Pappa2 deletion has sex- and age-specific effects on bone in mice

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Running title: Effects of *Pappa2* deletion on bone in mice

Abstract

Objective

In humans, loss-of-function mutations in the gene encoding pregnancy-associated pregnancy protein-A2 cause short stature and slightly reduced bone density. The goal of this study was to determine the effects of *Pappa2* deletion on bone in mice.

Design

Pappa2 deletion mice and littermate controls were culled at 10, 19 or 30 weeks of age and femurs were analysed by micro-computed tomography. Serum markers of bone turnover and insulin-like growth factor binding protein 5 (IGFBP-5), a proteolytic target of PAPP-A2, were measured by ELISA.

Results

At 10 and 19 weeks of age, *Pappa2* deletion mice had slightly reduced trabecular parameters, but by 19 weeks of age, female deletion mice had increased cortical tissue mineral density, and this trait was increased by a small amount in deletion mice of both sexes at 30 weeks. Cortical area fraction was increased in *Pappa2* deletion mice at all ages. Deletion of *Pappa2* increased circulating IGFBP-5 levels and reduced markers of bone turnover (PINP and TRACP 5b).

Conclusions

PAPP-A2 contributes to the regulation of bone structure and mass in mice, likely through control of IGFBP-5 levels. The net effect of changes in bone formation and resorption depend on sex and age, and differ between trabecular and cortical bone.

Keywords: bone, insulin-like growth factor, IGF, insulin-like growth factor binding protein, IGFBP, pappalysin, PAPP-A2

Introduction

Pregnancy-associated pregnancy protein-A2 (PAPP-A2) is a protease of insulin-like growth factor binding proteins (IGFBPs) [1] and therefore contributes to the regulation of insulin-like growth factor (IGF) availability [2]. Recently, loss-of-function mutations in the human *PAPPA2* gene were found to cause short stature and slightly reduced bone density [3,4], and these conditions were improved by treatment with IGF-I [5–7]. IGF-I is known to play important roles in bone physiology [8–10] while IGFBP-5 is one of the most abundant IGFBPs in bone [11] and is a target of PAPP-A2 [1]. IGFBP-5 influences bone mineral density (BMD) [12–14] by regulating IGF availability as well as through IGF-independent effects [15,16].

Study of the mechanisms by which PAPP-A2 influences skeletal growth and BMD will require animal models. In mice, postnatal skeletal growth is reduced by both constitutive and bone-specific deletion of *Pappa2* [17,18]. However, no effect of *Pappa2* deletion on BMD, measured by pQCT, was observed in 4 month old mice [19]. The goal of the present study was to examine the effects of constitutive *Pappa2* deletion on BMD at a range of ages using micro-computed tomography (micro-CT) to allow assessment of bone microarchitecture, and also to examine effects on circulating markers of bone turnover.

Methods

Mice

All work was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the SFU University Animal Care Committee (protocols 945-09, 1035-11 and 1188-11). Constitutive *Pappa2* deletion mice with a C57BL/6 background were generated as previously described [17,20]. Mice were collected at 10, 19 or 30 weeks of age. Peak BMD is achieved shortly before 19 weeks [21], but trabecular bone peaks around 6-8 weeks and declines thereafter [22]. Thus, 10 week mice have not yet achieved peak BMD but are close to maximum trabecular bone, 19 week mice have achieved peak BMD and show some trabecular bone loss, while 30 week mice have more bone loss and females are approaching reproductive senescence [23]. To generate the cohort collected at 10 weeks of age, mice heterozygous for the wild-type and deletion alleles ($Pappa2^{wt/KO}$) were paired to produce litters in which all three genotypes were present, and $Pappa2^{wt/wt}$ mice were used as controls for the homozygous deletion mice. To generate the cohorts collected at 19 and 30 weeks of age, mice heterozygous for the conditional (floxed) and deletion alleles ($Pappa2^{fl/KO}$) were paired to produce litters in which all three genotypes were present, and $Pappa2^{fl/fl}$ mice were used as controls; we have previously shown that postnatal growth does not differ between $Pappa2^{fl/fl}$ and $Pappa2^{wt/wt}$ mice [20]. Mice were genotyped by PCR using ear-clip tissue obtained at weaning, as previously described [20].

