Original Paper

Impact of Annexin A 7 Deficiency on FGF23 Plasma Concentrations

Anja T. Umbach Omar El-Attar Dong Luo Hajar Fakhri Rosi Bissinger
Florian Lang

Department of Cardiology, Vascular Medicine and Physiology, University of Tuebingen, Tuebingen, Germany

Key Words

FGF23 • 1,25(OH)₂D₃ • PTH • Corticosterone

Abstract

Background/Aims: The release of fibroblast growth factor FGF23, a powerful regulator of 1,25(OH)₂D₃ formation and mineral metabolism, is stimulated by store-operated Ca²⁺ entry (SOCE), which is accomplished by the pore forming Ca²⁺ release activated channel protein Orai1. Regulators of Orai1 and thus FGF23 release include serum & glucocorticoid inducible kinase SGK1, a kinase up-regulated by glucocorticosteroids. Some effects of glucocorticoids require the presence of annexin A7, such as suppression of prostaglandin E2 in gastric glands. The present study thus explored whether annexin A7 impacts on FGF23 plasma levels. **Methods:** Comparisons were made between gene targeted mice lacking functional annexin A7 (*Anx7^{-/-}*) and their wild type littermates (*Anx7^{+/+}*). Serum C-terminal-FGF23, intact FGF23, 1,25(OH)₂D₃ and PTH concentrations were measured by ELISA or EIA. The serum and urinary phosphate concentrations were measured by colorimetry, the serum Ca²⁺ concentration and the urinary Ca²⁺ concentration by flame photometry. **Results:** Serum C-terminal FGF23 levels and corticosterone levels were significantly higher and serum 1,25(OH)₂D₃ and PTH levels were significantly lower in *Anx7^{-/-}* than in *Anx7^{+/+}* mice. Water intake was slightly but significantly higher in *Anx7^{-/-}* mice than in *Anx7^{+/+}* mice. No significant difference was observed between *Anx7^{-/-}* and *Anx7^{+/+}* mice in urinary fluid excretion, plasma Ca²⁺ concentration, plasma phosphate concentration and urinary Ca²⁺ output. The urinary phosphate output was significantly lower in *Anx7^{-/-}* mice than in *Anx7^{+/+}* mice. **Conclusion:** Annexin A7 deficiency upregulates FGF23 plasma levels, an effect paralleled by increased corticosterone plasma levels, as well as decreased 1,25(OH)₂D₃ and PTH plasma levels.

© 2016 The Author(s)
Published by S. Karger AG, Basel

Introduction

FGF23 (fibroblast growth factor 23), a powerful regulator of calcium and phosphate metabolism [1, 2] decreases the formation of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) by down-regulation of renal 1 α hydroxylase (Cyp27b1) and fosters the inactivation of 1,25(OH)₂D₃ by up-regulation of 25-hydroxyvitamin D 24-hydroxylase (Cyp24a1) [3-6]. FGF23 thus lowers serum levels of 1,25(OH)₂D₃ [5-12], which in turn up-regulates renal and intestinal phosphate and calcium transport [13-16]. Beyond its effect on 1,25(OH)₂D₃ metabolism, FGF23 inhibits more directly renal tubular phosphate reabsorption [2, 5, 7, 8, 10-12, 17] thus enhancing renal phosphate elimination [5, 7, 8, 10-12]. FGF23 deficiency is thus followed by increased serum 1,25(OH)₂D₃, phosphate and calcium concentration, profuse tissue calcification, accelerated aging and reduction of lifespan [4, 12, 18-21].

Several effects of FGF23 require Klotho as a co-receptor [22-24]. Similar to FGF23 deficient mice, Klotho deficient mice suffer from tissue calcification, rapid aging and early death [22, 25-39], resulting from increased 1,25(OH)₂D₃ formation, enhanced renal tubular phosphate reabsorption and increased serum phosphate levels [40-42].

Stimulators of FGF23 release include 1,25(OH)₂D₃ [24, 43], PHEX (phosphate-regulating gene with homology to endopeptidase)[24], DMP-1 (dentin matrix protein or cyclin D binding myb-like protein 1) [24], sustained phosphate load even in the absence of hyperphosphatemia [24], increased extracellular Ca²⁺ concentration [24], parathyroid hormone [24], and lithium [44].

