

The P2Y₂ receptor controls BMD during development

Susanne Syberg¹, Maria Ellegard¹, Solveig Petersen¹, Jens-Erik Beck Jensen², Peter Schwarz^{1,3}, Niklas Rye Jørgensen¹

¹Research Center for Ageing and Osteoporosis, Departments of Diagnostics and Medicine M, Copenhagen University Hospital Glostrup, Denmark. ²Osteoporosis and Bone Metabolic Unit, Department of Endocrinology, Copenhagen University Hospital Hvidovre, Denmark. ³Faculty of Health Sciences, University of Copenhagen, Denmark.

INTRODUCTION

Nucleotides such as adenosine triphosphate (ATP) are released from bone cells, and subsequently act on P2 purinergic receptors. The P2Y₂ subtype is a G-protein coupled receptor expressed in osteoblasts and osteoclasts. *In vitro* activation of the P2Y₂ receptor in osteoblasts with nucleotides results in inhibition of alkaline phosphatase (ALP) production and of matrix mineralization. We have earlier shown that overexpression of the P2Y₂ receptor *in vivo* resulted in decreased bone mineral density (BMD), partially due to increased bone resorption, but also decreased formation (detected by serum bone markers and bone histomorphometry). The aim of this study was to investigate the osteogenic role of the P2Y₂ receptor in development and in two models known to induce bone loss.

METHODS

This study engaged 189 nullipara female P2Y₂ knockout (KO) mice and wild type siblings all with the BALB/cJ background. The Danish Animal Welfare Council approved all animal procedures in advance (2010/561-1788 and 2012/15-2935-00020).

The first sub-study examines the impact P2Y₂ gene KO on bone development from birth to nine months of age, with detection of body weight and DXA BMD every fourth week.

In the second sub-study mature animals of both genotypes were divided into groups to study bone loss following estrogen withdrawal and immobilization. These animals were followed for 12 weeks monitoring paralysis score, body weight and BMD.

Bone Mineral Measurements

Bone mineral measurements and body composition of the animals were determined on the PIXImus densitometer. Animals were fixed in a standard position, and measurements were performed sequentially, with duplicate determinations. Intraassay CV was 0.47% and interassay CV 0.73%. Due to the large mineral content in the skull, it was excluded from the calculations.

Bone strength measurements

Femoral neck and the 3-point bending test were performed after rehydration on a Lloyd material testing device LR50K (Lloyd Instruments, Fareham, UK). Load-deformation curves were generated and maximal load was recorded at a speed of 1 mm per minute with a 100 N load cell.

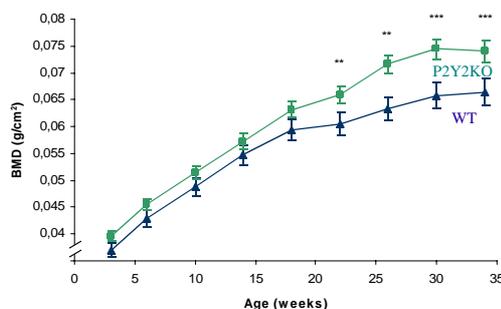


Figure 1. Whole body DXA measurements show significant differences from the age of 22 weeks (n=15-42). Data presented as mean ± SEM. ** = p<0.01, *** = p<0.001.

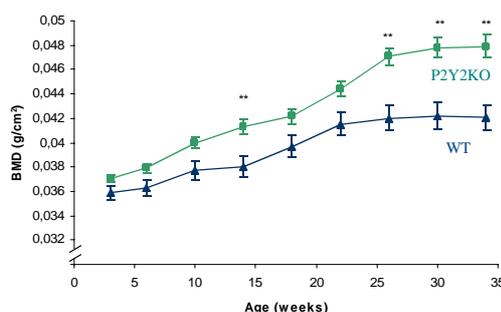


Figure 2. Femoral DXA measurements shows significant differences from the age of 26 weeks (n=15-42). Data presented as means ± SEM. * = p<0.05, ** = p<0.01.

