Loss of the phosphate transporter Slc20a2 leads to abnormal placentation and abundant placental calcification in the mouse.

Placenta Research Symposium
University of Washington
Turner Auditorium, D209
March 24th, 2016, 8:05am

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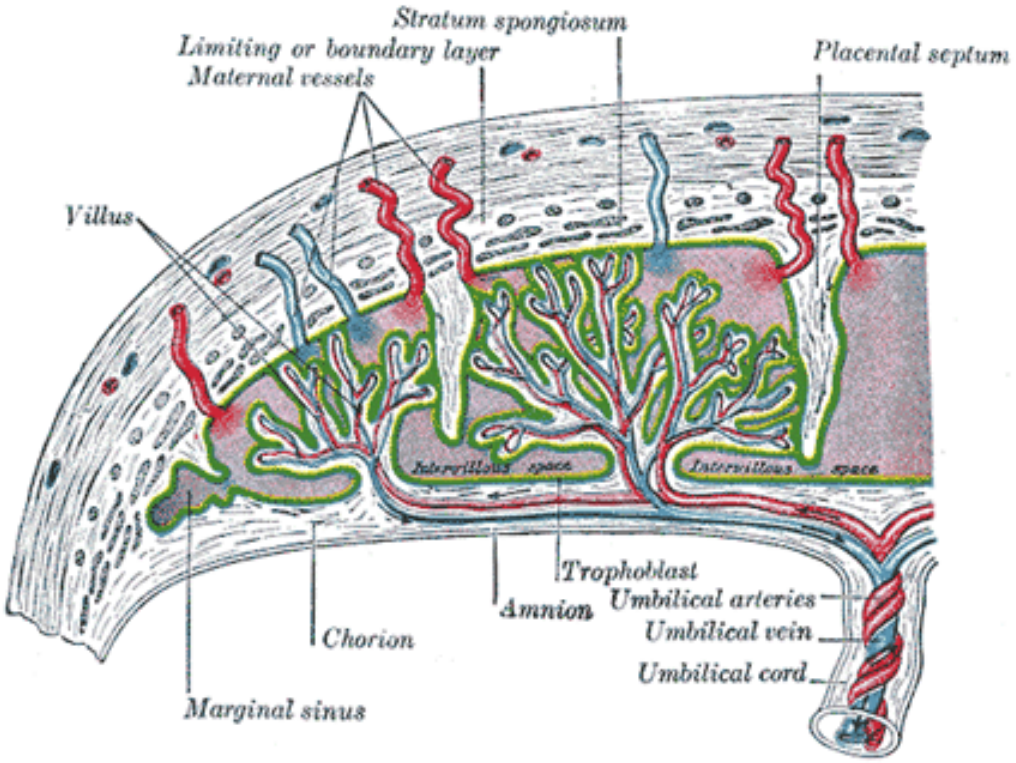
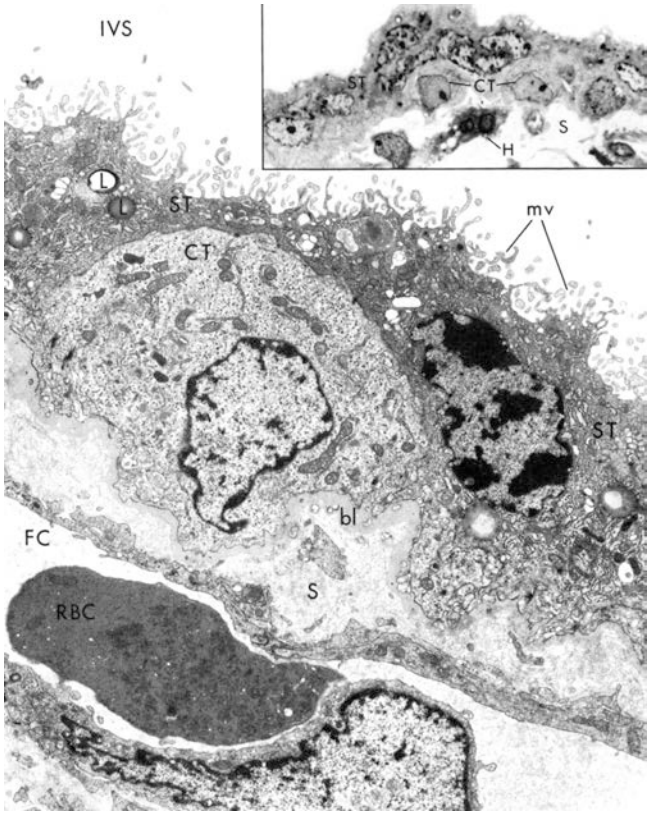
Major Functions of the Placenta

Functions

1. Facilitates transfer of nutrients and oxygen from mother to fetus
2. Facilitates removal of fetal waste products
3. Systemic modulation of maternal immune responses - trophoblast/maternal blood interaction
4. Fetomaternal tolerance - placenta/decidua interaction
5. Acts as an endocrine organ: synthesizes and secretes: hormones, neurotransmitters, vasoactive factors





Weisset al 1988, Adamson and Cross 2002, Erlebacher 2013, Iriyama 2015, Gray's Anatomy

Pregnancy and Preeclampsia

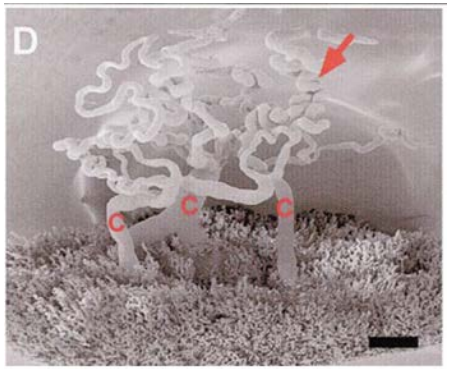
Maternal Physiological Adaptations

- Increased cardiac output
- Redistribution of blood flow
- Hyperventilation facilitating exchange of gases
- Insulin resistance (mobilizes glucose, amino acids, lipids
- Remodeling of the uterine spiral arteries