Cellular mechanisms governing FGF23 release include increase of cytosolic Ca²⁺ activity following stimulation of store operated Ca²⁺ entry (SOCE) [45-47]. Orai1, the pore forming channel protein accomplishing SOCE is upregulated by the serum & glucocorticoid inducible kinase SGK1 [48-51]. As SGK1 is upregulated by glucocorticoids [52], FGF23 may be sensitive to glucocorticoid action. As a matter of fact, glucocorticoids do increase FGF23 release[53, 54].

Modifiers of glucocorticoid effects include annexin A7 [55], a member of a large family of ubiquitously expressed Ca²⁺- and phospholipid-binding intracellular proteins[56-60], which are involved in the regulation of a wide variety of functions including inhibition of phospholipase A₂ [61, 62]. Gene targeted mice lacking functional annexin A7 suffer from excessive formation of PGE₂ [63], disorders of cardiac excitation [64, 65], enhanced glial cell proliferation [66], accelerated suicidal erythrocyte death [67] and resistance towards the stimulation of glucocorticoids on gastric acid secretion [55].

We hypothesized that SGK1 dependent regulation of FGF23 release could be sensitive to annexin A7. We thus explored whether annexin A7-deficiency impacts on FGF23 plasma concentrations.

Materials and Methods

Mice

All animal experiments were conducted according to the German law for the welfare of animals and were approved by the responsible authorities of the state of Baden-Württemberg (Regierungspräsidium Tübingen). Experiments were performed in gene-targeted mice lacking annexin A7 (*Anx7^{-/-}*) and in corresponding wild type mice (*Anx7^{+/+}*). Generation, properties and genotyping of *Anx7^{-/-}* mice were described earlier [64]. The male age-matched mice (9–15 weeks old) used for the experiments had free access to standard chow (SSniff, Soest, Germany) and tap water ad libitum.

Determination of serum and plasma hormone concentrations

To collect blood specimens, animals were lightly anesthetized and about 50 - 200 μ l of blood was withdrawn by puncturing the retro-orbital plexus [68]. Serum C-terminal FGF23 and serum intact FGF23 (Immutopics International, San Clemente, CA, USA), serum 1,25(OH)₂D₃ (IDS, Boldon, UK), plasma intact PTH (Immunotopics, San Clemente, USA), and serum corticosterone (IBL, Hamburg, Germany) concentrations were measured by

Fig. 1. Serum c-terminal and intact FGF23 levels in *Anx7^{-/-}* and *Anx7^{+/+}* mice. Arithmetic means $\pm$ SEM (n = 30–35) of serum (A) C-terminal FGF23 concentrations and (B) intact FGF23 concentration in mice lacking functional annexin A7 (*Anx7^{-/-}*, black bars) and their wild type littermates (*Anx7^{+/+}*, white bars). ***($p < 0.001$) indicates statistically significant difference from *Anx7^{+/+}* mice.

enzyme-linked immunosorbent assay (ELISA) or EIA according to the manufacturer's instructions. The serum phosphate and ionized Ca^{2+} concentrations were measured by a flame photometer (Eppendorf, Hamburg, Germany).

Measurement of urinary calcium and phosphate concentrations

For determination of urinary parameters, the mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) for 24 h collection of urine [69]. To assure quantitative urine collection, metabolic cages were siliconized, and urine was collected under water-saturated oil.

The urinary phosphate concentration was determined colorimetrically utilizing a commercial diagnostic kit (Roche Diagnostics, Mannheim, Germany). Urinary calcium was measured by flame photometry (Eppendorf, Hamburg, Germany).

Statistics

Data are provided as means $\pm$ SEM, n represents the number of independent experiments. All data were tested for significance using Student t -test. Only results with $p < 0.05$ were considered statistically significant.

Results

The present study compared FGF23 plasma levels and calcium phosphate metabolism in *Anx7^{-/-}* and *Anx7^{+/+}* mice. As illustrated in Fig. 1, serum C-terminal FGF23 levels were significantly higher in *Anx7^{-/-}* than in *Anx7^{+/+}* mice. Intact FGF23 serum levels similarly tended to be higher in *Anx7^{-/-}* than in *Anx7^{+/+}* mice, a difference, however, not reaching statistical significance.

