The need of a paradigm shift to better understand PiT1 and PiT2 biology

Response to *"Why is there no PiT1/SLC20A1 pathogenic variants yet linked to primary familial brain calcification?"*

Authors

Sarah Beck-Cormier, PhD[1,] , Laurent Beck, PhD[1 #]

Affiliation

[1] Université de Nantes, INSERM, UMR 1229, RMeS, ONIRIS, Nantes, F-44042 France.

Corresponding author

Dr Laurent BECK, PhD

INSERM U1229 - RMeS

Faculté de Chirurgie Dentaire

1 place Alexis Ricordeau, 44042 Nantes, FRANCE

email: laurent.beck@inserm.fr

Phone: +33 240 412 920

Disclosure statement

The authors declare that they have no conflict of interest

Dear Editor,

To our knowledge, there is no clear evidence for a role of PiT1 in physiological (*i.e.* skeletal) and pathophysiological (*i.e.* vascular) mineralization *in vivo*. Over the past 20 years, published experiments that have claimed a role for PiT1 in mineralization mechanisms have only been conducted in *in vitro* cell culture. An objective analysis of these publications shows that the conclusions drawn from these studies were mainly based on correlations rather than direct evidence. In addition, the use of phosphonoformic acid (PFA) to inhibit the phosphate (Pi) transport activity of PiT1 in these *in vitro* models has further increased confusion about the role of PiT1 in mineralization. Indeed, although PFA may inhibit type II renal Na-Pi transporters (Npt2a, b, c), it does not inhibit type III transporters (PiT1, 2), but rather the deposition of calcium-Pi in a process independent of any cellular activity that is similar to the physico-chemical mechanism of pyrophosphate [(1)]. Therefore, in order to better understand the biology of PiT1, it is necessary to consider the possibility that the pre-supposed role of PiT1 in mineralization mechanisms may have been based on partially biased observations. In line with this, several independent groups, including ours, have clearly demonstrated using genetically modified mice that PiT1 has no significant role in skeletal mineralization and vascular calcification *in vivo* [(2-4)]. In contrast, we and others have rather shown the involvement of PiT2 in these phenomena *in vivo* [(5,6)]. Therefore, when considering the recent *in vivo* studies rather than early *in vitro* observations, the absence of identified mutations in the *PiT1* gene in PFBC patients does not appear surprising.

The question raised by Drs. dos Santos-Junior and de Oliviera *"Why is there no PiT1 mutations in PFBC patients?"*, has its origin in the functional similarities between PiT1 and PiT2, and might be rephrased as follows: *"What does the absence of PiT1 mutations in PFBC patients teach us about the etiology of the disease and the biology of the PiT proteins?"*. By rephrasing in this way, we are driven to focus on the differences rather than the similarities between the two PiTs. A first obvious difference is that, unlike what is observed for PiT2, a loss-of-function mutation of PiT1 in mice is embryonic lethal [(7)]. Thus, if a loss-of-function mutation of PiT1 in humans were to be lethal, this would explain the absence of PiT1 mutation in PFBC patients, and the fact that only PiT2 is involved in the disease despite their similar Pi transport activity. Importantly, no PiT1 loss-of-function mutations have yet been found in humans in any genetic disease. The two studies from Simm *et al.* and Alonso *et al.* cited by Drs dos Santos-Junior and de Oliviera reported the existence of SNIPs in PiT1, as well as the role of the 2q13 chromosomal region, but without identifying loss-of-function mutations in PiT1.

In the event that PiT1 loss-of-function mutations are not lethal in humans and are identified in the future in genetic disorders other than PFBC, it would become clear that the etiology of PFBC could not be

explained solely by a defect in Pi transport activity, since it is shared identically by PiT1 and PiT2. In support for this possibility, although PFBC patients with PiT2 mutations have higher Pi levels in their cerebrospinal fluid, this is not necessarily the case for PFBC patients with mutations in other genes [8], challenging the systematic established link between Pi transport and mineralization mechanisms. In our recent study demonstrating the role of PiT2 in bone mineral quality and bone mechanical properties *in vivo* [5], we showed that PiT2-deficient osteoblasts had reduced Pi transport activity, but no mineralization defects *in vitro,* suggesting the possibility that Pi-transport independent mechanisms may be involved.

When studying the protein sequence differences between PiT1 and PiT2, we hypothesized 10 years ago that the roles of PiT1 in cell proliferation and apoptosis that were not shared by PiT2 may be explained by amino-acid sequence differences in the 4th intracellular loop. This loop displays only 34% protein sequence identity between PiT1 and PiT2, whereas the rest of the protein shares 75% identity. Using a two-hybrid screening strategy, we identified the protein partners of PiT1 and PiT2, which are distributed into two distinct and specific sets for each PiT. Using this data, we showed that the role of PiT1 in chondrocyte survival was due to its interaction with one specific partner (PDI), independently of its Pi transport function [4]. The same set of data was used to demonstrate a role of PiT1 in glucose metabolism independently of its Pi transport function, through its specific interaction with USP7 [9]. Finally, Ma *et al.* showed the role of PiT2 in neuronal growth by interacting with MAP1B, independently of its Pi transport function [10].