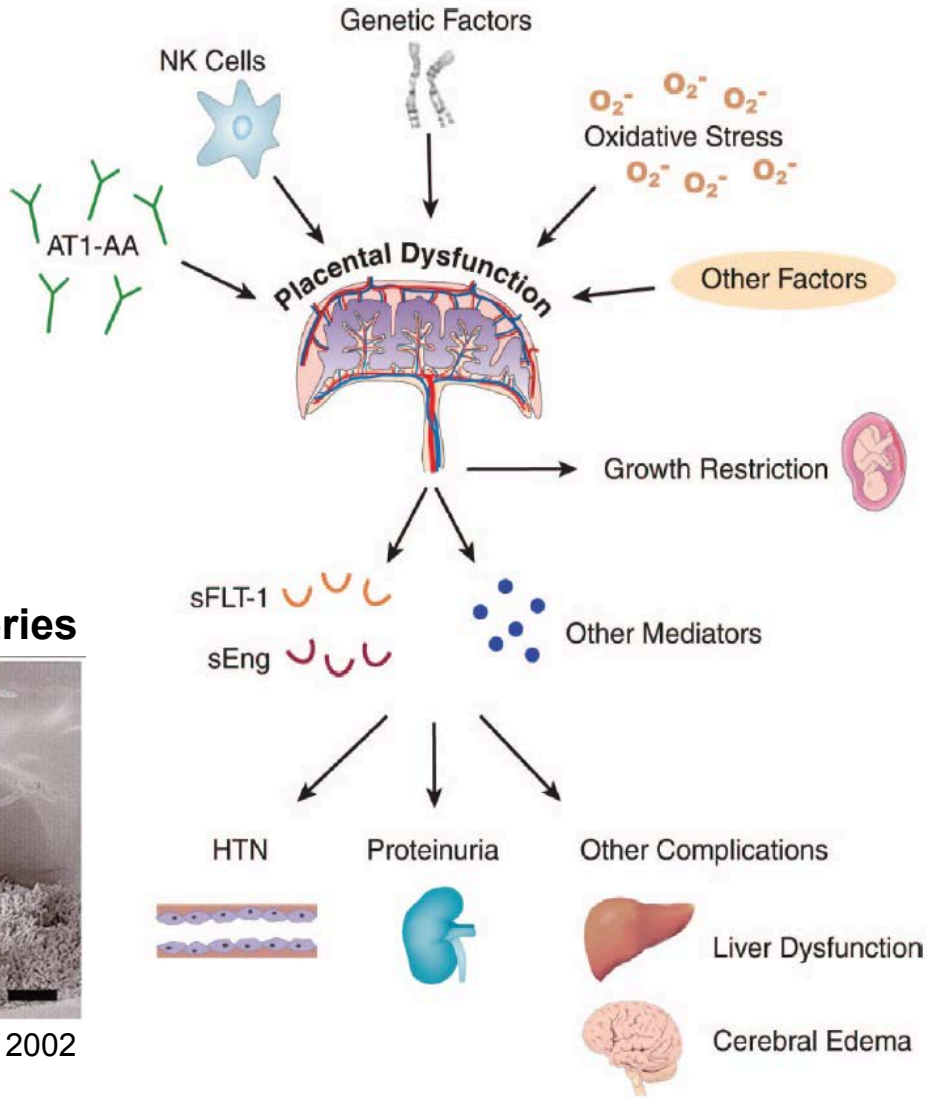
Preeclampsia

- Onset of hypertension and proteinuria or other clinical presentation after the 20th wk
- Spiral artery remodeling is frequently incomplete
- Resistance to vasospasm is compromised
- Blood pressure increases
- Cure: delivery of the placenta
- **HELLP or Eclampsia**

Mouse Spiral Arteries



Adamson 2002

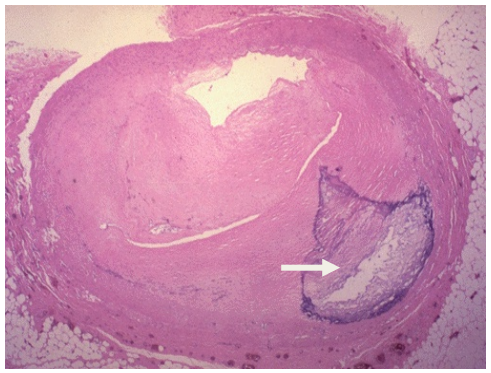


Diaz et al 2014 and Powe et al 2011

Vascular calcification and phosphate have emerging roles in cardiovascular health.

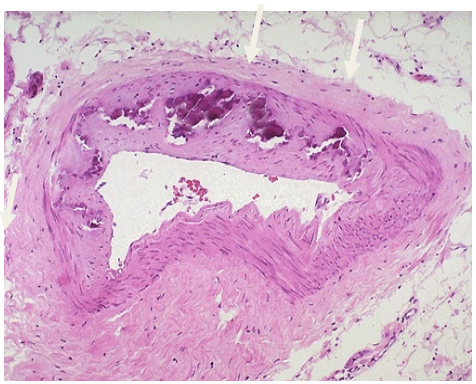
Vascular calcification is an independent **risk factor** for **cardiovascular morbidity**. The specific site of vascular calcification within the blood vessel dictates how it will impact the cardiovascular system.

Intimal Calcification



- Predictor of coronary artery disease and atherosclerotic plaque burden
- Associated with **sudden cardiac death**
- Calcification in fibrofatty lesions is associated with **vulnerable plaque morphology**

Medial Calcification



- Increased **aortic stiffness**
- Increased pulse wave velocity
- Increased **pulse pressure**
- Increased left ventricular hypertrophy

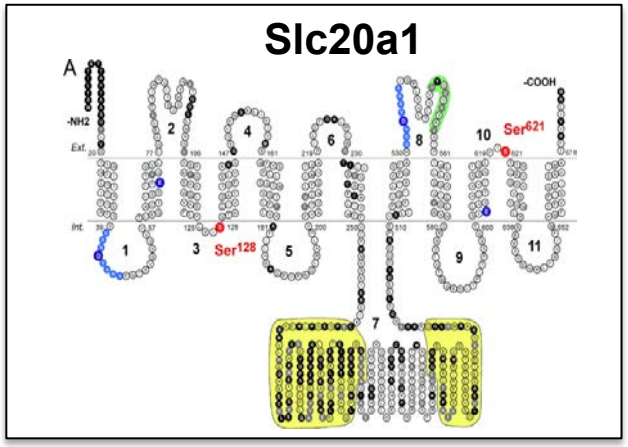
How does vascular calcification form?

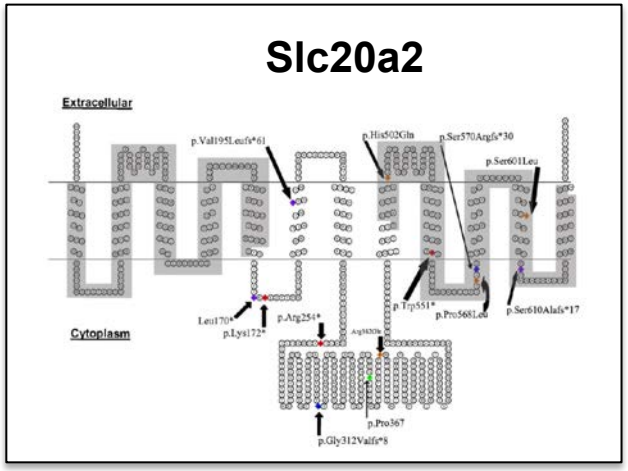
- Classic types of calcification: **physiological** (similar to bone), **dystrophic** (ischemia-related), or **metastatic** (supersaturated environment).
- Over the last ten years, VC researchers have found that this is an **oversimplification**.
- **High phosphate** induces calcification both in cell culture and in animal models.

Phosphate transporters import and export phosphoric acid (phosphate) and…

Phosphate

- An anion that contains elemental phosphorus (PO_4^{-3})
- Essential in cells (metabolism, signaling, cell structure)
- The majority of phosphate (~85%) is stored in bone (mobilized during pregnancy)
- Replaced through dietary consumption in adults
- Phosphate circulates through the blood stream as phosphoric acid (H_3PO_4)
- Moves against a concentration gradient to enter the cell
  - Serum: 0.8 to 1.5 mM
  - Intracellular: 100mM
- Transport is controlled by transmembrane sodium-dependent phosphate transporters
- Alteration of phosphate transporters can lead to calcium phosphate mineral ($Ca_5(PO_4)_3(OH)$) deposition
- Many Pi-transporters also transport other molecules: glucose, sodium, viral receptors

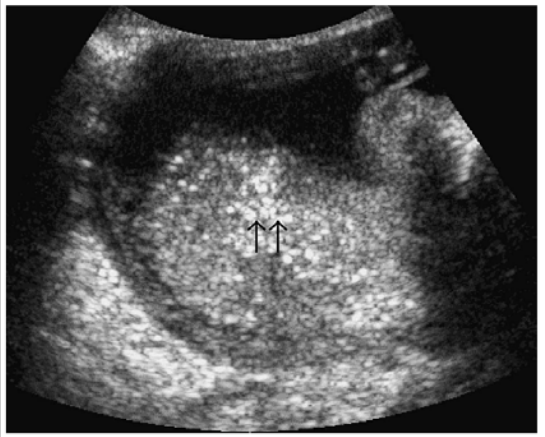




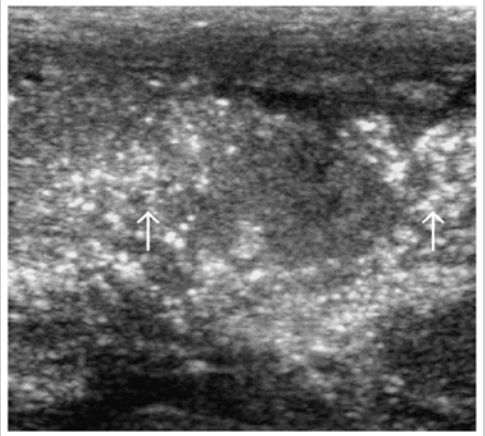
Placental Calcification

- Placental calcification literature is limited and discordant.
- Placental calcification may be a metastatic process[1].
- Calcification is a marker of viral infection (Rubella, CMV, HSV, VZV, ZIKV)[2,4].
- Preterm calcification has been associated with a higher incidence of adverse pregnancy outcomes[3].

