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Abstract: Background: The oxidative stress-responsive kinase 1 (OSR1) participates in the WNK-(with no K) kinase dependent regulation of renal salt excretion and blood pressure. Little is known, however, about the role of OSR1 in the regulation of further renal transport systems. The present study analyzed the effect of OSR1 on NaPiIIa, the major renal tubular phosphate transporter. Methods: Immunohistochemistry and confocal microscopy were employed to determine renal localization of OSR1 and NaPiIIa. To elucidate the effect of OSR on NaPiIIa activity, cRNA encoding NaPiIIa was injected into Xenopus oocytes with or without additional injection of cRNA encoding OSR1, and phosphate transport was estimated from phosphate-induced currents determined with dual electrode voltage clamp. To elucidate the in vivo significance of OSR1 serum phosphate and hormone concentrations as well as urinary phosphate output of mice carrying one allele of WNK-resistant OSR1 (osr1(tg/+)) were compared to the respective values of wild type mice (osr1(+/+)). Results: NaPiIIa and OSR1 were both expressed in proximal renal tubule cells. Coexpression of OSR1 significantly up-regulated phosphate-induced currents in NaPiIIa-expressing Xenopus oocytes. Despite decreased serum phosphate concentration urinary phosphate excretion was significantly increased and NaPiIIa protein abundance in the brush border membrane significantly reduced in osr1(tg/+)) mice as compared to osr1(+/+) mice. Serum PTH and calcitriol levels were similar in osr1(tg/+)) mice and in osr1(+/+)) mice, serum FGF23 concentration was, however, significantly higher in osr1(tg/+)) mice than in osr1(+/+)) mice. Conclusions: OSR1 is expressed in proximal renal tubules and participates in the regulation of FGF23 release and renal tubular phosphate transport.

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OSR1-sensitive renal tubular phosphate reabsorption

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Abstract

The oxidative stress-responsive kinase 1 (OSR1) participates in the WNK-(with no K) dependent regulation of renal salt excretion and blood pressure. Little is known, however, about the role of OSR1 in the regulation of further renal transport systems. The present study analyzed the effect of OSR1 on NaPiIIa, the major renal tubular phosphate transporter. Immunohistochemistry and confocal microscopy were employed to determine renal localization of OSR1 and NaPiIIa. To elucidate the effect of OSR on NaPiIIa activity, cRNA encoding NaPiIIa was injected into Xenopus oocytes with or without additional injection of cRNA encoding OSR1, and phosphate transport was estimated from phosphate-induced current determined with dual electrode voltage clamp. To elucidate the in vivo significance of OSR1 serum phosphate and hormone concentrations as well as urinary phosphate output of mice carrying one allele of WNK-resistant OSR1 (osr1<sup>tg/+</sup>) were compared to the respective values of wild type mice (osr1<sup>+/+</sup>). As a result, NaPiIIa and OSR1 were both expressed in proximal renal tubule cells. Coexpression of OSR1 significantly up-regulated phosphate-induced currents in NaPiIIa-expressing Xenopus oocytes. Despite a decreased serum phosphate concentration urinary phosphate excretion was significantly increased and NaPiIIa protein abundance in the brush border membrane significantly reduced in osr1<sup>tg/+</sup> mice as compared to osr1<sup>+/+</sup> mice. Serum PTH and calcitriol levels were similar in osr1<sup>tg/+</sup> mice and in osr1<sup>+/+</sup> mice, serum FGF23 concentration was, however, significantly higher in osr1<sup>tg/+</sup> mice than in osr1<sup>+/+</sup> mice. In conclusion, OSR1 is expressed in proximal renal tubules and participates in the regulation of renal tubular phosphate transport.

**Key words:** Na<sup>+</sup>, phosphate cotransporter, FGF23, bone density
Introduction

The oxidative stress-responsive kinase 1 (OSR1) participates in the signaling regulating transport during oxidative and osmotic stress\(^1\text{-}^6\). OSR1 is activated by the osmosensitive WNK (with no K) kinase isoforms WNK1 and WNK4\(^3\text{-}^7\text{,}^8\) and up-regulates the thiazide-sensitive \(\text{Na}^+\text{-Cl}^-\) cotransporter (NCC) and the furosemide-sensitive \(\text{Na}^+\text{,K}^+\text{-2Cl}^-\) cotransporters (NKCC1 and NKCC2), thus contributing to the regulation of cell volume, transepithelial transport, renal salt excretion, and GABA neurotransmission\(^1\text{-}^3\text{,}^9\text{-}^14\). Presumably due to their impact on renal tubular salt transport WNK1 and WNK4\(^2\text{-}^15\text{-}^18\) and OSR1\(^7\text{-}^19\text{-}^22\) participate in the regulation of blood pressure.

