Type III Sodium-Dependent Phosphate Transporter Encoded by Gene *Slc20a2* as a Hard Tissue Engineering Target

Philip A. Walczak[1], Shunsuke Yamada[2], Elizabeth M. Leaf[3], Timothy Cox[4], Kenichi Nagano[5], Roland Baron[5], Cecilia M. Giachelli[1,6]
[1]Dept. of Oral Health Sciences, School of Dentistry, University of Washington, Seattle, USA, [2]Kyushu University, Fukuoka, Japan, [3]Seattle Children's Research Institute, Seattle, USA, [4]University of Missouri-Kansas City, Kansas City, USA, [5]Harvard School of Dental Medicine, Boston, USA, [6]Dept. of Bioengineering, University of Washington, Seattle, USA

Inorganic Phosphate in Tissue Engineering

Increased use of intraosseous implants and bone grafting materials requires an understanding of many factors influencing their success. Inorganic phosphate (Pi) is an important factor in hard tissue mineralization and tissue regeneration: increased Pi availability by alkaline phosphatase adhesion on a fibrin scaffold was shown to significantly increase bone formation over an untreated fibrin scaffold in mouse calvarial defects (1). Our lab was the first to report that phosphate transporter *Slc20a2* KO mice exhibit stunted growth and decreased bone mineral density compared to WT (2). **Here we extend these studies to further investigate the role of *Slc20a2* in regulating hard tissue differentiation and mineralization to provide tools for tissue engineering applications.**

Objectives

1. Investigate the role of PiT-2 in bone and tooth formation *in vivo.*
2. Investigate the effects of PiT-2 knockout (KO) on bone mineralization through histomorphometry.

Methods

Mice: C57Bl/6NTac-Slc20a2<tm1a (EUCOMM)Wtsi>/Ieg (Slc20a2) mice were used to generate PiT-2 WT and KO littermates (0.98% Pi diet). Calcein labelled tibias, femurs, and vertebrae used for histomorphometry.

MicroCT: MicroCT scans were obtained (34.42 µm resolution, 60kV, 170 µA, 0.5mm Al filter, 120ms exposure, rotation step of 0.6*, 180 scans, 3 frame averaging). Raw data reconstruction was completed using accompanying software for the microCT scanner.

Histomorphomtetry: Undecalcified 4-µm-thick sections were obtained by microtome. Three consecutive sections were Von Kossa stained, unstained for fluorescence labeling, and 3% Toluidine Blue stained.

Bone histomorphometric analysis was performed in the lumbar vertebra at 200X magnification in a 1.35 mm high x 1.3 mm wide region located 400 µm away from the growth plate using open source semi-automated bone histomorphmetry software.

The structural parameters were obtained by calculating an average of 2 separate measurements of consecutive sections. The structural, dynamic, and cellular parameters were calculated and expressed according to the standardized nomenclature (3).

Mineralized Bone Volume Decreases With *Slc20a2* Deficiency

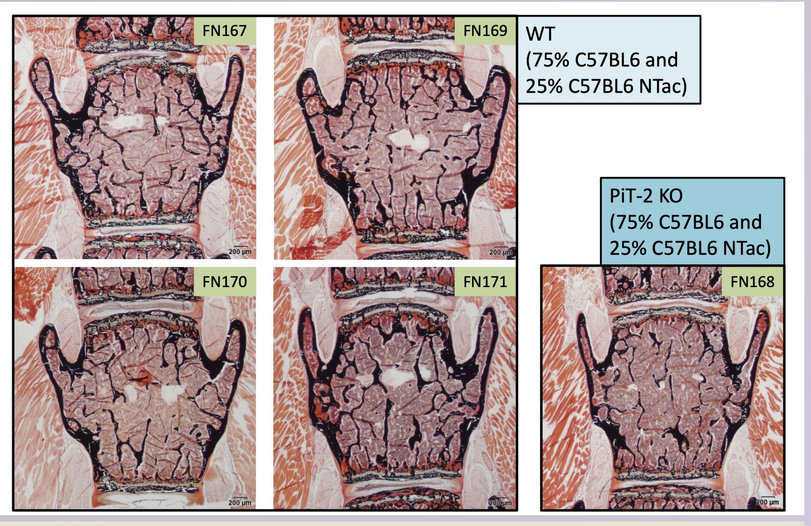

Figure 2: Von Kossa stained PiT-2 WT and KO mouse vertebrae imaged at 20x magnification showing decreased trabeculation and relative bone volume in PiT-2 KO female mice compared to PiT-2 WT female mice.