Chronic Placentitis



CMV



Bailao et al 2005

ZIKV

Mlaker et al 2016

References: 1. Demer et al 2001, 2. Bailao et al 2005, 3. Chen et al 2011 4. Mlaker et al 2016.

Preterm placental calcification may be associated with adverse outcomes.

Table 2 Pregnancy outcomes of the women in the three study groups

Outcome	Preterm calcification 28-32wks n=63	Term calcification 32-36wks n=192	Not Calcified 28-36wks n=521	P
Maternal outcome				
Postpartum hemorrhage				0.047*
Yes	6 (9.5)	5 (2.6)	15 (2.9)	
No	57 (90.5)	187 (97.4)	506 (97.1)	
Placental abruption				0.007**
Yes	4 (6.3)	1 (0.5)	3 (0.6)	
No	59 (93.7)	191 (99.5)	518 (99.4)	
Maternal transfer to ICU				0.004**
Yes	4 (6.3)	1 (0.5)	2 (0.4)	
No	59 (93.7)	191 (99.5)	519 (99.6)	
Fetal outcome				
Preterm birth				0.007**
Yes	9 (14.3)	7 (3.6)	20 (3.8)	
No	54 (85.7)	185 (96.4)	501 (96.2)	
Low birth weight				< 0.001***
Yes	14 (22.2)	9 (4.7)	30 (5.8)	
No	49 (77.8)	183 (95.3)	491 (94.2)	
Low Apgar score†				0.006**
Yes	6 (9.5)	3 (1.6)	8 (1.5)	
No	57 (90.5)	189 (98.4)	513 (98.5)	
Neonatal death				0.024*
Yes	3 (4.8)	1 (0.5)	2 (0.4)	
No	60 (95.2)	191 (99.5)	519 (99.6)	

Chen et al 2011

Phosphate Transport and Vascular Calcification in Pregnancy

Research Questions:

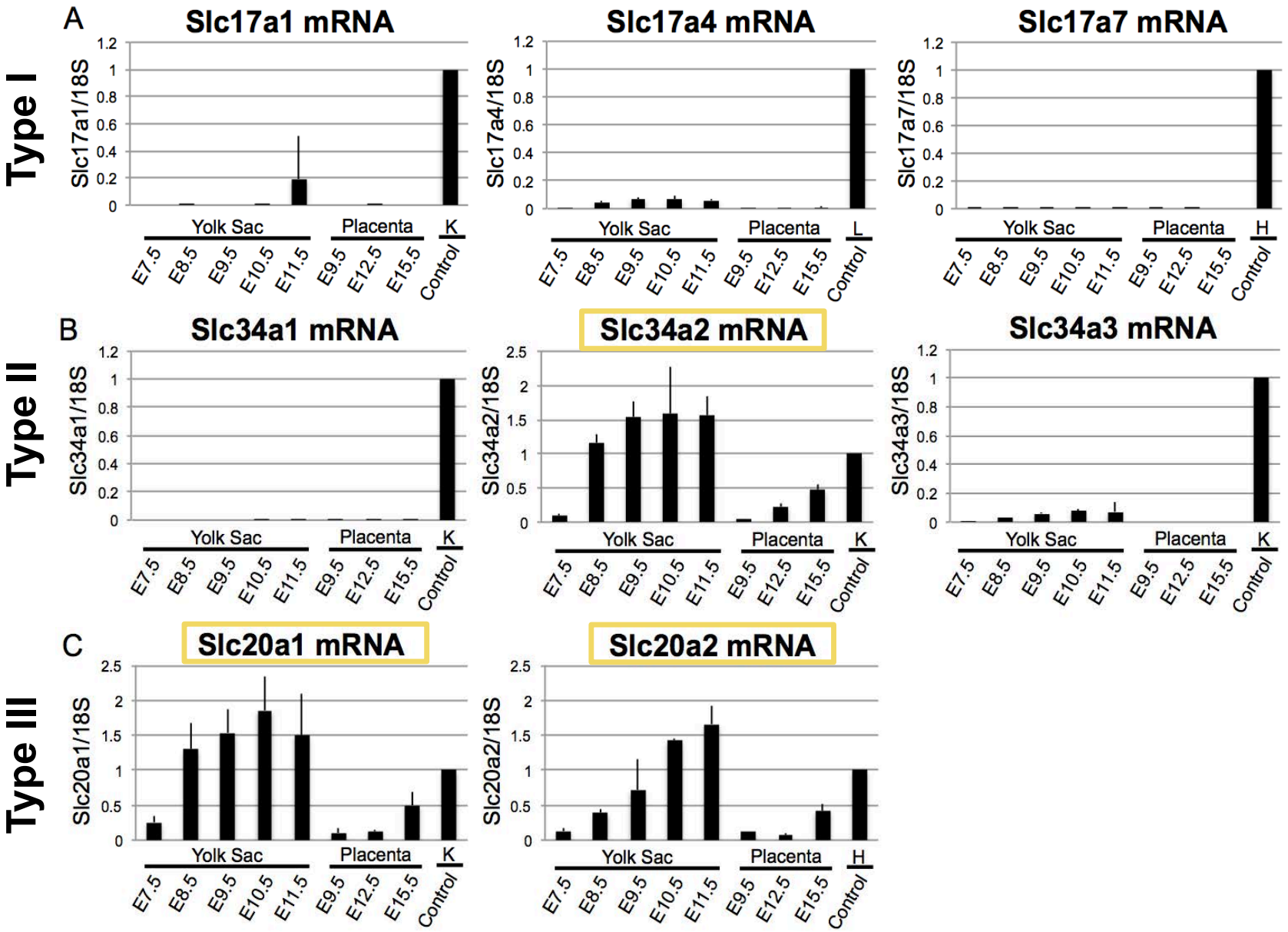
1. How does phosphate get to the developing embryo?
2. What VC mechanism(s) promote placental calcification?
3. What impact does placental calcification have on placental function?

Maternal-fetal phosphate transport: What is known

- The human embryo obtains ~16-30g of phosphorus during development
- Transport occurs against a concentration gradient
- Placental Pi transport parameters match those of type III sodium-dependent transporters: Slc20a1 and Slc20a2
  - Moderated by parathyroid hormone, pH, and sodium availability
  - Each Pi molecule is accompanied by at least 2 sodium molecules.

References: Brunette et al 1986; Lajeunesse and Brunette 1988; Brunette et al. 1989; Stulc 1997; Schauberger and Pitkin 1997; Nishimura et al. 2008; Beck et al. 2009; Hsu et al. 2012, Yang et al. 2014.