Micro-computed tomography

Following sacrifice, mice were stored frozen at -20°C. Mice were later thawed, the skin and internal organs were removed, and the carcasses were exposed to dermestid beetles for removal of soft tissue. Femurs were measured using calipers and regions proportional to 5% of the total

length of bone were used to measure trabecular parameters (in the distal metaphysis) and cortical characteristics (at the mid-shaft). Bones were scanned using micro-CT with an isotropic voxel size of 7.4µm (Scanco Medical µCT100, Switzerland; 70kVp, 114 µA, 100 ms integration time). For trabecular bone, the region of interest was proximal to the distal growth plate. The region of interest for cortical bone was immediately distal to the third trochanter (where the cross-section of the bone appears round/oval rather than the shape of a tear drop). Measures of trabecular bone microarchitecture included bone volume within the region of interest (BV, mm^3), total volume of the region of interest (TV, mm^3), bone volume fraction (BV/TV, %), trabecular number (Tb.N, mm^{-1}), trabecular thickness (Tb.Th, µm), and trabecular separation (Tb.Sp, mm) [24]. Measures of cortical bone morphology included total cross-sectional area (Tt.Ar, mm^2), cortical bone area (Ct.Ar, mm^2), cortical area fraction (Ct.Ar/Tt.Ar, %), average cortical thickness (Ct.Th, µm), cortical porosity (Ct.Po, %) and tissue mineral density (TMD, mg calcium hydroxyapatite (HA)/cm^3) [24].

Serum PINP and TRACP 5b

We used ELISA to measure serum levels of a marker of osteoblast activity (bone formation), N-terminal propeptide of type I procollagen (PINP) (AC-33F1, IDS Immunodiagnostics), and a marker of osteoclast number (bone resorption), tartrate-resistant acid phosphatase form 5b (TRACP 5b) (SB-TR103, IDS Immunodiagnostics) in a subset of constitutive *Pappa2* deletion females at 6 and 19 weeks of age. We also measured serum IGFBP-5 at 19 and 30 weeks by ELISA (DY578, R&D Systems).

Statistical analyses

Data were analysed using general linear models (proc GLM, SAS, version 9.4) including effects of genotype, sex and the sex*genotype interaction term to test for sex-specific effects [25]. Where the interaction was significant, differences between genotypes were tested within each sex using the ESTIMATE statement (proc GLM). Since PINP and TRACP 5b were measured in the same individuals at two different ages, these data were analysed by repeated measures analyses (proc MIXED, SAS, Version 9.4). Values of PINP, TRACP 5b and IGFBP-5 were log-transformed prior to analyses because the distributions were skewed, with a few large values.

Results and Discussion

Regions of interest were selected as a proportion of total bone length, and so were slightly smaller in *Pappa2* deletion mice at all ages because their bones were shorter (Tables 1-3). At 10 weeks of age, there were no sex-specific effects of *Pappa2* deletion (no significant genotype*sex interactions). *Pappa2* deletion increased cortical area fraction and reduced trabecular thickness (Table 1). At 19 weeks of age, *Pappa2* deletion increased cortical area fraction, cortical thickness, and cortical TMD in females only. In contrast, *Pappa2* deletion reduced trabecular bone volume fraction in males but not females, and there was a marginally non-significant reduction in trabecular thickness in both sexes (Table 2). At 30 weeks of age, there were no significant genotype*sex interactions, and *Pappa2* deletion increased cortical area fraction and cortical TMD in both sexes, with no effects on trabecular parameters (Table 3). Although previous work using a different technique (pQCT) found no effect of *Pappa2* deletion on BMD

in 4 month old mice [19], this previous analysis did not examine cortical and trabecular bone separately. At 19 weeks (~4.5 months), we observed contrasting effects of *Pappa2* deletion on cortical and trabecular bone that might have been obscured if these two compartments had been analysed together.

Since PAPP-A2 cleaves IGFBP-5, serum levels of IGFBP-5 were expected to be higher in *Pappa2* deletion mice, and this was observed at 19 and 30 weeks (19 weeks: $F_{1,35} = 8.4$, $P = 0.007$; 30 weeks: $F_{1,35} = 4.0$, $P = 0.054$; Fig. 1), although the difference was marginally non-significant at 30 weeks. We have previously found serum IGFBP-5 to be elevated in *Pappa2* deletion mice at 6 weeks of age [20]. IGFBP-5 levels were higher in females than in males (19 weeks: $F_{1,35} = 9.7$, $P = 0.004$; 30 weeks: $F_{1,35} = 11.9$, $P = 0.002$; Fig. 1), but there was no significant interaction between sex and genotype (19 weeks: $F_{1,35} = 1.26$, $P = 0.27$; 30 weeks: $F_{1,35} = 0.1$, $P = 0.82$; Fig. 1). IGFBP-5 levels were significantly higher at 19 weeks than at 30 weeks ($F_{1,74} = 27.0$, $P < 0.0001$; Fig. 1) when analyzing the ages together and including effects of genotype, sex, genotype*sex interaction and age.

Markers of bone formation (PINP) and bone resorption (TRACP 5b) were lower in *Pappa2* deletion mice (PINP: $F_{1,18} = 6.6$, $P = 0.02$; TRACP 5b: $F_{1,18} = 5.4$, $P = 0.03$; Fig. 2). PINP levels were higher at 6 weeks than 19 weeks ($F_{1,18} = 975.5$, $P < 0.0001$), while TRACP 5b showed the opposite pattern ($F_{1,17} = 117.8$, $P < 0.0001$). However, there was no interaction between age and genotype (PINP: $F_{1,18} = 0.62$, $P = 0.44$; TRACP 5b: $F_{1,17} = 0.1$, $P = 0.83$).