Nothing is known about a role of OSR1 in other nephron segments and in other renal tubular transport systems. The present study thus explored whether OSR1 influences the activity of the \(\text{Na}^+\)-coupled phosphate transporter NaPiIIa (SLC34A1), which is the most important carrier accomplishing renal tubular phosphate transport across the apical brush border membrane of proximal renal tubules\(^23\text{-}^25\). Renal tubular phosphate reabsorption is tightly regulated by dietary phosphate intake, acid-base status, parathyroid hormone, \(1,25\text{-}\text{(OH)}_2\text{ vitamin D}_3\), FGF23, insulin and insulin-like growth factor IGF1\(^26\text{-}^32\). Signaling known to regulate NaPiIIa includes the protein kinases A and C, ERK1/2, Klotho and the PI3K/PKB/GSK3 kinase cascade\(^33\text{-}^40\).

The present study addressed the putative role of OSR1 in the regulation of NaPiIIa. Coexpression of OSR1 enhanced phosphate-induced currents reflecting electrogenic phosphate transport in NaPiIIa-expressing \textit{Xenopus} oocytes. Immunohistochemistry and confocal microscopy revealed coexpression of OSR1 and NaPiIIa in proximal renal tubules. Urinary phosphate excretion was higher and serum phosphate concentration lower in heterozygous OSR1 knockin mice resistant to WNK-mediated activation (\textit{osr}^I+) than in respective wild type mice (\textit{osr}^+/+). Thus, the observations point to a role of OSR1 in the regulation of renal tubular phosphate transport.
Materials and Methods

In vitro expression

For generation of cRNA, constructs were used encoding wild type human NaPiIIa and human OSR1. The cRNA was generated as described previously. The constructs encoding wild type and catalytically-inactive D164A OSR1 were kindly provided by Dario Alessi (University of Dundee, UK). For electrophysiology, Xenopus oocytes were prepared as previously described. Fifteen ng of NaPiIIa cRNA were injected on the first day and 10 ng OSR1 cRNA on the second day into Xenopus oocytes. All experiments were performed at room temperature 3 days after the second injection. Two electrode voltage-clamp recordings were performed at a holding potential of -50 mV. The data were filtered at 10 Hz and recorded with a Digidata A/D-D/A converter and Chart V.4.2 software for data acquisition and analysis (Axon Instruments). The control solution (superfusate/ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2 and 5 mM HEPES, pH 7.4. One mM phosphate was added to induce NaPiIIa-dependent currents. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s.

Animals

All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities. Blood was drawn, urine collected or tissue isolated from sex- and age-matched 3-8-month-old heterozygous OSR1 knockin mice (osr1tg/+), and respective wild type mice (osr1+/+), kindly provided by Dario Alessi. As described earlier, in the knockin mice the T-loop Thr residue in OSR1 (Thr185) was mutated to Ala to prevent activation by WNK isoforms. Homozygous OSR1 knockin mice (osr1tg/tg) are not viable. Mice had free access to control diet (sniff, Soest, Germany) containing 7000 mg/kg phosphorus or to phosphate-depleted diet (Altromin, Lage, Germany) containing 131 mg/kg phosphate and to tap drinking water ad libitum. To obtain serum, mice were anaesthetized with diethylether (Roth, Karlsruhe, Germany) and blood was withdrawn into capillaries by puncturing the retrobulbar plexus.

To determine creatinine clearance, urinary flow rate and urinary excretion of phosphate, the mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) as described previously. They were allowed a 2 days habituation period during which food and water intake, urinary flow rate and phosphate excretion were recorded every day to ascertain that the mice were adapted to the new environment. Subsequently, 24 h collection of urine was performed for three consecutive days in order to obtain the urinary parameters. This procedure was repeated under low-phosphate diet. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil.