Candidate Maternal-Fetal Na-Dependent Pi Transporters

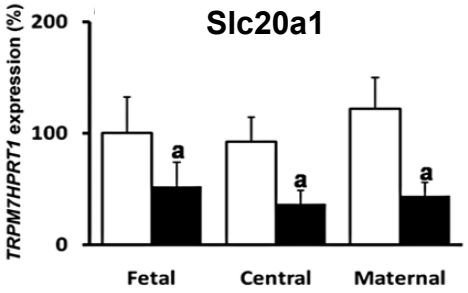


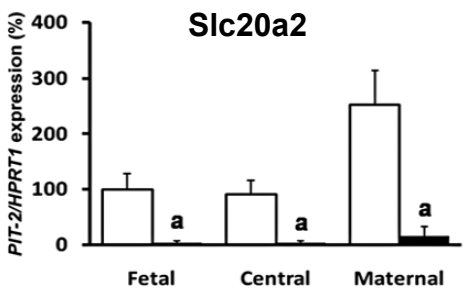
All three candidate transporters display developmental phenotypes.

- **Slc34a2 null**: embryonic lethal from unknown causes (Shibasaki 2009).
- **Slc20a1 null**: embryonic lethal possibly due to yolk sac vascular remodeling defect (Wallingford et al. 2014).
- **Slc20a2 null**: develop neurovascular and placental calcification, abnormal placental vascular remodeling, fetal growth restriction, subviability, maternal morbidity (Wallingford et al 2016a, 2016b).

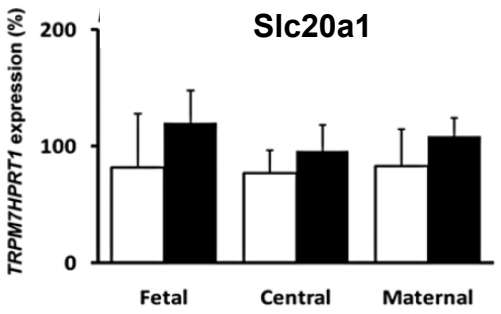
Slc20a1 & Slc20a2 Levels Are Altered in PE (Yang et al 2014)

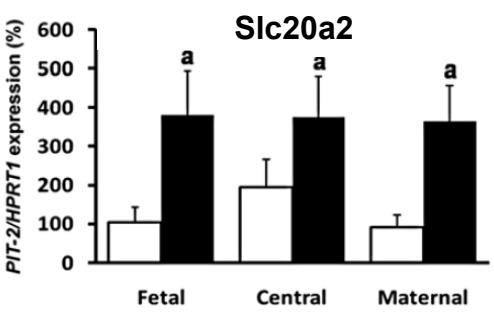
Less than 36wks



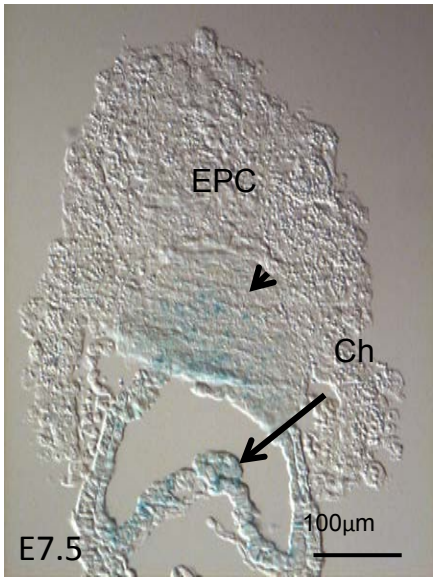


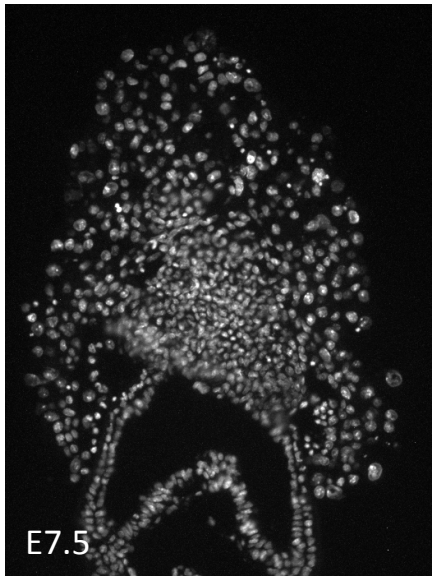
More than 36wks

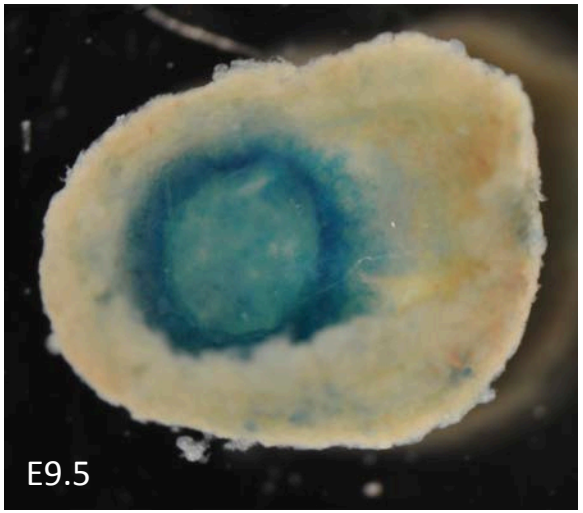


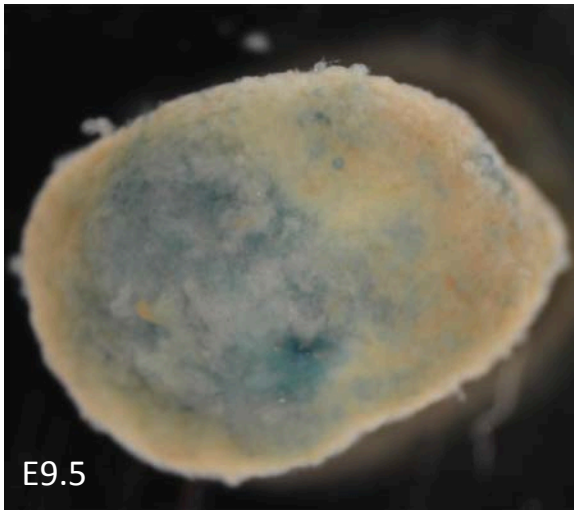


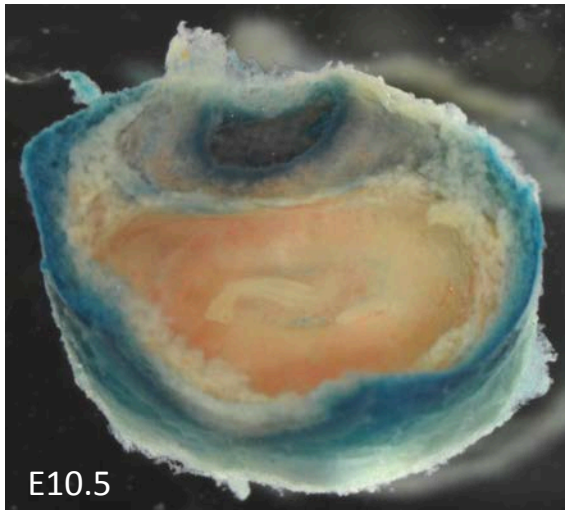
Slc20a2 is expressed during mouse placentation.