Pappa2 deletion reduced markers of bone formation and resorption and the net effect of these changes depended on age. At younger ages, *Pappa2* deletion mice had slightly impaired trabecular parameters, but by 19 weeks of age, female deletion mice had very modest improvement in cortical TMD, and this trait was increased in both sexes by 30 weeks. The increases in cortical area fraction seen in *Pappa2* deletion mice at all ages may reflect subtle changes in bone morphology, as previously described for the mandible and pelvic girdle [17].

Increased IGFBP-5 concentrations, either at the local level or in circulation, represent a likely mechanism underlying the effects of *Pappa2* deletion. The effects of IGFBP-5 on bone are controversial [11]. IGFBP-5 overexpression reduced BMD in young mice but not in older animals [12,14]. In contrast, daily injections of IGFBP-5 increased BMD in ovariectomized mice [13]. Thus, in healthy young mice, increasing IGFBP-5 may reduce BMD by reducing IGF-I availability, as observed in human children with loss-of-function mutations in *PAPPA2* [3]. In contrast, in older or ovariectomized mice, when bone formation is reduced, an increase in IGFBP-5 may exert beneficial effects through IGF-independent mechanisms. While deletion of *Pappa2*'s paralog, *Pappa*, impaired bone density in mice at 2-12 months of age [26], PAPP-A cleaves IGFBP-4 as well as IGFBP-5, and so it is possible that the beneficial effects of increased IGFBP-5 were outweighed by reduced IGF-I availability due to increased IGFBP-4 and -5 levels.

In conclusion, the present study shows that, in addition to its effects on the linear growth of bones [17,18], PAPP-A2 also plays sex- and age-specific roles in the regulation of bone mass in mice.

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Declaration of interest

The authors have no competing interests.

References

[1] M.T. Overgaard, H.B. Boldt, L.S. Laursen, L. Sottrup-Jensen, C.A. Conover, C. Oxvig, Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase, J. Biol. Chem. 276 (2001) 21849–21853.

[2] R.C. Bunn, J.L. Fowlkes, Insulin-like growth factor binding protein proteolysis, Trends Endocrinol. Metab. 14 (2003) 176–181. doi:10.1016/S1043-2760(03)00049-3.

[3] A. Dauber, M.T. Munoz-Calvo, V. Barrios, H.M. Domene, S. Kloverpris, C. Serra-Juhe, V. Desikan, J. Pozo, R. Muzumdar, G.A. Martos-Moreno, F. Hawkins, H.G. Jasper, C.A. Conover, J. Frystyk, S. Yakar, V. Hwa, J.A. Chowen, C. Oxvig, R.G. Rosenfeld, L.A. Perez-Jurado, J. Argente, Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability, EMBO Mol. Med. 8 (2016) 363–374. doi:10.15252/emmm.201506106.

[4] J. Argente, L.A. Pérez-Jurado, History and clinical implications of PAPP-A2 in human growth: When reflecting on idiopathic short stature leads to a specific and new diagnosis, Growth Horm. IGF Res. 40 (2018) 17–19. doi:10.1016/j.ghir.2018.04.001.

[5] C. Cabrera-Salcedo, T. Mizuno, L. Tyzinski, M. Andrew, A.A. Vinks, J. Frystyk, H. Wasserman, C.M. Gordon, V. Hwa, P. Backeljauw, A. Dauber, Pharmacokinetics of IGF-1 in PAPP-A2-Deficient Patients, Growth Response, and Effects on Glucose and Bone Density, J. Clin. Endocrinol. Metab. 102 (2017) 4568–4577. doi:10.1210/jc.2017-01411.

[6] M.T. Muñoz-Calvo, V. Barrios, J. Pozo, J.A. Chowen, G.Á. Martos-Moreno, F. Hawkins, A. Dauber, H.M. Domené, S. Yakar, R.G. Rosenfeld, L.A. Pérez-Jurado, C. Oxvig, J. Frystyk, J. Argente, Treatment with recombinant human insulin-like growth factor-1 improves growth in patients with PAPP-A2 deficiency, J. Clin. Endocrinol. Metab. 101 (2016) 3879–3883. doi:10.1210/jc.2016-2751.

[7] F.G. Hawkins-Carranza, M.T. Muñoz-Calvo, G. Martos-Moreno, G. Allo-Miguel, L. Del Río, J. Pozo, J.A. Chowen, L.A. Pérez-Jurado, J. Argente, rhIGF-1 Treatment Increases Bone Mineral Density and Trabecular Bone Structure in Children with PAPP-A2

Deficiency, Horm. Res. Paediatr. 89 (2018) 200–204. doi:10.1159/000486336.