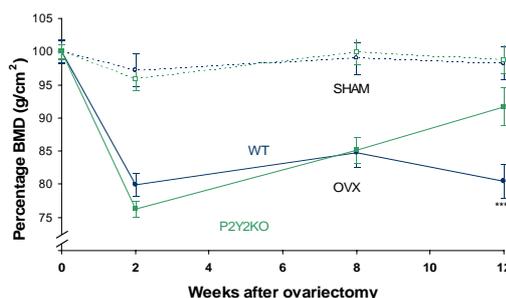


Figure 3. Baseline corrected femoral DXA measurements shows significant decrease in BMD following ovariectomy (week 20, n=11). Data presented as mean ± SEM.

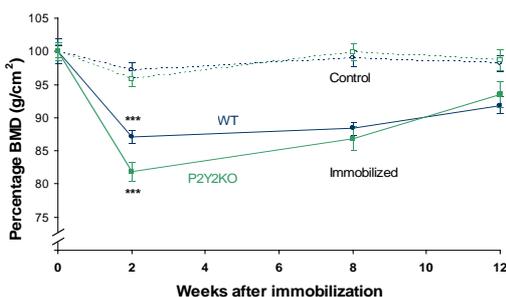


Figure 4. Baseline corrected femoral DXA measurements shows significant differences following immobilization (n=12-15). Data presented as means ± SEM. * = p<0.05, ** = p<0.01, *** = p<0.001.

RESULTS

From 3 weeks of age and until 34 weeks of age P2Y₂KO mice had higher BMD than WT, but only significant from age 22 and up (fig. 1). We found no difference in weight or fat percentage between the two genotypes. When narrowing the region of interest down to the femoral region, only a difference was found at age 14, 26, 30 and 34 – again P2Y₂KO had significantly higher BMD (fig. 2).

Bone strength measurements revealed that P2Y₂KO femoral midshafts had equal strength with WT (Table 1). However the femoral neck of P2Y₂KO showed decreased strength determined from the load-displacement curve (decreased maximal load and decreased energy absorption).

By challenging the P2Y₂ receptor in two models known to induce bone loss, both immobilization and ovariectomy lead to decreased BMD, but only P2Y₂KO were able to significantly regain some of the lost bone (fig. 3, 4).

Table 1: Results from mechanical testing of femurs (34 weeks).
Results are presented as means ± SEM. * = p<0.05

Three-point-bending of femoral midshaft			
	WT	P2Y2KO	p
Maximal Load (N)	30.1 ± 1.3	28.3 ± 1.2	ns
Stiffness (N/mm)	205.8 ± 7.8	205.9 ± 6.3	ns
Energy absorbed (N·mm)	7.13 ± 0.61	7.27 ± 0.59	ns
Vertical test of femoral neck			
	WT	P2Y2KO	p
Maximal Load (N)	13.2 ± 0.9	10.6 ± 0.8	0.019
Stiffness (N/mm)	55.65 ± 5.1	60.8 ± 4.3	ns
Energy absorbed (N·mm)	1.64 ± 0.23	1.25 ± 0.16	0.046

CONCLUSION

In conclusion, P2Y₂ receptor knockout in mice have an increased BMD (whole body and femoral) at mature age (week 22/26 and older). They are susceptible to bone loss induced by both OVX and immobilization but regain their bone density faster than the wild type mice.

The former is consistent with previously published *in vivo* effects of P2Y₂ receptor inactivation as seen in the P2Y₂ knockout mice¹. Further studies will show whether P2Y₂ receptor inhibition could increase bone mass and thus be a potential pharmacological target for treatment of bone loss.

REFERENCES

¹Orriss IR, Utting JC, Brandao-Burch A, Colston K, Grubb BR, Burnstock G, Arnett TR. Extracellular nucleotides block bone mineralization *in vitro*: evidence for dual inhibitory mechanisms involving both P2Y₂ receptors and pyrophosphate. *Endocrinology* 2007 Sep;148(9):4208-16.