At least in theory, the increased FGF23 level in *Anx7^{-/-}* mice could have resulted from enhanced stimulation by $1,25(\text{OH})_2\text{D}_3$ and/or PTH. Thus the serum concentrations of $1,25(\text{OH})_2\text{D}_3$ and of PTH were determined. As illustrated in Fig. 2A, serum $1,25(\text{OH})_2\text{D}_3$ levels were significantly lower in *Anx7^{-/-}* than in *Anx7^{+/+}* mice. Similarly, serum PTH levels were significantly lower in *Anx7^{-/-}* than in *Anx7^{+/+}* mice (Fig. 2B).

In order to test whether annexin A7 deficiency affects glucocorticoid release, plasma corticosterone levels were determined. As illustrated in Fig. 3, serum corticosterone levels were significantly higher in *Anx7^{-/-}* mice than in *Anx7^{+/+}* mice.

Further experiments explored whether the differences in hormone levels between *anx7^{-/-}* and *Anx7^{+/+}* mice affected plasma concentrations and urinary output of Ca^{2+} and/or phosphate. To this end, the animals were placed in metabolic cages. As illustrated in Fig. 4, water intake was slightly but significantly higher in *Anx7^{-/-}* mice than in *Anx7^{+/+}* mice (Fig. 4A). No significant difference was observed in urinary fluid excretion (Fig. 4B), food intake (Fig. 4C), and body weight (Fig. 4D) between *Anx7^{-/-}* and *Anx7^{+/+}* mice.

Fig. 2. 1,25(OH)₂D₃ and PTH levels in *anx7*^{-/-} and *anx7*^{+/+} mice. Arithmetic means ± SEM (n = 11-13) of serum (A) 1,25(OH)₂D₃ levels and (B) intact PTH levels in mice lacking functional annexin A7 (*Anx7*^{-/-}, black bars) and their wild type littermates (*Anx7*^{+/+}, white bars). * (p < 0.05) indicates statistically significant difference from *Anx7*^{+/+} mice.

Fig. 3. Serum corticosterone levels in *anx7*^{-/-} and *anx7*^{+/+} mice. Arithmetic means ± SEM (n = 6-7) of serum corticosterone concentrations in mice lacking functional annexin A7 (*Anx7*^{-/-}, black bars) and their wild type littermates (*Anx7*^{+/+}, white bars). * (p < 0.05) indicates statistically significant difference from *Anx7*^{+/+} mice.

Fig. 4. Water intake, food intake and urinary fluid excretion in *anx7*^{-/-} and *anx7*^{+/+} mice. Arithmetic means ± SEM of (A) water intake (n = 33/group; upper left), (B) urinary fluid excretion (n = 40/group; upper right), (C) food intake (n = 33/group; lower left) and (D) body weight (Bwt) (n = 40/group; lower right) in mice lacking functional annexin A7 (*Anx7*^{-/-}, black bars) and their wild type littermates (*Anx7*^{+/+}, white bars). ** (p < 0.01) indicates statistically significant difference from *Anx7*^{+/+} mice.

As illustrated in Fig. 5, Ca²⁺ plasma concentration (Fig. 5A) was similar in *Anx7*^{-/-} mice and *Anx7*^{+/+} mice. Urinary Ca²⁺ output (Fig. 5B) tended to be lower in *Anx7*^{-/-} than in *Anx7*^{+/+} mice, a difference, however, not reaching statistical significance. Moreover, no significant difference was observed between *Anx7*^{-/-} and *Anx7*^{+/+} mice in plasma phosphate concentration (Fig. 5C). The urinary phosphate output was, however, significantly lower in *Anx7*^{-/-} mice than in *Anx7*^{+/+} mice (Fig. 5D).

Discussion

The present observations reveal that annexin A7 deficiency leads to enhanced plasma FGF23 concentration, which is paralleled by increased plasma corticosterone levels, significantly decreased serum $1,25(\text{OH})_2\text{D}_3$ and PTH levels, significantly enhanced water intake and significantly decreased urinary phosphate output.