[8] K.E. Govoni, D.J. Baylink, S. Mohan, The multi-functional role of insulin-like growth factor binding proteins in bone, Pediatr. Nephrol. 20 (2005) 261–268. doi:10.1007/s00467-004-1658-y.

[9] S. Mohan, C. Richman, R.Q. Guo, Y. Amaar, L.R. Donahue, J. Wergedal, D.J. Baylink, Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and -independent mechanisms, Endocrinology. 144 (2003) 929–936. doi:10.1210/en.2002-220948.

[10] S. Yakar, O. Isaksson, Regulation of skeletal growth and mineral acquisition by the GH/IGF-1 axis: Lessons from mouse models, Growth Horm. IGF Res. 28 (2016) 26–42. doi:10.1016/j.ghir.2015.09.004.

[11] A. Mukherjee, P. Rotwein, Insulin-like growth factor binding protein-5 in osteogenesis: Facilitator or inhibitor?, Growth Horm. IGF Res. 17 (2007) 179–185.

[12] D.A.M. Salih, S. Mohan, Y. Kasukawa, G. Tripathi, F.A. Lovett, N.F. Anderson, E.J. Carter, J.E. Wergedal, D.J. Baylink, J.M. Pell, Insulin-like growth factor-binding protein-5 induces a gender-related decrease in bone mineral density in transgenic mice, Endocrinology. 146 (2005) 931–940. doi:10.1210/en.2004-0816.

[13] D.L. Andress, IGF-binding protein-5 stimulates osteoblast activity and bone accretion in ovariectomized mice, Am. J. Physiol. Metab. 281 (2001) E283–E288.

[14] R.D. Devlin, Z. Du, V. Buccilli, V. Jorgetti, E. Canalis, Transgenic mice overexpressing insulin-like growth factor binding protein-5 display transiently decreased osteoblastic function and osteopenia, Endocrinology. 143 (2002) 3955–3962. doi:10.1210/en.2002-220129.

[15] S. Mohan, D.J. Baylink, IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms, J. Endocrinol. 175 (2002) 19–31. doi:10.1677/joe.0.1750019.

[16] N. Miyakoshi, C. Richman, Y. Kasukawa, T.A. Linkhart, D.J. Baylink, S. Mohan, Evidence that IGF-binding protein-5 functions as a growth factor, J. Clin. Invest. 107 (2001) 73–81. doi:10.1172/JCI10459.

[17] J.K. Christians, D.R. de Zwaan, S.H.Y. Fung, Pregnancy Associated Plasma Protein A2 (PAPP-A2) Affects Bone Size and Shape and Contributes to Natural Variation in Postnatal Growth in Mice, PLoS One. 8 (2013) e56260. doi:10.1371/journal.pone.0056260.

[18] N. Amiri, J.K. Christians, PAPP-A2 expression by osteoblasts is required for normal postnatal growth in mice, Growth Horm. IGF Res. 25 (2015) 274–80. doi:10.1016/j.ghir.2015.09.003.

[19] C.A. Conover, H.B. Boldt, L.K. Bale, K.B. Clifton, J.A. Grell, J.R. Mader, E.J. Mason, D.R. Powell, Pregnancy-Associated Plasma Protein-A2 (PAPP-A2): Tissue Expression and Biological Consequences of Gene Knockout in Mice, Endocrinology. 152 (2011) 2837–2844.

[20] J.K. Christians, A.K. Bath, N. Amiri, Pappa2 deletion alters IGFBPs but has little effect on glucose disposal or adiposity, Growth Horm. IGF Res. 25 (2015) 232–239. doi:http://dx.doi.org/10.1016/j.ghir.2015.07.001.

[21] W.G. Beamer, L.R. Donahue, C.J. Rosen, D.J. Baylink, Genetic variability in adult bone density among inbred strains of mice, Bone. 18 (1996) 397–403. doi:10.1016/8756-3282(96)00047-6.

[22] V. Glatt, E. Canalis, L. Stadmeyer, M.L. Bouxsein, Age-Related Changes in Trabecular Architecture Differ in Female and Male C57BL/6J Mice, J. Bone Miner. Res. 22 (2007) 1197–1207. doi:10.1359/jbmr.070507.

[23] L.M. Franks, J. Payne, The influence of age on reproductive capacity in C57BL mice., J. Reprod. Fertil. 21 (1970) 563–565.

[24] M.L. Bouxsein, S.K. Boyd, B.A. Christiansen, R.E. Guldberg, K.J. Jepsen, R. Mueller, Guidelines for Assessment of Bone Microstructure in Rodents Using Micro-Computed Tomography, J. Bone Miner. Res. 25 (2010) 1468–1486. doi:10.1002/jbmr.141.