The phosphate concentration was determined colorimetrically utilizing a commercial diagnostic kit (Roche Diagnostics, Mannheim, Germany). The urinary concentration of Ca2+ was measured by flame photometry (Eppendorf, Hamburg, Germany). The creatinine concentration in urine was determined using the Jaffe reaction (Labor und Technik, Berlin, Germany). The serum intact parathormone concentration was measured using an ELISA kit (Immunotopics, San Clemante, USA) as was the concentration of 1,25(OH)2D3 (IDS Diagnostics, Frankfurt/Main, Germany) and of FGF23 (Immunotopics).

Brush border membrane vesicles (BBMV) preparation and immunoblotting

BBMVs were prepared from mouse kidney cortex and outer medulla using the Mg2+ precipitation technique as described previously. The total protein concentration was measured using the Bio–Rad Protein Assay kit (Bio–Rad, Hercules, CA, USA). BBMVs were stored at −80°C until further use. Five micrograms of renal brush border membrane proteins were solubilized in loading buffer containing dithiothreitol (DTT) and separated on 8% polyacrylamide gels. For immunoblotting, the proteins were transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA, USA). After blocking with 5% milk powder in Tris-buffered saline/0.1% Tween-20 for 60 min, the
blots were incubated with the primary antibodies: rabbit polyclonal anti-NaPiIIa (1:6000) and mouse monoclonal anti-β-actin antibody (42 kD; Sigma, St. Louis, MO; 1:5000) either for 2 h at room temperature or overnight at 4°C. Membranes were then incubated for 1 h at room temperature with secondary goat anti-rabbit or donkey anti-mouse antibodies 1:5000 linked to alkaline phosphatase (Promega, Mannheim, Germany) or to horseradish peroxidase (HRP) (Amersham, Freiburg, Germany). The protein signal was detected with the appropriate substrates (Millipore, Schwalbach, Germany) using the DIANA III-chemiluminescence detection system (Raytest, Straubenhardt, Germany). All images were analysed using the software Advanced Image Data Analyser AIDA, Raytest to calculate the protein of interest/β-actin ratio.

**Western blotting of whole kidney**

To determine protein abundance in renal tissue, mice were anesthetized with diethylether. The kidneys were removed and immediately shock-frozen in liquid nitrogen. Renal tissue was homogenised with an electric homogenizer at 4°C in lysis buffer (54.6 mM HEPES; 2.69 mM Na₄P₂O₇; 360 mM NaCl; 10% [vol/vol] Glycerol; 1% [vol/vol] NP40) containing phosphatase and protease inhibitors (Complete mini, Roche, Mannheim, Germany). Homogenates were clarified by centrifugation at 20000 g for 20 min. Total protein (100 µg) was separated by SDS-PAGE (10% Tris-Glycine), transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany), blocked for 3 h in blocking buffer (5% fat-free milk in Tris-buffered saline (TBS) containing 0.1% Tween), and incubated overnight with an anti Klotho antibody (kindly provided by Akiko Saito from the Kyowa Hakko Kirin Co., Ltd., Japan) (1:1.000 in 5% fat free milk in TBS). After incubation with a horseradish peroxidase-conjugated anti-rat secondary antibody (Cell Signaling, Germany), the bands were visualized with enhanced chemiluminescence according to the manufacturer's instructions. Homogenates were also probed with a primary GAPDH (Cell Signaling) antibody as loading control. Densitometric analysis of Klotho and GAPDH was performed using Quantity One software (Bio-Rad Laboratories).

**Immunhistochemistry**

For immunofluorescence microscopic detection of NaPiIIa and OSR in proximal tubule immunolabeling was performed on cryostat or paraffin sections of 5 µm thickness, blocked with 5% milk powder in PBS, incubated for 2 h with primary antibodies (sheep anti-OSR antibody [1:500 dilution; University of Dundee] and rabbit anti-NaPiIIa [1:400 dilution; kind gift of J. Biber, Zurich]), followed by secondary antibodies (Cy3-coupled donkey-anti sheep IgG and Cy2-coupled swine-anti rabbit IgG [Dianova]). Specificity of the double-staining procedures was controlled by parallel incubation of consecutive sections, each incubated only with one single probe. Fluorescence signals were detected by confocal laser scanning microscopy (LSM 510 META; Carl Zeiss, Jena, Germany) with a Plan Neofluar 100×/1.3 oil objective.58,59

**Statistics**

Data are provided as means ± SEM, n represents the number of independent experiments. All data were tested for significance using unpaired Student’s t-test or ANOVA, as appropriate. Only results with p < 0.05 were considered statistically significant.
Results

Immunohistochemistry was employed to explore whether OSR1 is expressed in proximal renal tubules. As illustrated in Fig. 1, OSR1 protein could indeed be detected in the proximal nephron. Concomitant labelling of the proximal tubular phosphate transporter NaPiIIa revealed co-localization of the kinase with the carrier in a subset of proximal tubules (Fig. 1).