The increased FGF23 formation in *Anx7^{-/-}* mice could possibly be due to increased plasma corticosterone levels, as, similar to mineralocorticoids [45] glucocorticoids are known to up-regulate FGF23 release [53, 54]. Glucocorticoids and mineralocorticoids are presumably effective by up-regulating the

serum & glucocorticoid inducible kinase (SGK1), a kinase participating in the regulation of FGF23 formation [45]. SGK1 is in turn at least in part effective by increasing store operated Ca^{2+} entry [45-47].

The enhanced FGF23 plasma level in *Anx7^{-/-}* mice explains the decreased plasma $1,25(\text{OH})_2\text{D}_3$ levels, as FGF23 downregulates 1α hydroxylase, CYP27b1 and thus $1,25(\text{OH})_2\text{D}_3$ formation [3, 5]. For its effect, FGF23 requires Klotho as a co-receptor [22, 70]. As a negative feedback, $1,25(\text{OH})_2\text{D}_3$ stimulates the release of FGF23 [71].

The significantly decreased urinary phosphate output in *Anx7^{-/-}* mice is presumably a result of decreased formation of $1,25(\text{OH})_2\text{D}_3$, a stimulator of intestinal phosphate transport [13]. FGF23, on the other hand, was expected to trigger phosphaturia as it decreases renal tubular phosphate reabsorption by inhibiting $1,25(\text{OH})_2\text{D}_3$ formation and Na^+ -coupled phosphate transport in proximal renal tubules [2, 5]. It must further be kept in mind that renal Ca^{2+} and phosphate excretion are modified by glucocorticoids [72-75].

Conclusion

The present observations reveal that lack of annexin A7 results in increased plasma FGF23 levels, an effect possibly in part due to enhanced corticosterone release, and

Fig. 5. Serum calcium and phosphate concentration and urinary calcium and phosphate excretion in *anx7^{-/-}* and *anx7^{+/+}* mice. Arithmetic means $\pm$ SEM of (A) serum Ca^{2+} concentration (n = 28-29/group; upper left), (B) urinary Ca^{2+} excretion (n = 25-26/group; upper right), (C) serum phosphate concentration (n = 20-21/group; lower left) and (D) urinary phosphate excretion (n = 20/group; lower right) in mice lacking functional annexin A7 (*Anx7^{-/-}*, black bars) and their wild type littermates (*Anx7^{+/+}*, white bars). *(p < 0.05) indicates statistically significant difference from *Anx7^{+/+}* mice.

presumably accounting for decreased serum $1,25(\text{OH})_2\text{D}_3$ and PTH levels as well as decreased urinary phosphate output.

Disclosure Statement

All authors disclose that they have not any potential conflict of interest.

Acknowledgements