[25] E.H. Chin, J.K. Christians, When are sex-specific effects really sex-specific?, J. Dev. Orig. Health Dis. 6 (2015) 438–442. doi:10.1017/S2040174415001348.

[26] S.J. Tanner, T.E. Hefferan, C.J. Rosen, C.A. Conover, Impact of pregnancy-associated plasma protein-A deletion on the adult murine skeleton, J. Bone Miner. Res. 23 (2008) 655–662.

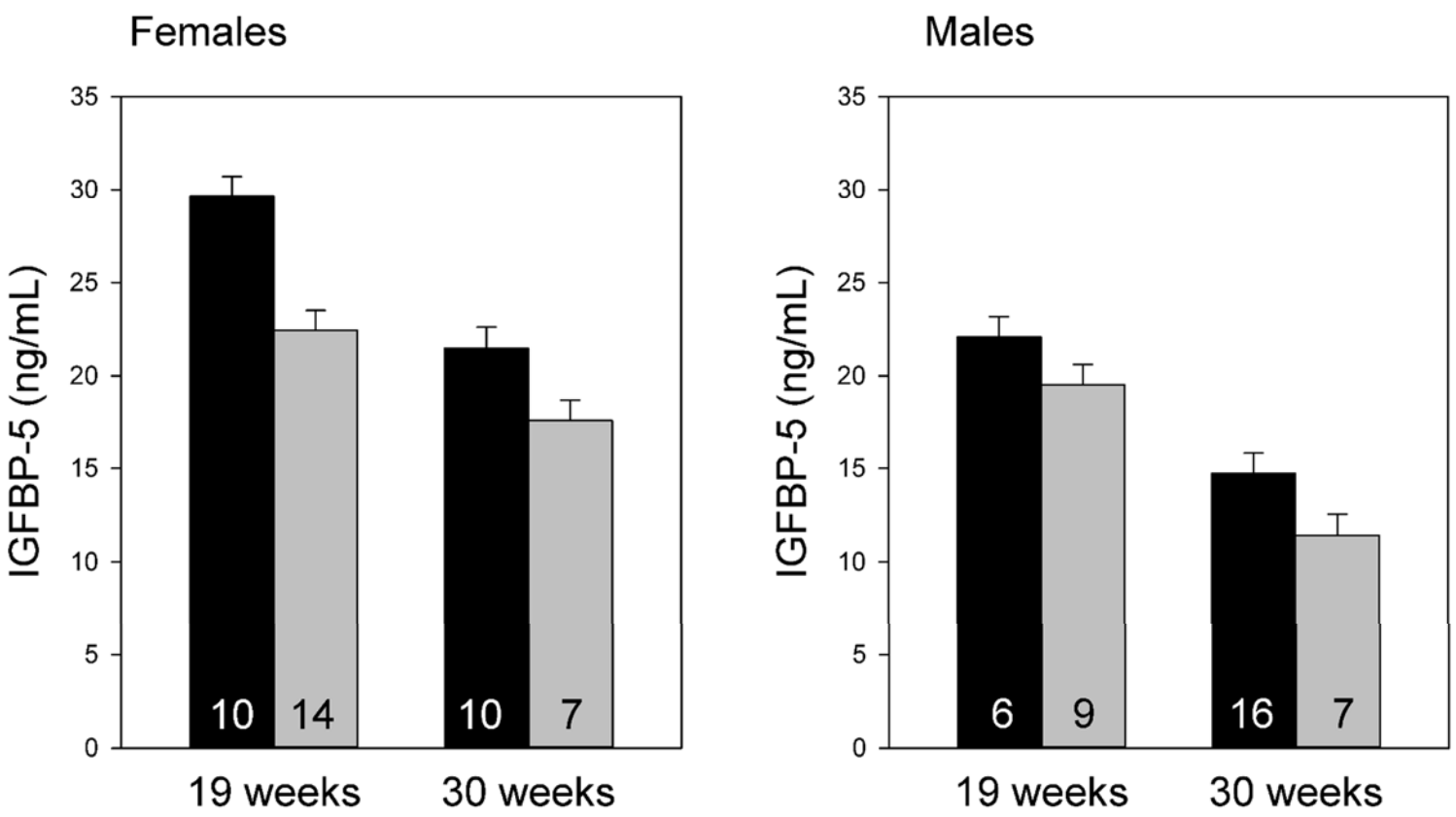

Figure 1. Effects of *Pappa2* deletion on serum IGFBP-5 levels at 19 and 30 weeks of age (black bars: *Pappa2* deletion mice; grey bars: controls). Values are least squares means ± standard error from general linear models including effects of genotype, sex, and the genotype*sex interaction. Data were log-transformed prior to analyses and back-transformed for graphical presentation. Sample sizes are shown above the x-axis.