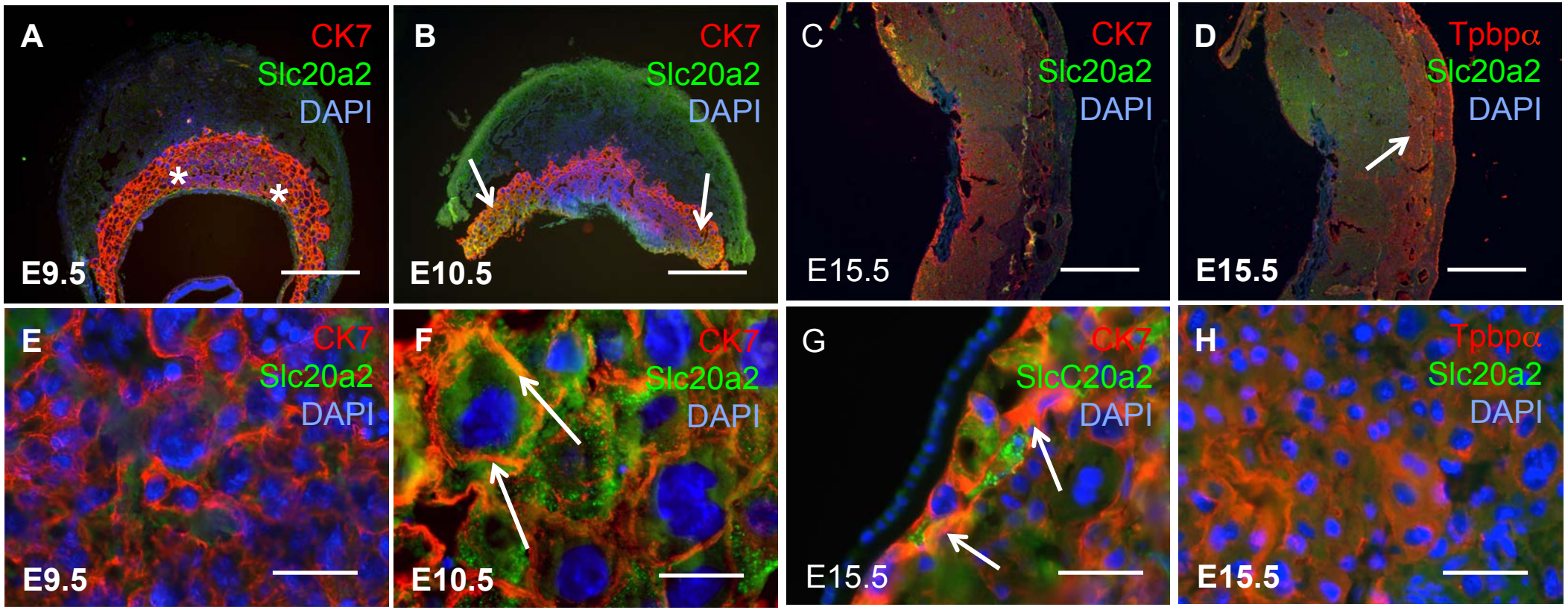






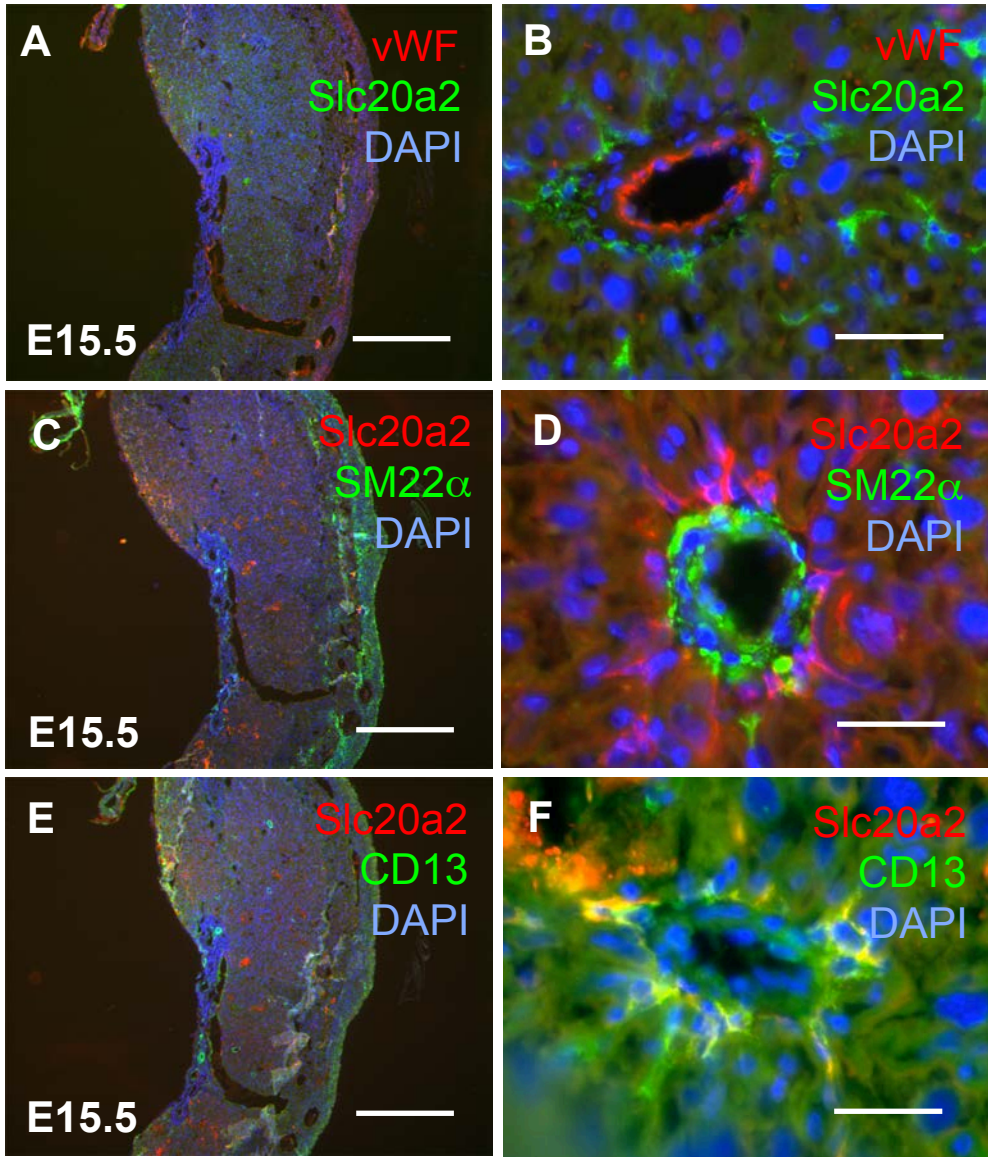
Slc20a2 is expressed at the mouse chorionic plate.

During placentation, Slc20a2 is expressed in several cell types, including specific populations of trophoblasts.

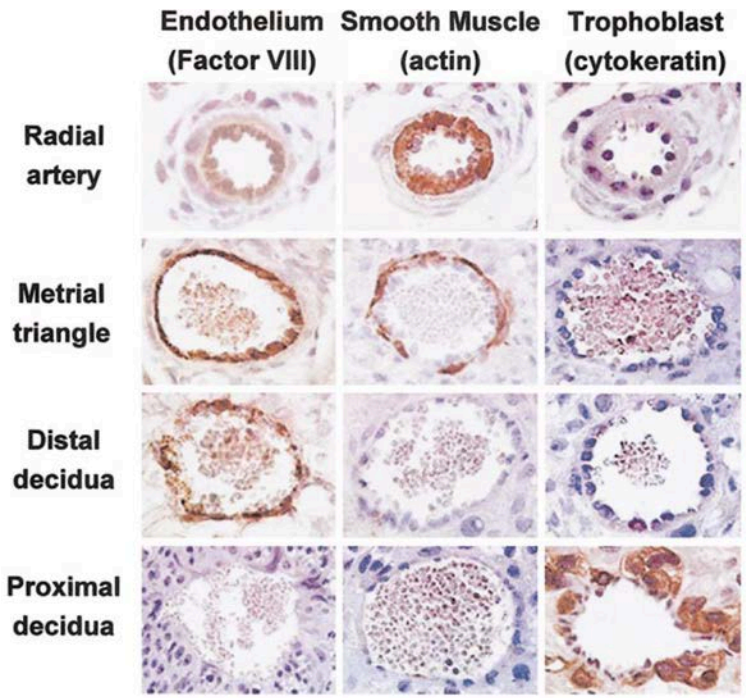


Wallingford et al 2016, Reproductive Biology

Slc20a2 is detected in CD13 positive cells in metrial triangle vessels.