By interacting with numerous and specific proteins in different organs, the biology of the PiT proteins is much more complex than just being Pi transporters. It is therefore urgent to change the paradigm concerning the biology of PiTs, no longer considering them as Pi transporters only, but as multi-functional proteins. This paradigm shift would allow, for instance, to focus on the involvement of their protein partners in the etiology of certain diseases, such as PFBC, and thus more easily explain the observed differences between PiT1 and PiT2.

1. Villa-Bellosta R, Sorribas V. Phosphonoformic acid prevents vascular smooth muscle cell calcification by inhibiting calcium-phosphate deposition. Arterioscler Thromb Vasc Biol. 2009;29(5):761–6.

2. Bourgine A, Pilet P, Diouani S, Source S, Lesoeur J, Beck-Cormier S, Khoshniat S, Weiss P, Friedlander G, Guicheux J, Beck L. Mice with hypomorphic expression of the sodium-phosphate cotransporter PiT1/Slc20a1 have an unexpected normal bone mineralization. PLoS ONE. 2013;8(6):e65979.

3. Crouthamel MH, Lau WL, Leaf EM, Chavkin NW, Wallingford MC, Peterson DF, Li X, Liu Y, Chin MT, Levi M, Giachelli CM. Sodium-Dependent Phosphate Cotransporters and Phosphate-Induced Calcification of Vascular Smooth Muscle Cells: Redundant Roles for PiT-1 and PiT-2. Arterioscler Thromb Vasc Biol. 2013;33(11):2625–32.

4. Couasnay G, Bon N, Devignes C-S, Sourice S, Bianchi A, Véziers J, Weiss P, Elefteriou F, Provot S, Guicheux J, Beck-Cormier S, Beck L. PiT1/Slc20a1 Is Required for Endoplasmic Reticulum Homeostasis, Chondrocyte Survival, and Skeletal Development. J Bone Miner Res. 2018;34(2):387–98.

5. Beck-Cormier S, Lelliott CJ, Logan JG, Lafont DT, Merametdjian L, Leitch VD, Butterfield NC, Protheroe HJ, Croucher PI, Baldock PA, Gaultier-Lintia A, Maugars Y, Nicolas G, Banse C, Normant S, Magne N, Gérardin E, Bon N, Sourice S, Guicheux J, Beck L, Williams GR, Bassett JHD. Slc20a2, Encoding the Phosphate Transporter PiT2, Is an Important Genetic Determinant of Bone Quality and Strength. J Bone Miner Res. 2019;17(12):e3691.

6. Yamada S, Wallingford MC, Borgeia S, Cox TC, Giachelli CM. Loss of PiT-2 results in abnormal bone development and decreased bone mineral density and length in mice. Biochem Biophys Res Commun. 2018;495(1):553–9.

7. Beck L, Leroy C, Beck-Cormier S, Forand A, Salaün C, Paris N, Bernier A, Urena-Torres P, Prié D, Ollero M, Coulombel L, Friedlander G. The phosphate transporter PiT1 (Slc20a1) revealed as a new essential gene for mouse liver development. PLoS ONE. 2010;5(2):e9148.

8. Hozumi I, Kurita H, Ozawa K, Furuta N, Inden M, Sekine S-I, Yamada M, Hayashi Y, Kimura A, Inuzuka T, Seishima M. Inorganic phosphorus (Pi) in CSF is a biomarker for SLC20A2-associated idiopathic basal ganglia calcification (IBGC1). Journal of the Neurological Sciences. 2018;338:150-4.

9. Forand A, Koumakis E, Rousseau A, Sassier Y, Journe C, Merlin J-F, Leroy C, Boitez V, Codogno P, Friedlander G, Cohen I. Disruption of the Phosphate Transporter Pit1 in Hepatocytes Improves Glucose Metabolism and Insulin Signaling by Modulating the USP7/IRS1 Interaction. Cell Reports. 2016;16:1-13.

10. Ma X-X, Li X, Yi P, Wang C, Weng J, Zhang L, Xu X, Sun H, Feng S, Liu K, Chen R, Du S, Mao X, Zeng X, Zhang L-Y, Liu M, Tang B-S, Zhu X, Jin S, Liu J-Y. PiT2 regulates neuronal outgrowththrough interaction withmicrotubule-associated protein 1B. Sci Rep. 2017;17:1–13.