Slc20a2 KO Mice Exhibit Blunted Incisors

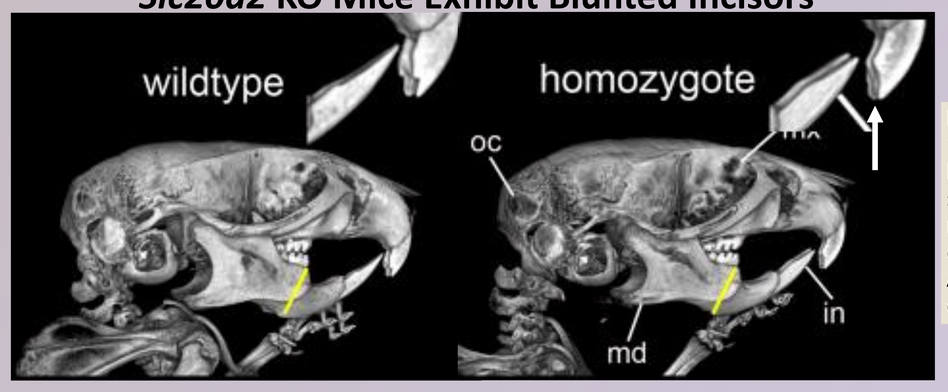

Figure 1: microCT images from 10 week old PiT-2 KO homozygous mouse (right) showing narrower angle of mandible and blunted incisors compared to the WT (left). Arrow points to KO incisors exhibiting blunting.

Slc20a2 KO Decreases Osteoblast Number and Function

Parameter	Wild Type (n=9)	*Slc20a2* Knockout (n=6)	p value (t test)
BV/TV (%)	11.9±2.56	8.84±1.29	0.0194*
Tb.N (/mm)	3.10±0.38	2.75±0.41	0.1343
Tb.Th (µm)	37.7±4.45	33.3±1.65	0.0540
MAR (µm/day)	1.78±0.13	1.17±0.05	<0.001*
BFR/BV (%/day)	4.33±0.68	3.38±0.38	0.0084
BFR/TV (%/day)	0.31±0.09	0.29±0.03	<0.001*
N.Ob/T.Ar (/mm²)	95.6±23.3	56.9±17.2	0.0041*
OV/TV (%)	0.32±0.08	0.19±0.11	0.0245*
N.Oc/T.Ar (/mm²)	24.6±2.59	23.6±4.71	0.5963
ES/BS (%)	3.94±1.06	3.81±1.22	0.8290

Table 1: Histomorphometric analysis of bone parameters (3) of 16-week old female PiT-2 WT and KO mouse vertebrae.