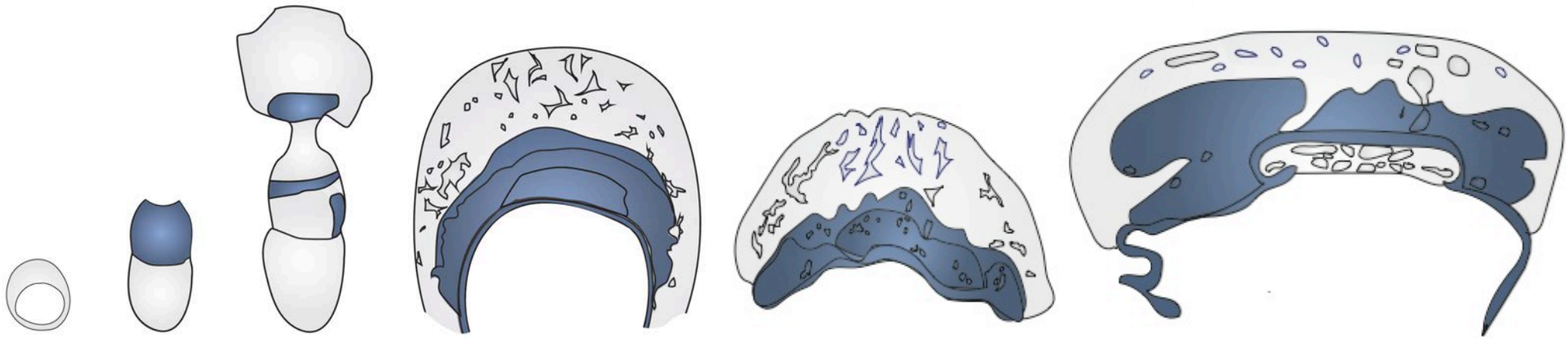


Wallingford et al 2016, Reproductive Biology



E13.5 Adamson et al. 2002

Summary of Slc20a2 Expression - Mouse

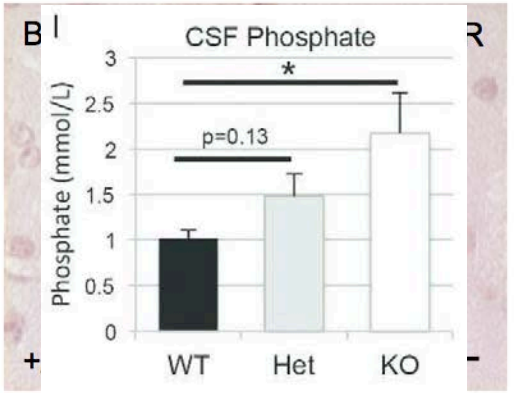


E3.5	E5.5	E7.5	E9.5	E10.5	E15.5
ND	EXE	1. EPC 2. chorion 3. allantois	1. parietal endoderm, laminin+ 2. fetal endothelial cells, vWF+ 3. labyrinth pericytes, CD13+	1. trophoblast at chorionic plate, CK7+ 2. pericytes in decidua, CD13+	1. trophoblast at chorionic plate, CK7+ 2. trilaminar trophoblast cells, laminin+ 3. pericytes in decidua, CD13+

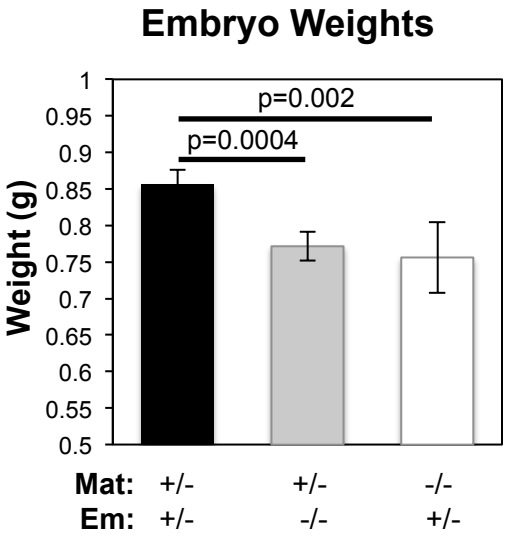
Slc20a2 KO mice are subviable.

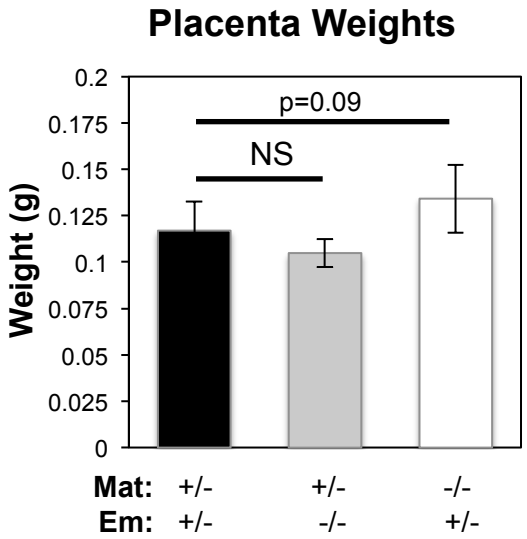
Animals Generated by Slc20a2 Heterozygous Intercrosses

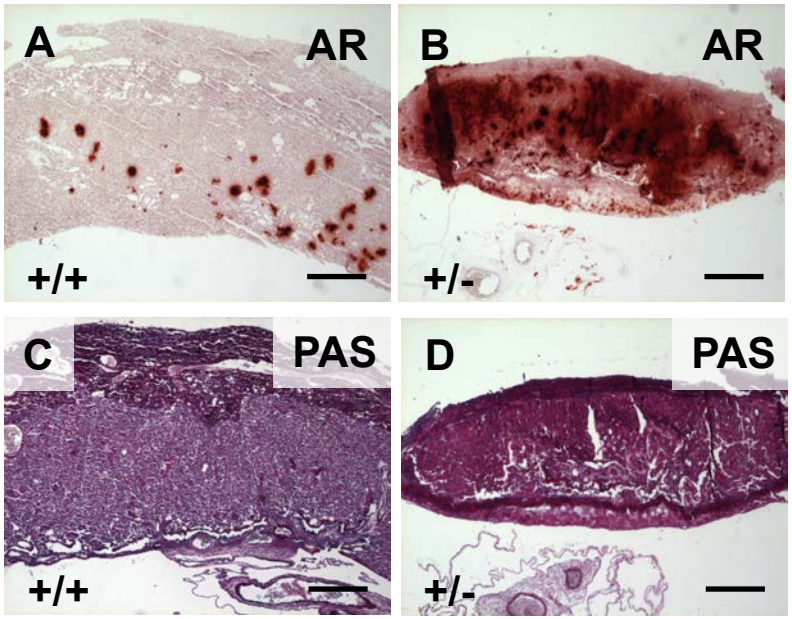
Parameter	Slc20a2 +/+	Slc20a2 +/-	Slc20a2 -/-	Total
Expected (%)	25	50	25	100
Expected Number of Animals	42	84	42	168
Experimental (%)	37	49	14	100
Experimental Number of Animals	42	55	16	113
Lost prior to weaning (%)	0	35	62	N/A