To possibly disclose an effect of OSR1 on NaPiIIa, cRNA encoding NaPiIIa was injected into Xenopus oocytes with or without cRNA encoding OSR1 and electrogenic phosphate transport determined by the dual-electrode voltage-clamp technique. Addition of phosphate (1 mM) to the bath induced only a small inward current in non-injected or water-injected Xenopus oocytes, indicating that Xenopus oocytes do not express endogeneous electrogenic phosphate transport (Fig. 2). In Xenopus oocytes expressing NaPiIIa, phosphate induced an inward current (I_p) consistent with electrogenic entry of Na^+ together with phosphate (Fig. 2). As illustrated in Fig. 2, I_p was significantly enhanced by additional coexpression of OSR1. In contrast, coexpression of the inactive mutant D164A-OSR1 did not significantly modify I_p in NaPiIIa-expressing Xenopus oocytes (Fig. 2).

In order to elucidate the in vivo significance of OSR1-sensitive renal tubular phosphate transport, experiments were performed in OSR1 knockin mice (osr1^{tg/+}), which were heterozygously carrying a WNK-insensitive T185A-OSR1 mutant. The animals were compared to respective wild type mice (osr1^{+/-}). On a standard diet, urinary phosphate excretion was significantly higher in osr1^{tg/+} mice than in osr1^{+/-} mice (Fig. 3). A low-phosphate diet decreased the urinary phosphate excretion in both, osr1^{tg/+} and osr1^{+/-} mice (Fig. 3). During the low-phosphate-diet, however, urinary phosphate excretion was still significantly higher in osr1^{tg/+} mice than in osr1^{+/-} mice (Fig. 3).

In theory, the phosphaturia of osr1^{tg/+} mice could have been due to an increased serum phosphate concentration. Conversely, phosphaturia due to decreased renal tubular phosphate transport should result in a decrease of serum phosphate concentration. Thus, serum phosphate concentration was determined. As illustrated in Fig. 4, the serum phosphate concentration was significantly lower in osr1^{tg/+} mice than in osr1^{+/-} mice. Thus, the phosphaturia of osr1^{tg/+} mice was not due to hyperphosphatemia but obviously resulted in hypophosphatemia. Dietary phosphate depletion decreased the serum phosphate concentration in both, osr1^{tg/+} mice and in osr1^{+/-} mice and further enhanced the difference between the genotypes.

To study whether the partial OSR1 deficiency results in lower apical expression of NaPiIIa, BBMVs from the proximal tubule were retrieved and NaPiIIa protein expression determined. As shown in Fig. 5, BBMVs from osr1^{tg/+} mice indeed expressed NaPiIIa to a significantly lesser extent than BBMVs from osr1^{+/-} mice.

At least in theory, the decreased renal tubular phosphate transport could have resulted from altered hormone levels. Thus, the serum concentration of parathyroid hormone (PTH), calcitriol (1,25(OH)_{2}D_{3}), and fibroblast growth factor 23 (FGF23) were determined. As shown in Fig. 6, the serum concentrations of PTH (Fig. 6A) and of 1,25(OH)_{2}D_{3} (Fig. 6B) were similar in osr1^{tg/+} mice and in osr1^{+/-} mice. The serum FGF23 concentration was, however, significantly higher in osr1^{tg/+} mice than in osr1^{+/-} mice (Fig. 6C). Dietary phosphate depletion resulted in a significant increase in the 1,25(OH)_{2}D_{3} concentration of both, osr1^{tg/+} mice and osr1^{+/-} mice and tended to decrease the serum concentrations of PTH and FGF23, effects, however not reaching statistical significance (Fig. 6).

