

## SHORT COMMUNICATION

### TNAP UPREGULATION IN VASCULAR SMOOTH MUSCLE CELLS IS SUFFICIENT TO CAUSE MEDIAL VASCULAR CALCIFICATION

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Medial vascular calcification (MVC) is a pathological condition common to variety of diseases, including chronic kidney disease, diabetes, obesity, generalised arterial calcification of infancy, arterial calcification due to deficiency of CD73, Kawasaki Disease and Keutel Syndrome. While the genetic and physiological causes of MVC differ between these diseases, several of them share the common feature of tissue-nonspecific alkaline phosphatase (TNAP) upregulation. To investigate whether TNAP expression is sufficient to cause MVC, we developed a conditional knockin mouse model that overexpresses human TNAP under the control of the vascular smooth muscle cell specific *Tagln* promoter. As early as seven days of age, male mice showed strong alkaline phosphatase activity in the aorta and, by 14 days, distinct aortic calcification was visible by X-ray. Further X-rays and micro-computed tomography at 30 days showed extensive calcification of the aorta and renal, carotid and coronary arteries. Heart weight measurements showed cardiac hypertrophy at 14 days that became progressively worse at 30 days. Survival curves showed that most male mice died between 25 and 60 days of age. Serum alka-

line phosphatase activity was approximately five and 22 times normal at days 14 and 30, respectively. No change in serum phosphate was detected at either age, and while serum calcium was normal at 14 days, it was slightly lower than controls at 30 days of age. No difference in serum pyrophosphate levels were detected at either age, implying that it is the local rather than systemic levels of pyrophosphate that are important for inhibition of calcification. Gene expression analysis showed upregulation of classical markers of MVC (*Bmp2* and *Opn*) and osteoblast markers, (*Runx2*, *Alpl*, *Phospho1*, *PiT-1*, *Enpp1*, *Ank* and *Col1a1*) and a decrease in expression of the smooth muscle marker *Tagln*. This data indicates that altering the local Pi/PPi balance is sufficient to initiate the transdifferentiation of smooth muscle cells, a hallmark of MVC. The expression levels of a variety of genes related to phosphate and pyrophosphate metabolism and/or transport were also altered. Overall, the data from this mouse model indicate that TNAP overexpression is sufficient to cause calcification and suggest that TNAP may be a critical mediator in a variety of ectopic calcification disorders.