The authors acknowledge the technical assistance of E. Faber and the meticulous preparation of the manuscript by L. Subasic. The study was supported by the Deutsche Forschungsgemeinschaft (La 315/15-1 and Fo 695/1-1 and Fo 695/2-1).

References

- 1 Hori M, Shimizu Y, Fukumoto S: Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology* 2011;152:4-10.
- 2 Marsell R, Jonsson KB: The phosphate regulating hormone fibroblast growth factor-23. *Acta Physiol (Oxf)* 2010;200:97-106.
- 3 Gattineni J, Twombly K, Goetz R, Mohammadi M, Baum M: Regulation of Serum $1,25(\text{OH})_2\text{Vitamin D}_3$ levels by Fibroblast Growth Factor 23 is Mediated by FGF Receptors 3 and 4. *Am J Physiol Renal Physiol* 2011;301:F371-F377.
- 4 Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T: Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest* 2004;113:561-568.
- 5 Shimada T, Yamazaki Y, Takahashi M, Hasegawa H, Urakawa I, Oshima T, Ono K, Kakitani M, Tomizuka K, Fujita T, Fukumoto S, Yamashita T: Vitamin D receptor-independent FGF23 actions in regulating phosphate and vitamin D metabolism. *Am. J Physiol Renal Physiol* 2005;289:F1088-F1095.
- 6 Inoue Y, Segawa H, Kaneko I, Yamanaka S, Kusano K, Kawakami E, Furutani J, Ito M, Kuwahata M, Saito H, Fukushima N, Kato S, Kanayama HO, Miyamoto K: Role of the vitamin D receptor in FGF23 action on phosphate metabolism. *Biochem J* 2005;390:325-331.
- 7 Bai X, Miao D, Li J, Goltzman D, Karaplis AC: Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. *Endocrinology* 2004;145:5269-5279.
- 8 Bai XY, Miao D, Goltzman D, Karaplis AC: The autosomal dominant hypophosphatemic rickets R176Q mutation in fibroblast growth factor 23 resists proteolytic cleavage and enhances in vivo biological potency. *J Biol Chem* 2003;278:9843-9849.
- 9 Saito H, Kusano K, Kinosaki M, Ito H, Hirata M, Segawa H, Miyamoto K, Fukushima N: Human fibroblast growth factor-23 mutants suppress Na^{+} -dependent phosphate co-transport activity and $1\alpha,25$ -dihydroxyvitamin D_3 production. *J Biol Chem* 2003;278:2206-2211.
- 10 Segawa H, Kawakami E, Kaneko I, Kuwahata M, Ito M, Kusano K, Saito H, Fukushima N, Miyamoto K: Effect of hydrolysis-resistant FGF23-R179Q on dietary phosphate regulation of the renal type-II Na/Pi transporter. *Pflugers Arch* 2003;446:585-592.
- 11 Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T: Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia. *Proc Natl Acad Sci USA* 2001;98:6500-6505.
- 12 Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S, Yamashita T: FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 2004;19:429-435.