Wallingford et al 2016, Brain Pathology

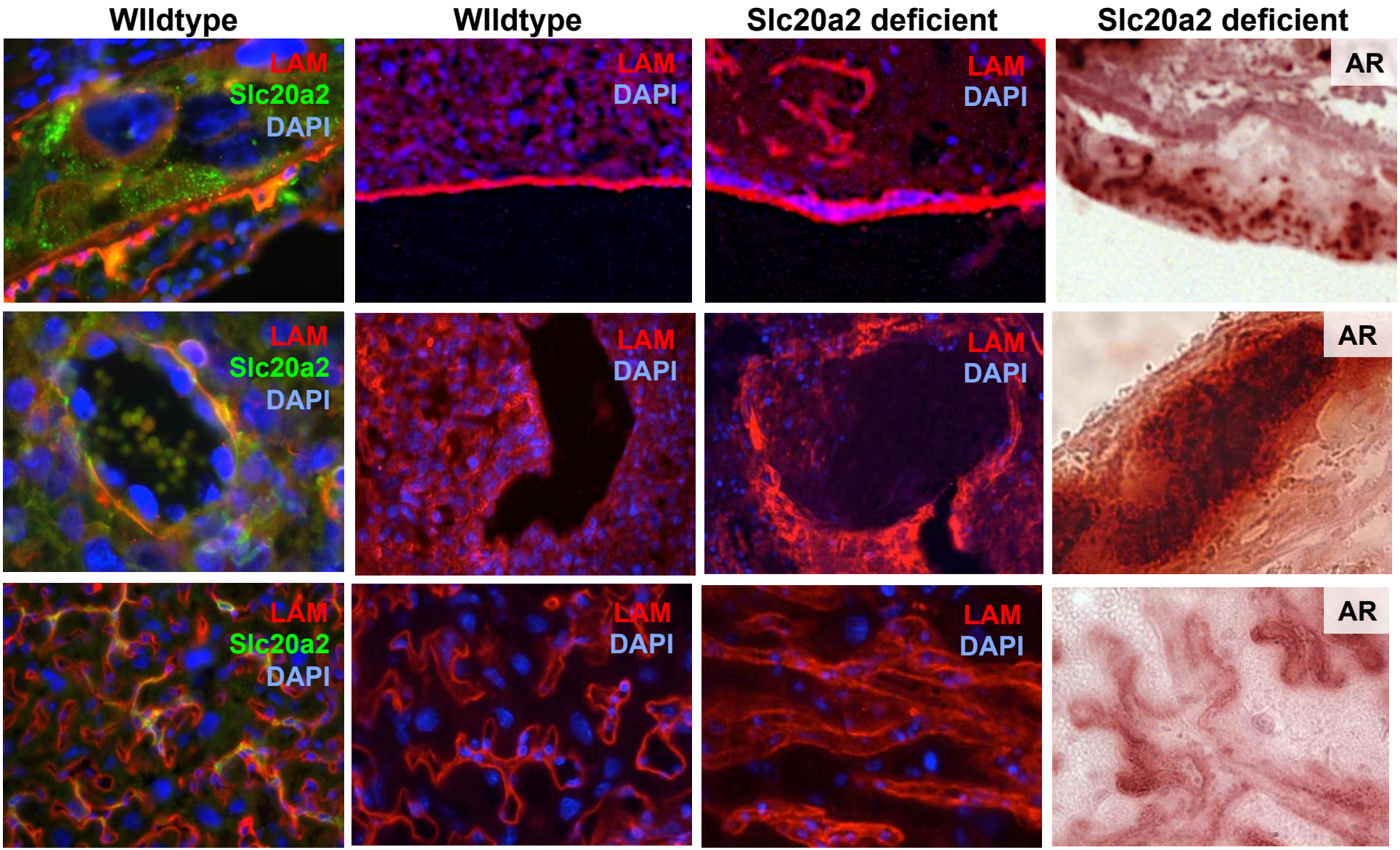




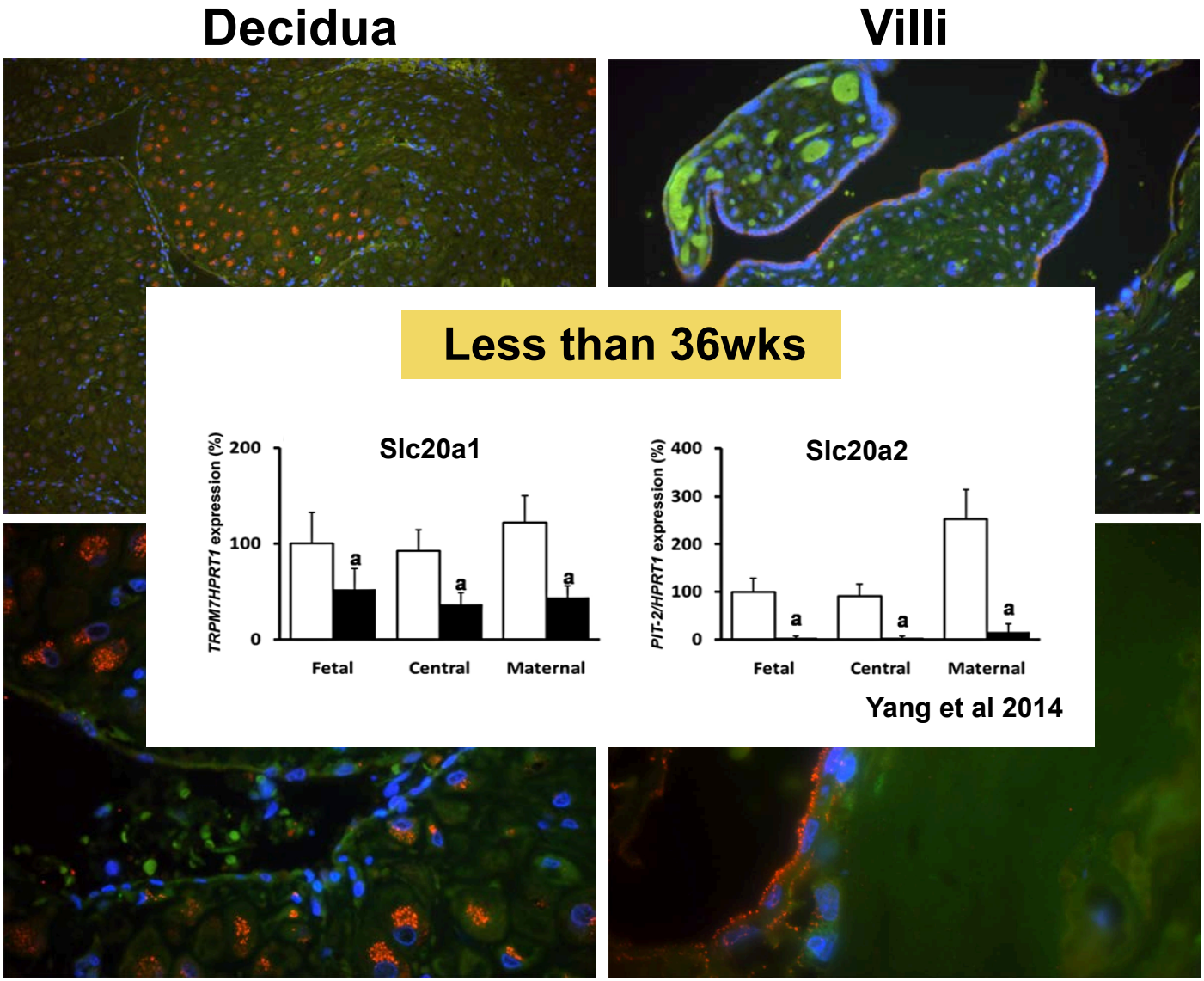


Wallingford et al 2016, Reproductive Biology

What is the mechanism of Slc20a2-mediated calcification inhibition?



Slc20a2 is expressed in human placenta (28-33wks).