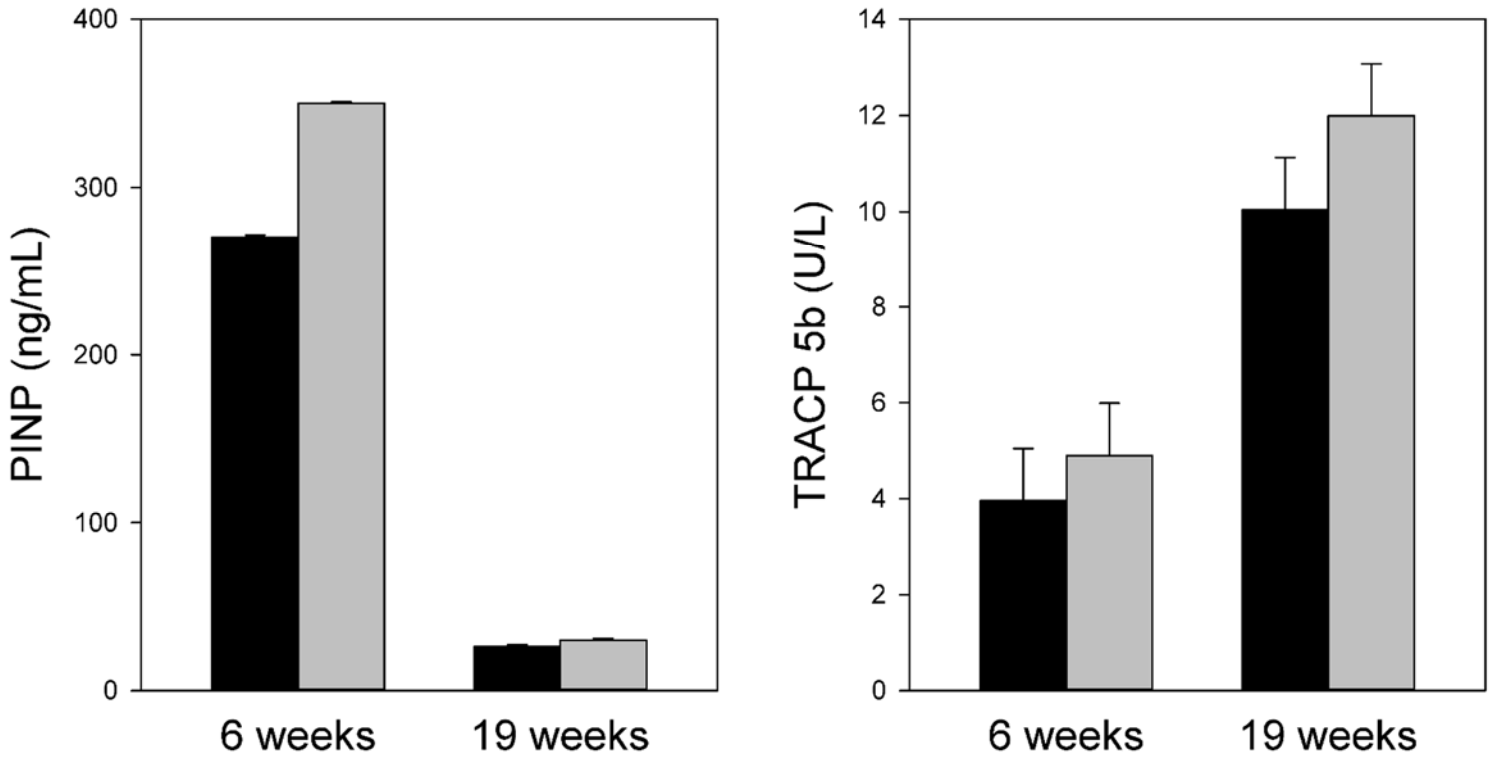

Figure 2. Effects of *Pappa2* deletion on serum PINP and TRACP 5b levels in females at 6 and 19 weeks of age (black bars: *Pappa2* deletion mice; grey bars: controls). Levels were measured in the same individuals at two different ages and values are least squares means ± standard error from a repeated measures analyses including effects of genotype, age, and the genotype*age interaction. Data were log-transformed prior to analyses and back-transformed for graphical presentation. N = 10 females per genotype.

Table 1. Bone parameters at 10 weeks of age. Values are least squares means ± standard error from a general linear model including effects of genotype, sex, and the genotype*sex interaction. Traits where the effect of genotype is significant are shown in bold.