- 13 Brown AJ, Finch J, Slatopolsky E: Differential effects of 19-nor-1,25-dihydroxyvitamin D(2) and 1,25-dihydroxyvitamin D(3) on intestinal calcium and phosphate transport. *J Lab Clin Med* 2002;139:279-284.
- 14 Miyamoto K, Segawa H, Ito M, Kuwahata M: Physiological regulation of renal sodium-dependent phosphate cotransporters. *Jpn J Physiol* 2004;54:93-102.
- 15 Murer H, Hernando N, Forster I, Biber J: Proximal tubular phosphate reabsorption: molecular mechanisms. *Physiol Rev* 2000;80:1373-1409.
- 16 Segawa H, Kaneko I, Yamanaka S, Ito M, Kuwahata M, Inoue Y, Kato S, Miyamoto K: Intestinal Na-P(i) cotransporter adaptation to dietary P(i) content in vitamin D receptor null mice. *Am J Physiol Renal Physiol* 2004;287:F39-F47.
- 17 Larsson T, Marsell R, Schipani E, Ohlsson C, Ljunggren O, Tenenhouse HS, Juppner H, Jonsson KB: Transgenic mice expressing fibroblast growth factor 23 under the control of the $\alpha 1(I)$ collagen promoter exhibit growth retardation, osteomalacia and disturbed phosphate homeostasis. *Endocrinology* 2003;145:3094.
- 18 Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, Drueke TB, Massy ZA, Group. EUTEW: FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int* 2012;23:2017-2025.
- 19 Mackenzie NC, MacRae VE: The role of cellular senescence during vascular calcification: a key paradigm in aging research. *Curr Aging Sci* 2011;4:128-136.
- 20 Schoppet M, Hofbauer LC, Brinselle-Schmal N, Varennes A, Goudable J, Richard M, Hawa G, Chapurlat R, Szulc P: Serum Level of the Phosphaturic Factor FGF23 Is Associated with Abdominal Aortic Calcification in Men: The STRAMBO Study. *J Clin Endocrinol Metab* 2012;97:E575-E583.
- 21 Sitara D, Razzaque MS, Hesse M, Yoganathan S, Taguchi T, Erben RG, Juppner H, Lanske B: Homozygous ablation of fibroblast growth factor-23 results in hyperphosphatemia and impaired skeletogenesis, and reverses hypophosphatemia in PheX-deficient mice. *Matrix Biol* 2004;23:421-432.
- 22 Kuro-o M: Overview of the FGF23-Klotho axis. *Pediatr Nephrol* 2010;25:583-590.
- 23 Kuro-o M: Klotho. *Pflugers Arch* 2010;459:333-343.
- 24 Hu MC, Shiizaki K, Kuro O, Moe OW: Fibroblast growth factor 23 and klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 2013;75:503-533.
- 25 Bernheim J, Bencherit S: The potential roles of FGF23 and Klotho in the prognosis of renal and cardiovascular diseases. *Nephrol Dial Transplant* 2011;26:2433-2438.
- 26 Carpinelli MR, Wise AK, Burt RA: Vitamin D-deficient diet rescues hearing loss in Klotho mice. *Hear Res* 2011;275:105-109.
- 27 Fukuchi Y: The aging lung and chronic obstructive pulmonary disease: similarity and difference. *Proc Am Thorac Soc* 2009;6:570-572.
- 28 Kempe DS, Ackermann TF, Fischer SS, Koka S, Boini KM, Mahmud H, Foller M, Rosenblatt KP, Kuro O, Lang F: Accelerated suicidal erythrocyte death in Klotho-deficient mice. *Pflugers Arch* 2009;458:503-512.
- 29 Kosakai A, Ito D, Nihei Y, Yamashita S, Okada Y, Takahashi K, Suzuki N: Degeneration of mesencephalic dopaminergic neurons in klotho mouse related to vitamin D exposure. *Brain Res* 2011;1382:109-117.
- 30 Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 1997;390:45-51.
- 31 Kuro O: Klotho and betaKlotho. *Adv Exp Med Biol* 2012;728:25-40.
- 32 Maltese G, Karalliedde J: The putative role of the antiageing protein klotho in cardiovascular and renal disease. *Int J Hypertens* 2012;2012:757469.
- 33 Nikolov IG, Mozar A, Drueke TB, Massy ZA: Impact of disturbances of calcium and phosphate metabolism on vascular calcification and clinical outcomes in patients with chronic kidney disease. *Blood Purif* 2009;27:350-359.
- 34 Ohnishi M, Razzaque MS: Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging. *FASEB J* 2010;24:3562-3571.
- 35 Saito Y, Nakamura T, Ohyama Y, Suzuki T, Iida A, Shiraki-Iida T, M K-o, Nabeshima Y, Kurabayashi M, Nagai R: In vivo klotho gene delivery protects against endothelial dysfunction in multiple risk factor syndrome. *Biochem Biophys Res Commun* 2000;276:767-772.