As FGF23 signaling depends on Klotho, additional experiments were performed to elucidate whether partial OSR deficiency modifies renal Klotho expression. To this end, renal Klotho protein was determined in osr1^{tg/+} mice and osr1^{+/-} mice (Fig. 7). As a result, no significant difference of Klotho protein abundance could be observed between osr1^{tg/+} mice and osr1^{+/-} mice.
Discussion

The present observations reveal that oxidative stress-responsive kinase 1 (OSR1) is expressed in proximal renal tubules and stimulates renal tubular phosphate transport. Moreover, WNK resistance of OSR1 led to phosphaturia. Accordingly, renal tubular phosphate reabsorption was decreased and thus renal phosphate excretion increased in OSR1 knockin mice carrying one allele of WNK-insensitive T185A-OSR1 mutant (osr1tg) as compared to the respective wild type mice (osr1+/+).

The electrophysiological recordings with the two-electrode voltage clamp in Xenopus oocytes heterologously expressing NaPiIIa with and without OSR1 pointed to a stimulatory effect of OSR1 on NaPiIIa activity. Along those lines, partial deficiency of OSR1 activity in osr1tg/+ mice led to lower NaPiIIa surface abundance in brush border membrane vesicles isolated from the proximal tubule.

The phosphaturia of osr1tg/+ mice was not secondary to an increased serum phosphate concentration, which was actually decreased. In theory, the phosphaturia osr1tg/+ could result from an increased serum level of PTH, a hormone increasing urinary phosphate output by stimulation of internalization and subsequent degradation of NaPiIIa. The PTH serum concentration was, however, not significantly different between osr1tg+ and osr1+/+ mice. This observation, does not rule out minor alterations of the serum PTH level. Renal tubular phosphate transport and serum phosphate concentration could further be modified by calcitriol (1,25(OH)2D3). The serum 1,25(OH)2D3 concentration was, however, not significantly different between osr1tg+ mice and osr1+/+ mice. The renal 1α-hydroxylase and thus the formation of 1,25(OH)2D3 are stimulated by PTH and by cellular phosphate depletion. The present observations confirm the increase in serum 1,25(OH)2D3 following dietary phosphate depletion and confirm the decrease of urinary phosphate output in phosphate-depleted animals.

Our study further shows that the FGF23 serum concentration was higher in osr1tg+ mice than in osr1+/+ mice. FGF23 is known to be up-regulated by 1,25(OH)2D3 and in turn to decrease the formation of 1,25(OH)2D3. In view of the serum 1,25(OH)2D3 levels, the slightly but significantly enhanced FGF23 serum level in osr1tg/+ mice cannot be explained by 1,25(OH)2D3-dependent stimulation of FGF23 expression. FGF23 is secreted by osteoblasts and decreases renal tubular phosphate transport. The increased serum FGF23 concentrations could thus contribute to the phosphaturia of the osr1tg/+ mice. Since FGF23 formation is stimulated by PTH, increased phosphate intake and hyperphosphatemia, the enhanced FGF23 serum level of osr1tg/+ mice cannot be explained by renal phosphate loss and hypophosphatemia. In theory, the enhanced FGF23 release in osr1tg/+ mice could be secondary to the effect of OSR1 on NaCl-co-transporter NCC. The carrier is expressed in osteoblasts, fosters bone differentiation and increases bone mineral density. OSR1 activates and phosphorylates NCC. Alternatively, OSR1 could be effective through inhibition of renal NCC or by more directly modulating FGF23 release in osteoblasts. Further studies are warranted addressing the mechanisms causing the elevated FGF23 plasma concentration in osr1tg/+ mice.

The effect of FGF23 on renal cells requires expression of Klotho, which thus similarly influences phosphate homeostasis. The determination of total renal Klotho protein expression did, however not reveal any significant differences between osr1tg/+ mice and osr1+/+ mice.