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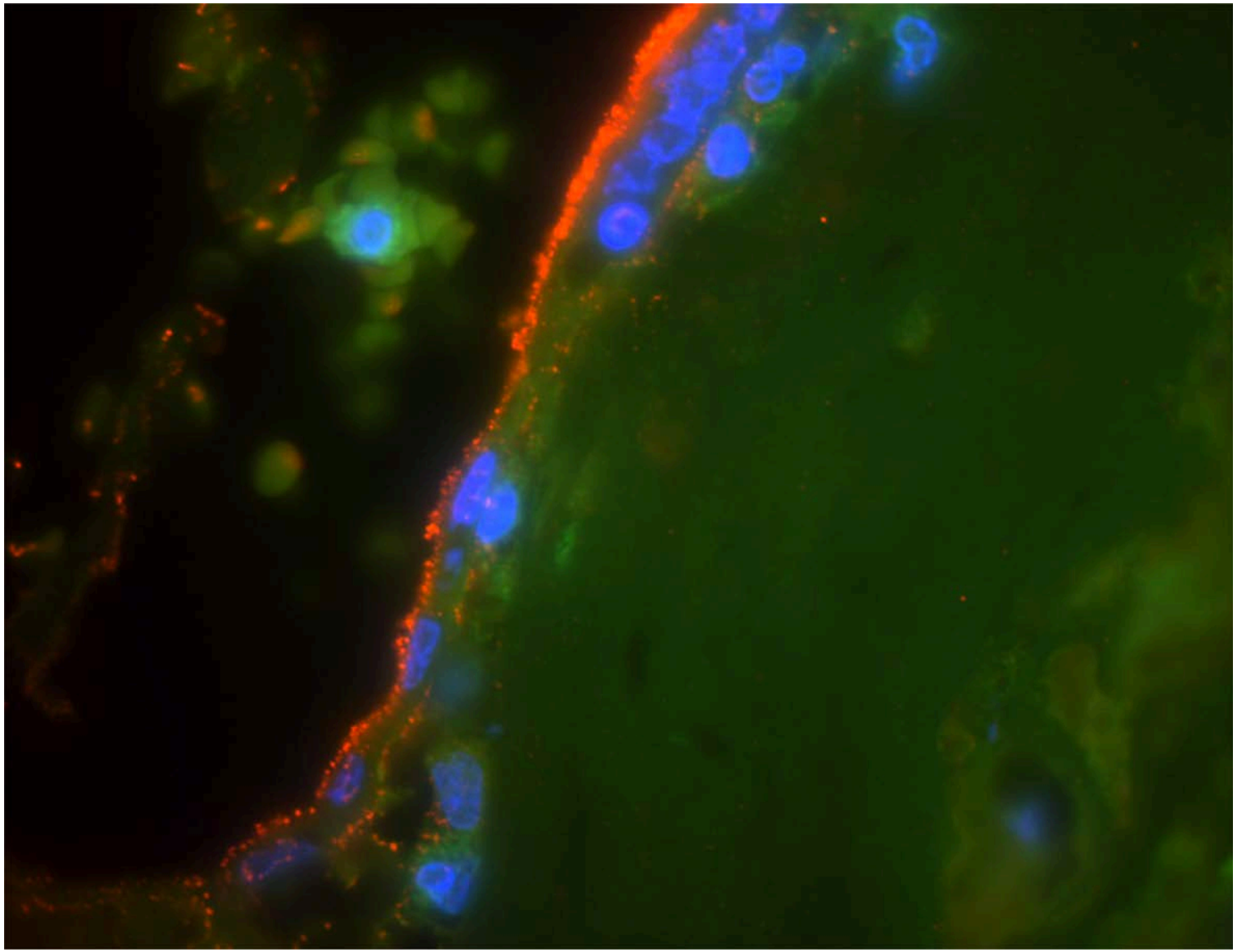
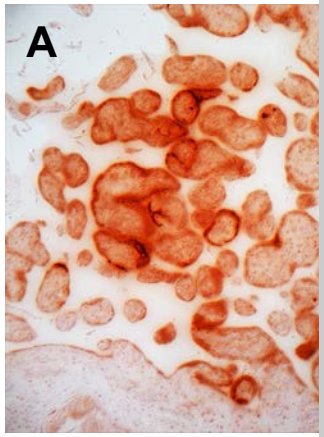
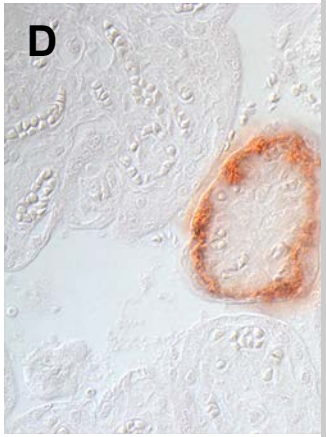


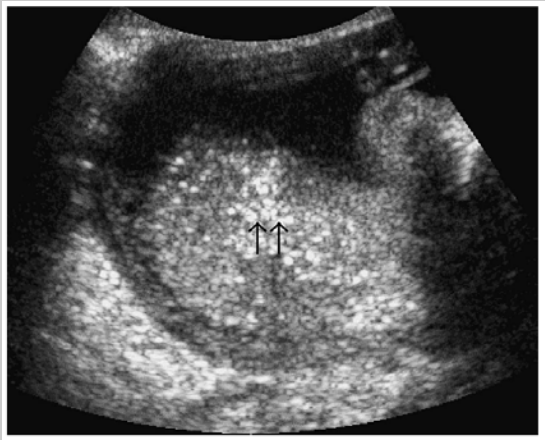
There are three distinct types of calcification in human placenta (28-33wks).

Syncytial

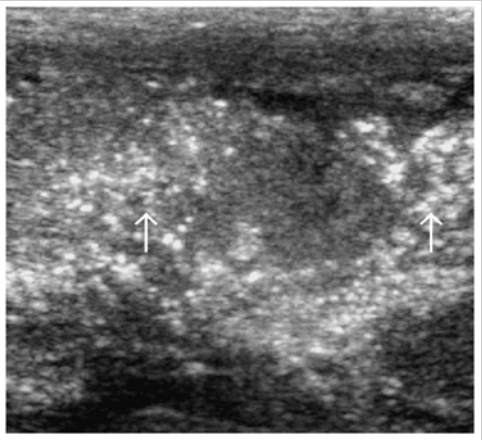




Chronic Placentitis



CMV

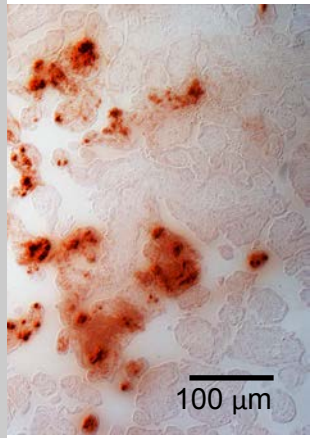


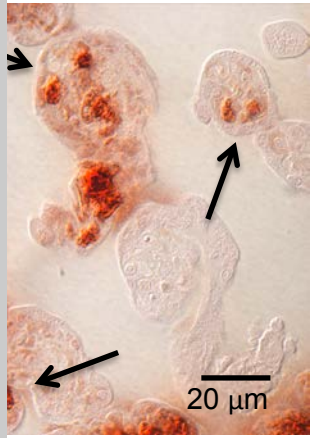
Bailao et al 2005

ZIKV

Mlaker et al 2016

apillaries





Summary and Future Work

Summary

1. Phosphate dysregulation can cause vascular calcification.
2. Placental calcification may associate with adverse pregnancy outcomes.
3. Slc20a1 and Slc20a2 are altered in preeclampsia.
4. Slc20a2 plays an anti-calcific role in the placenta.
5. The Slc20a2 null mouse is the first-ever placental calcification model.
6. Slc20a2 is abundant in human synCt cells
7. There are three distinctive types of placental calcification in human villi.

Conclusions

1. Candidate mouse placental Pi transporters include: Slc34a2, Slc20a1, Slc20a2.
2. Optimized imaging systems are needed to accurately assess and understand clinical placental calcification.

Future Directions

1. Determine biological relevance of Slc34a2, Slc20a1 and Slc20a2 and maternal-fetal phosphate transport.
2. Test hypothetical mechanisms of placental vascular calcification.
3. Evaluate how placental calcification impacts placental function.

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