- 36 Shimada T, Takeshita Y, Murohara T, Sasaki K, Egami K, Shintani S, Katsuda Y, Ikeda H, Nabeshima Y, Imaizumi T: Angiogenesis and vasculogenesis are impaired in the precocious-aging klotho mouse. *Circulation* 2004;110:1148-1155.
- 37 Takeshita K, Fujimori T, Kurotaki Y, Honjo H, Tsujikawa H, Yasui K, Lee JK, Kamiya K, Kitaichi K, Yamamoto K, Ito M, Kondo T, Iino S, Inden Y, Hirai M, Murohara T, Kodama I, Nabeshima Y: Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. *Circulation* 2004;109:1776-1782.
- 38 Torres PU, Prie D, Molina-Bletty V, Beck L, Silve C, Friedlander G: Klotho: an antiaging protein involved in mineral and vitamin D metabolism. *Kidney Int* 2007;71:730-737.
- 39 Tuohimaa P: Vitamin D and aging. *J Steroid Biochem Mol Biol* 2009;114:78-84.
- 40 Yoshida T, Fujimori T, Nabeshima Y: Mediation of unusually high concentrations of 1,25-dihydroxyvitamin D in homozygous klotho mutant mice by increased expression of renal 1 α -hydroxylase gene. *Endocrinology* 2002;143:683-689.
- 41 Dermaku-Sopjani M, Sopjani M, Saxena A, Shojaiefard M, Bogatkov E, Alesutan I, Eichenmuller M, Lang F: Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of Klotho. *Cell Physiol Biochem* 2011;28:251-258.
- 42 Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, Razzaque MS, Rosenblatt KP, Baum MG, Kuro-o M, Moe OW: Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J* 2010;24:3438-3450.
- 43 Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, Bouillon R, Carmeliet G: Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* 2006;116:3150-3159.
- 44 Fakhri H, Pathare G, Fajol A, Zhang B, Bock T, Kandolf R, Schleicher E, Biber J, Foller M, Lang UE, Lang F: Regulation of mineral metabolism by lithium. *Pflügers Arch* 2014;466:467-475.
- 45 Zhang B, Umbach AT, Chen H, Yan J, Fakhri H, Fajol A, Salker MS, Spichtig D, Daryadel A, Wagner CA, Foller M, Lang F: Up-regulation of FGF23 release by aldosterone. *Biochem Biophys Res Commun* 2016;470:384-390.
- 46 Zhang B, Yan J, Schmidt S, Salker MS, Alexander D, Foller M, Lang F: Lithium- Sensitive Store-Operated Ca²⁺ Entry in the Regulation of FGF23 Release. *Neurosignals* 2015;23:34-48.
- 47 Zhang B, Yan J, Umbach AT, Fakhri H, Fajol A, Schmidt S, Salker MS, Chen H, Alexander D, Spichtig D, Daryadel A, Wagner CA, Foller M, Lang F: NF κ B-sensitive Orai1 expression in the regulation of FGF23 release. *J Mol Med (Berl)* 2016;94:557-566.
- 48 Borst O, Schmidt EM, Munzer P, Schonberger T, Towhid ST, Elvers M, Leibrock C, Schmid E, Eyleinstein A, Kuhl D, May AE, Gawaz M, Lang F: The serum- and glucocorticoid-inducible kinase 1 (SGK1) influences platelet calcium signaling and function by regulation of Orai1 expression in megakaryocytes. *Blood* 2012;119:251-261.
- 49 Eyleinstein A, Gehring EM, Heise N, Shumilina E, Schmidt S, Sztejn K, Munzer P, Nurbaeva MK, Eichenmuller M, Tyran L, Regel I, Foller M, Kuhl D, Soboloff J, Penner R, Lang F: Stimulation of Ca²⁺-channel Orai1/STIM1 by serum- and glucocorticoid-inducible kinase 1 (SGK1). *FASEB J* 2011;25:2012-2021.
- 50 Eyleinstein A, Schmidt S, Gu S, Yang W, Schmid E, Schmidt EM, Alesutan I, Sztejn K, Regel I, Shumilina E, Lang F: Transcription factor NF- κ B regulates expression of pore-forming Ca²⁺ channel unit, Orai1, and its activator, STIM1, to control Ca²⁺ entry and affect cellular functions. *J Biol Chem* 2012;287:2719-2730.
- 51 Lang F, Shumilina E: Regulation of ion channels by the serum- and glucocorticoid-inducible kinase SGK1. *FASEB J* 2013;27:3-12.
- 52 Lang F, Stournaras C: Serum and glucocorticoid inducible kinase, metabolic syndrome, inflammation, and tumor growth. *Hormones (Athens)* 2013;12:160-171.
- 53 Quarles LD: A systems biology preview of the relationships between mineral and metabolic complications in chronic kidney disease. *Semin Nephrol* 2013;33:130-142.
- 54 Ramon I, Kleynen P, Valsamis J, Body JJ, Karmali R: Hypophosphatemia related to paraneoplastic Cushing syndrome in prostate cancer: cure after bilateral adrenalectomy. *Calcif Tissue Int* 2011;89:442-445.
- 55 Pasham V, Rotte A, Mia S, Alesutan I, Chatterjee S, Hosseinzadeh Z, Bhandaru M, Noegel AA, Lang F: Annexin 7 in the regulation of gastric acid secretion. *Cell Physiol Biochem* 2013;32:1643-1654.