PiT-2 Localizes in Mineralizing Tissues in Odontogenesis

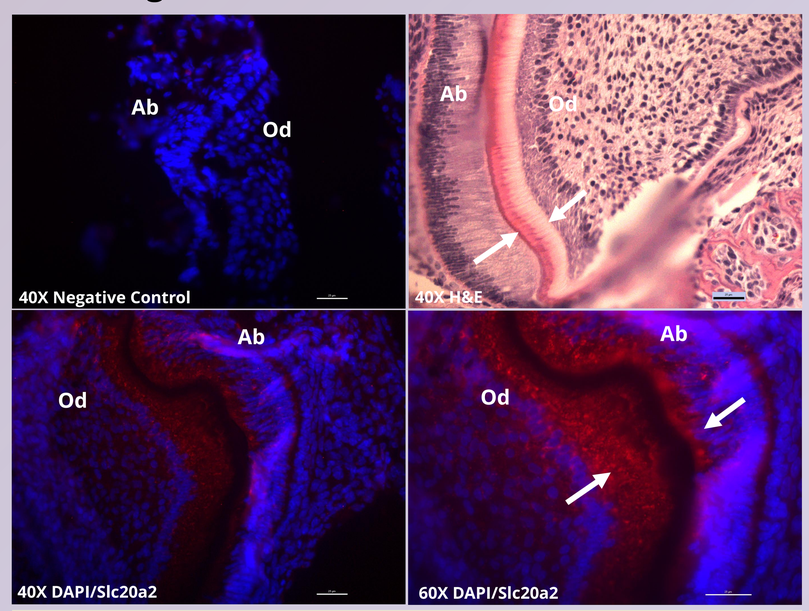

Figure 3: PiT-2 WT P3 stained maxillary incisors. Upper Right: hematoxylin and eosin staining showing collagen rich predentin and enamel matrix (arrows). Upper Left: DAPI and IF negative control (no primary antibody). Lower Left and Right: DAPI/Slc20a2 staining showing Slc20a2 localization in newly mineralizing dentin and enamel (arrows). Legend: Ab = Ameloblast; Od = Odontoblast. 25 µm scale bars.

Slc20a2 KO Decreases Relative Enamel Volume in Molars

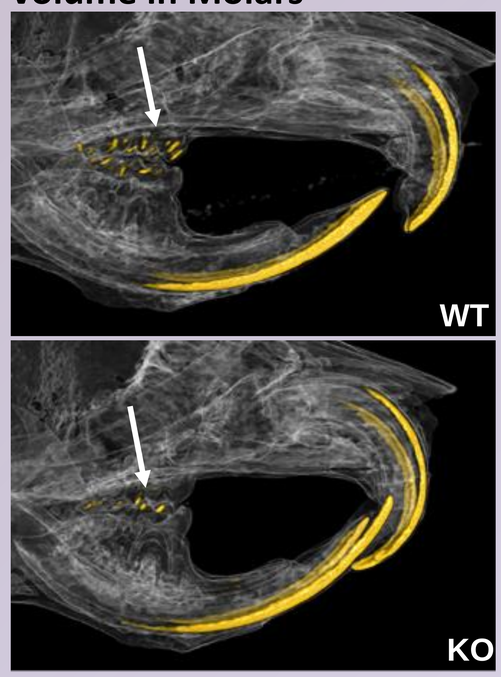

Figure 4: microCT images from PiT-2 KO homozygous mouse (lower) showing decreased enamel mineralization in the molars using false coloring compared to WT littermate (upper). Arrows point to molars with decreased mineralization.

PiT-2 Is The Predominant Pi Transporter In Vascular Matrix Vesicles

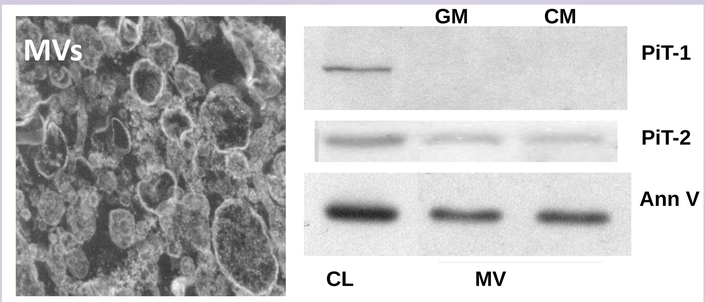

Figure 5: Matrix vesicles (MV) or cell lysates (CL) were isolated from mineralizing human VSMCs and examined by Western blotting for PiT-1, PiT-2, and Annexin V (Ann V). GM=growth medium, CM=mineralizing media. Left - MVs visualized by TEM.

Works Cited

1. Osathanon T. *Biomaterials*. 2009;30(27):4513-21
2. Yamada S. *Biochem Biophys Res Commun.* 2018;495(1):553-559.
3. Dempster DW. *J Bone Miner Res*. 2013;28(1):2–17.
4. Merametdjian L. *J Dent Res.* 2018;97(2):209-217.
5. Beck-Cormier S. *J Bone Miner Res.* 2019;34(6):1101-1114.

Acknowledgements

- NIH R35 HL139602 to CG
- NIH NIDCR NRSA F30DE029094 to PW
- Morton-Awamura Pediatric Dentistry/Oral Biology Leadership in Children's Oral Health Research Endowed Fellowship to PW