	Females		Males		Genotype*sex		Genotype		Sex	
	Pappa2$^{KO/KO}$	*Pappa2*$^{wt/wt}$	*Pappa2*$^{KO/KO}$	*Pappa2*$^{wt/wt}$						
Sample size	9	6	9	7	$F_{1,27}$	P	$F_{1,27}$	P	$F_{1,27}$	P
Mass at cull (g)	**16.3 ± 0.5**	**19.0 ± 0.6**	**22.2 ± 0.5**	**24.5 ± 0.6**	0.1	0.73	22.5	**0.0001**	115.3	0.0001
Femur length (mm)	**13.9 ± 0.1**	**14.3 ± 0.1**	**14.5 ± 0.1**	**14.8 ± 0.1**	0.3	0.58	9.2	**0.005**	21.6	0.0001
Trabecular										
TV (mm^3)	**1.000 ± 0.035**	**1.208 ± 0.043**	**1.345 ± 0.035**	**1.572 ± 0.039**	0.0	0.81	32.8	**0.0001**	87.2	0.0001
BV (mm^3)	0.055 ± 0.012	0.057 ± 0.014	0.147 ± 0.012	0.185 ± 0.013	2.0	0.17	2.6	0.12	76.5	0.0001
BV/TV (%)	5.4 ± 0.6	4.7 ± 0.8	10.9 ± 0.6	11.6 ± 0.7	1.0	0.34	0.0	0.98	76.4	0.0001
Tb.N (mm^{-1})	4.3 ± 0.2	4.1 ± 0.2	5.3 ± 0.2	5.1 ± 0.2	0.1	0.76	1.0	0.32	36.5	0.0001
Tb.Th (µm)	**32 ± 1**	**33 ± 1**	**37 ± 1**	**40 ± 1**	2.6	0.12	5.1	**0.03**	51.6	0.0001
Tb.Sp (mm)	0.24 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.0	0.94	1.2	0.29	36.1	0.0001
Cortical										
Tt.Ar (mm^2)	**1.20 ± 0.04**	**1.42 ± 0.04**	**1.49 ± 0.04**	**1.72 ± 0.04**	0.0	0.84	35.2	**0.0001**	59.0	0.0001
Ct.Ar (mm^2)	**0.54 ± 0.02**	**0.58 ± 0.02**	**0.66 ± 0.02**	**0.73 ± 0.02**	0.8	0.39	8.1	**0.008**	47.3	0.0001
Ct.Ar/Tt.Ar (%)	**45 ± 1**	**41 ± 1**	**44 ± 1**	**42 ± 1**	4.2	0.051	26.8	**0.0001**	1.4	0.25
Ct.Th (µm)	155 ± 3	149 ± 4	167 ± 3	166 ± 3	0.9	0.35	1.1	0.31	21.7	0.0001
Ct.Po (%)	8.1 ± 0.2	8.4 ± 0.3	7.7 ± 0.2	8.0 ± 0.3	0.0	0.91	1.5	0.23	2.9	0.10
TMD (mg HA/cm^3)	1153 ± 7	1158 ± 8	1140 ± 7	1123 ± 8	2.4	0.14	0.7	0.40	11.0	0.003

Table 2. Bone parameters at 19 weeks of age. Values are least squares means ± standard error from a general linear model including effects of genotype, sex, and the genotype*sex interaction. Where the genotype*sex interaction is significant, the difference between genotypes has been tested within each sex. Traits where the effect of genotype is significant are shown in bold. In some cases, the genotype*sex interaction is significant and the effect of genotype is significant within both sexes, indicating that the magnitude of the effect differs between the sexes.

	Females		Males		Genotype*sex		Genotype		Sex	
	Pappa2$^{KO/KO}$	*Pappa2*$^{fl/fl}$	*Pappa2*$^{KO/KO}$	*Pappa2*$^{fl/fl}$						
Sample size	10	14	6	9	$F_{1,35}$	P	$F_{1,35}$	P	$F_{1,35}$	P
Mass at cull (g)	**18.8 ± 0.6**	**21.1 ± 0.4**	**23.8 ± 0.9**	**26.7 ± 0.6**	0.2	0.64	14.9	**0.0008**	61.8	0.0001
Femur length (mm)	14.2 ± 0.1	14.4 ± 0.1	**14.4 ± 0.1**	**14.9 ± 0.1**	4.7	**0.037**	18.3	0.0001	14.2	0.0006
Trabecular										
TV (mm^3)	**0.771 ± 0.040**	**1.063 ± 0.034**	**1.110 ± 0.051**	**1.662 ± 0.042**	9.5	**0.004**	100.4	0.0001	123.6	0.0001
BV (mm^3)	0.010 ± 0.006	0.015 ± 0.005	**0.059 ± 0.007**	**0.122 ± 0.006**	20.5	**0.0001**	28.7	0.0001	149.7	0.0001
BV/TV (%)	1.3 ± 0.3	1.4 ± 0.3	**5.3 ± 0.4**	**7.3 ± 0.4**	6.5	**0.015**	7.8	0.009	183.7	0.0001
Tb.N (mm^{-1})	2.8 ± 0.1	2.7 ± 0.1	3.9 ± 0.1	3.7 ± 0.1	0.3	0.56	2.0	0.17	87.6	0.0001
Tb.Th (µm)	27 ± 2	34 ± 2	40 ± 3	43 ± 3	0.4	0.54	3.6	0.07	16.0	0.0003
Tb.Sp (mm)	0.36 ± 0.01	0.37 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.1	0.83	0.9	0.36	70.0	0.0001
Cortical										
Tt.Ar (mm^2)	**1.34 ± 0.03**	**1.64 ± 0.03**	**1.77 ± 0.04**	**2.17 ± 0.04**	1.6	0.22	94.4	**0.0001**	178.6	0.0001
Ct.Ar (mm^2)	**0.69 ± 0.01**	**0.75 ± 0.01**	**0.79 ± 0.02**	**0.93 ± 0.01**	7.9	**0.008**	48.5	0.0001	92.8	0.0001
Ct.Ar/Tt.Ar (%)	**52 ± 1**	**46 ± 1**	45 ± 1	43 ± 1	11.7	**0.002**	43.3	0.0001	68.2	0.0001
Ct.Th (µm)	**192 ± 2**	**183 ± 2**	184 ± 3	192 ± 3	10.5	**0.003**	0.0	0.89	0.1	0.82
Ct.Po (%)	5.6 ± 0.2	5.9 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	5.1	**0.03**	0.8	0.38	0.5	0.48
TMD (mg HA/cm^3)	**1257 ± 6**	**1241 ± 5**	1215 ± 7	1222 ± 6	3.9	0.06	0.6	0.44	27.5	0.0001