In conclusion, OSR1 participates in the regulation of FGF23 release and renal tubular phosphate transport. OSR1 stimulates NaPiIIa, and partial loss of OSR1 activity leads to phosphaturia and increased formation of FGF23. The present observations thus disclose a completely novel, powerful element in the regulation of mineral metabolism and a novel functional role of oxidative stress-responsive kinase 1.
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Statement of competing financial interests

The authors declare that they have no competing financial interests to disclose.
References


Figure legends

Fig. 1. Distribution of the oxidative stress responsive kinase 1 (OSR1) in wild type (osr1+/+) kidneys.
A. Confocal images showing double-labeling of OSR1 (green signal) and NaPiIIa (red signals) in proximal tubules from wild type kidneys. Merged images demonstrate co-localization of OSR1 and NaPiIIa in a subset of proximal tubules, whereas some proximal tubules express either OSR1 or NaPiIIa.
B. Higher resolution images demonstrating co-localization of OSR1 (green signal in the apical cellular compartment) and NaPiIIa (red signal in the brushborder membrane) in proximal tubules from wild type kidney; nuclei are counterstained in blue on the merged image.

Fig. 2: Coexpression of OSR1 up-regulates electrogenic phosphate transport in NaPiIIa-expressing Xenopus oocytes
A. Representative original tracings showing phosphate (1 mM)-induced current (I_P) in Xenopus oocytes injected with water (a) as well as expressing NaPiIIa without (b) or with additional coexpression of wild type OSR1(c) or inactive mutant D164AOSR1 (d).
B. Arithmetic means ± SEM (n = 17-62) of phosphate (1 mM)-induced current (I_P) in Xenopus oocytes injected with water (H2O), or expressing NaPiIIa without (2nd bar) or with additional coexpression of wild type OSR1 (3rd bar) or of the inactive mutant D164AOSR1 (4th bar). *** (p<0.001) indicates statistically significant difference from the absence of OSR1. ### (p<0.001) indicates statistically significant difference between wild type OSR1 and mutant D164AOSR1.

Fig. 3: Urinary phosphate excretion in osr1+/+ and osr1 tg/+ mice
Arithmetic means ± SEM (n = 14-16) of urinary phosphate excretion in heterozygous OSR1 knockin mice (osr1tg/+, black bars) and wild type mice (osr1+/+, white bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05), ** (p<0.01) indicate significant difference from osr1+/+ mice, ### (p<0.001) indicates significant difference from normal diet.

Fig. 4: Serum phosphate concentration in osr1+/+ and osr1 tg/+ mice
Arithmetic means ± SEM (n = 14-16) of the serum phosphate concentration in heterozygous OSR1 knockin mice (osr1tg/+, black bars) and wild type mice (osr1+/+, white bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05), ** (p<0.01) indicate significant difference from osr1+/+ mice, # (p<0.05), ## (p<0.01) indicate significant difference from normal diet.

Fig. 5: Protein abundance of renal sodium-dependent phosphate cotransporter NaPiIIa in the brush border membrane in kidneys from osr1+/+ and osr1 tg/+ mice
Western blot for NaPiIIa. The membrane was stripped and reprobed for β-actin to control for loading. Densitometry was performed and the ratio of the protein of interest over β-actin calculated. Bar graphs present data as arithmetic means ± SEM (n = 10). *** indicates significant difference from osr1+/+ mice (Student’s t-test, p<0.001).

Fig. 6: Serum concentration of PTH, 1,25(OH)2D3, and FGF23 in osr1+/+ and osr1 tg/+ mice
Arithmetic means ± SEM (n = 8-11) of serum PTH (A), 1,25(OH)2D3 (B), and FGF23 (C) in osr1+/+ mice (white bars) and osr1tg/+ mice (black bars) under normal diet (left bars) and following dietary phosphate depletion (right bars). * (p<0.05) indicates significant difference from osr1+/+ mice, ### (p<0.001) indicates significant difference from normal diet.

Fig. 7 Abundance of Klotho protein in the kidneys from osr1+/+ and osr1 tg/+ mice
Original Western blots (upper panel) and arithmetic means ± SEM (n = 10) of the Klotho over GAPDH density ratio in osr1+/+ mice (white bar) and osr1tg/+ mice (black bar).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

NaPi-IIa

β-actin

+/-
tg/+

NaPi-IIa/β-actin

***

+/

tg/+
Figure 6

A

- Normal diet
- Low phosphate diet

B

- Normal diet
- Low phosphate diet
Figure 6

C

- Normal diet
- Low phosphate diet

Comparison of serum FGF 23 levels between +/+ and tg/+ groups under normal diet and low phosphate diet conditions.
Klotho Protein Expression in OSR1 mice

Figure 7