMM)Wtsi>/Ieg (Slc20a2+/−) mice were purchased from the European Mouse Mutant Archive (EMMA). Wildtype (Slc20a2+/+) C57Bl/6 mice were purchased from Jackson labs (Sacramento, CA) and from Taconic labs (Hudson, NY). At least 3 animals/genotype were analyzed for each experiment; specific animal numbers are noted throughout.

2.1.2. Human specimens

Placentas were collected with IRB approval from the University of Washington, Seattle; all subjects provided written informed consent for placental collection. Preeclampsia was defined by standard clinical criteria [14]. Gestational ages were between 33 and 40 weeks, specifically: 33 weeks 2 days, 35 weeks and 0 days, and 39 weeks 1 day.

2.2. Western blotting

Kidney and placental lysates were collected and treated with a protease inhibitor cocktail (Roche). Lysates were denatured at 90 °C in Laemli Buffer containing β-Mercaptoethanol, and run in a 10% SDS Page gel. 40 μg of protein was loaded per well. Proteins were then transferred to a PVDF membrane, and blots were blocked with 5% milk. Slc20a2 antibody (provided by Dr. Moshe Levi, UC) was used at a concentration of 3.2 μg/mL and the primary antibody was detected with Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 111-035-144) at 3 μg/mL. Western Lighting Plus ECL (PerkinElmer), was used to detect HRP signal. In order to determine loading control values, the western blot was stripped with Restore Plus Western Blot Stripping Buffer (Thermo Scientific PI-46430) and reprobed with β-actin (Abcam ab8227). Densitometry was performed with ImageJ.

2.3. RNA extraction, cDNA synthesis, and qPCR

Placenta tissue RNA was extracted using the RNeasy Mini Kit according to the manufacturer's directions (Qiagen, 74106). cDNA was made with 1000ug of total RNA per sample using the Omniscript Reverse Transcriptase kit (Qiagen, 205113). TaqMan probes conjugated with a fluorochrome reporter (FAM) tag at the 5′-end and an MGB quencher at the 3′-end were used to assess expression levels. Amplification and detection were carried out in 96-well optical plates on an ABI Prism 7000 Sequence Detection System (Applied Biosystems), with TaqMan Universal PCR 2X master mix (Life Technologies, 4305719) in a final volume of 20 μL per reaction. Each reaction was carried out at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Results were analyzed with the manufacturer's software, SDS 1.1 (Applied Biosystems). Gene mRNA expression was normalized to the housekeeping gene 18S (Life Technologies, Cat. No. 4308329) using the quantitative method ($2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = [C_T^{gene} - C_T^{18S}]_{treated} - [C_T^{gene} - C_T^{18S}]_{control}$). The following Life Technologies Taqman Assays were used: Slc20a2 (Mm00660204_mH), IL-6 (Mm00446190_m1), Il-10 (Mm00439614_m1), Arg1 (Mm00475988_m1), TNF (Mm00443258_m1).

2.4. Histology

2.4.1. Fixation, embedding and sectioning

Samples were dissected and fixed in 4% paraformaldehyde (PFA)/PBS overnight at 4 °C. After whole mount images were