Table 3. Bone parameters at 30 weeks of age. Values are least squares means ± standard error from a general linear model including effects of genotype, sex, and the genotype*sex interaction. Traits where the effect of genotype is significant are shown in bold.

	Females		Males		Genotype*sex		Genotype		Sex	
	Pappa2$^{KO/KO}$	*Pappa2*$^{fl/fl}$	*Pappa2*$^{KO/KO}$	*Pappa2*$^{fl/fl}$						
Sample size	10	7	16	7	$F_{1,36}$	P	$F_{1,36}$	P	$F_{1,36}$	P
Mass at cull (g)	**22.4 ± 0.8**	**25.4 ± 0.9**	**28.1 ± 0.6**	**30.9 ± 0.9**	0.0	0.95	13.6	**0.0008**	49.1	0.0001
Femur length (mm)	**14.6 ± 0.1**	**14.9 ± 0.1**	**14.6 ± 0.1**	**15.0 ± 0.1**	1.0	0.32	13.0	**0.0009**	0.1	0.72
Trabecular										
TV (mm^3)	**1.227 ± 0.063**	**1.503 ± 0.075**	**1.673 ± 0.050**	**2.039 ± 0.075**	0.5	0.51	23.3	**0.0001**	54.5	0.0001
BV (mm^3)	0.040 ± 0.017	0.052 ± 0.020	0.197 ± 0.013	0.215 ± 0.020	0.0	0.87	0.7	0.40	80.3	0.0001
BV/TV (%)	3.3 ± 0.7	3.4 ± 0.9	11.6 ± 0.6	10.2 ± 0.9	0.9	0.34	0.6	0.46	91.0	0.0001
Tb.N (mm^{-1})	2.7 ± 0.1	2.6 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	0.2	0.68	0.0	0.97	165.8	0.0001
Tb.Th (µm)	44 ± 2	45 ± 2	49 ± 1	44 ± 2	3.4	0.07	1.3	0.27	1.45	0.24
Tb.Sp (mm)	0.38 ± 0.01	0.38 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.0	0.88	0.0	0.98	188.7	0.0001
Cortical										
Tt.Ar (mm^2)	**1.40 ± 0.05**	**1.74 ± 0.06**	**1.94 ± 0.04**	**2.27 ± 0.06**	0.0	0.87	37.1	**0.0001**	96.9	0.0001
Ct.Ar (mm^2)	**0.73 ± 0.02**	**0.82 ± 0.02**	**0.82 ± 0.01**	**0.90 ± 0.02**	0.0	0.86	19.0	**0.0001**	20.8	0.0001
Ct.Ar/Tt.Ar (%)	**52 ± 1**	**47 ± 1**	**42 ± 1**	**40 ± 1**	2.4	0.13	18.1	**0.0001**	92.9	0.0001
Ct.Th (µm)	198 ± 3	194 ± 4	178 ± 3	173 ± 4	0.0	0.92	1.4	0.25	30.0	0.0001
Ct.Po (%)	5.3 ± 0.1	5.6 ± 0.2	6.3 ± 0.2	6.6 ± 0.2	0.0	0.91	1.6	0.21	22.6	0.0001
TMD (mg HA/cm^3)	**1266 ± 6**	**1257 ± 7**	**1217 ± 5**	**1196 ± 7**	0.9	0.34	6.0	**0.02**	79.7	0.0001