- 56 Camors E, Monceau V, Charlemagne D: Annexins and Ca²⁺ handling in the heart. *Cardiovasc Res* 2005;65:793-802.
- 57 Gerke V, Moss SE: Annexins: from structure to function. *Physiol Rev* 2002;82:331-371.
- 58 Monastyrskaya K, Babiychuk EB, Draeger A: The annexins: spatial and temporal coordination of signaling events during cellular stress. *Cell Mol Life Sci* 2009;66:2623-2642.
- 59 Monastyrskaya K, Babiychuk EB, Hostettler A, Wood P, Grewal T, Draeger A: Plasma membrane-associated annexin A6 reduces Ca²⁺ entry by stabilizing the cortical actin cytoskeleton. *J Biol Chem* 2009;284:17227-17242.
- 60 Raynal P, Pollard HB: Annexins: the problem of assessing the biological role for a gene family of multifunctional calcium- and phospholipid-binding proteins. *Biochim Biophys Acta* 1994;1197:63-93.
- 61 Russo-Marie F: Macrophages and the glucocorticoids. *J Neuroimmunol* 1992;40:281-286.
- 62 Yoshizaki H, Tanabe S, Arai K, Murakami A, Wada Y, Ohkuchi M, Hashimoto Y, Maki M: Effects of calphobindin II (annexin VI) on procoagulant and anticoagulant activities of cultured endothelial cells. *Chem Pharm Bull (Tokyo)* 1992;40:1860-1863.
- 63 Lang PA, Kasinathan RS, Brand VB, Duranton C, Lang C, Koka S, Shumilina E, Kempe DS, Tanneur V, Akel A, Lang KS, Foller M, Kun JF, Kremsner PG, Wesselborg S, Laufer S, Clemen CS, Herr C, Noegel AA, Wieder T, Gulbins E, Lang F, Huber SM: Accelerated clearance of Plasmodium-infected erythrocytes in sickle cell trait and annexin-A7 deficiency. *Cell Physiol Biochem* 2009;24:415-428.
- 64 Herr C, Smyth N, Ullrich S, Yun F, Sasse P, Hescheler J, Fleischmann B, Lasek K, Brixius K, Schwinger RH, Fassler R, Schroder R, Noegel AA: Loss of annexin A7 leads to alterations in frequency-induced shortening of isolated murine cardiomyocytes. *Mol Cell Biol* 2001;21:4119-4128.
- 65 Schrickel JW, Brixius K, Herr C, Clemen CS, Sasse P, Reetz K, Grohe C, Meyer R, Tiemann K, Schroder R, Bloch W, Nickenig G, Fleischmann BK, Noegel AA, Schwinger RH, Lewalter T: Enhanced heterogeneity of myocardial conduction and severe cardiac electrical instability in annexin A7-deficient mice. *Cardiovasc Res* 2007;76:257-268.
- 66 Clemen CS, Herr C, Hovelmeyer N, Noegel AA: The lack of annexin A7 affects functions of primary astrocytes. *Exp Cell Res* 2003;291:406-414.
- 67 Lang E, Lang PA, Shumilina E, Qadri SM, Kucherenko Y, Kempe DS, Foller M, Capasso A, Wieder T, Gulbins E, Clemen CS, Herr C, Noegel AA, Huber SM, Lang F: Enhanced eryptosis of erythrocytes from gene-targeted mice lacking annexin A7. *Pflugers Arch* 2010;460:667-676.
- 68 Foller M, Kempe DS, Boini KM, Pathare G, Siraskar B, Capuano P, Alesutan I, Sopjani M, Stange G, Mohebbi N, Bhandaru M, Ackermann TF, Judenhofer MS, Pichler BJ, Biber J, Wagner CA, Lang F: PKB/SGK-resistant GSK3 enhances phosphaturia and calciuria. *J Am Soc Nephrol* 2011;22:873-880.
- 69 Boini KM, Bhandaru M, Mack A, Lang F: Steroid hormone release as well as renal water and electrolyte excretion of mice expressing PKB/SGK-resistant GSK3. *Pflugers Arch* 2008;456:1207-1216.
- 70 Bai X, Dinghong Q, Miao D, Goltzman D, Karaplis AC: Klotho ablation converts the biochemical and skeletal alterations in FGF23 (R176Q) transgenic mice to a Klotho-deficient phenotype. *Am J Physiol Endocrinol Metab* 2009;296:E79-E88.
- 71 Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, Bouillon R, Carmeliet G: Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* 2006;116:3150-3159.
- 72 Friedlander G: Regulation of renal phosphate handling: recent findings. *Curr Opin Nephrol Hypertens* 1996;5:316-320.
- 73 Pennisi P, Trombetti A, Rizzoli R: Glucocorticoid-induced osteoporosis and its treatment. *Clin Orthop Relat Res* 2006;443:39-47.
- 74 Prie D, Beck L, Urena P, Friedlander G: Recent findings in phosphate homeostasis. *Curr Opin Nephrol Hypertens* 2005;14:318-324.
- 75 Rojas-Vega L, Gamba G: Mini-review: regulation of the renal NaCl cotransporter by hormones. *Am J Physiol Renal Physiol* 2016;310:F10-14.