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GENETIC DISEASES OF RENAL PHOSPHATE HANDLING

Carsten A Wagner, Isabel Rubio-Aliaga, Jürg Biber, Nati Hernando

Institute of Physiology and Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

Corresponding author
Carsten A. Wagner
Institute of Physiology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
Switzerland
Phone: +41-44-63 55023
Fax: +41-44-63 56814
E-mail: Wagnerca@access.unizh.ch

ABSTRACT

Renal control of systemic phosphate homeostasis is critical as evident from inborn and acquired diseases causing renal phosphate wasting. At least three transport proteins are responsible for renal phosphate reabsorption: NAPI-IIa (SLC34A1), NAPI-IIc (SLC34A3), and PIT-2 (SLC20A2). These transporters are highly regulated by various cellular mechanisms and factors including acid-base status, electrolyte balance, and hormones such as dopamine, glucocorticoids, growth factors, vitamin D_3, parathyroid hormone, and FGF23. Whether renal phosphate wasting is caused by inactivating mutations in the *NAPI-IIa* transporter is controversial. Mutations in the *NAPI-IIc* transporter cause hereditary hypophosphatemic rickets with hypercalciuria. Besides the primary inherited defects there are also inherited defects in major regulators of phosphate homeostasis that lead to alterations in phosphate handling. Autosomal dominant hypophosphatemic rickets is due to *FGF23* mutations leading to resistance against its own degradation. Similarly, inactivating mutations in the *PHEX* gene, which causes FGF23 inactivation, cause X-linked hypophosphatemia due to renal phosphate losses. In contrast, mutations in *GALNT3*, responsible for O-glycosylation of FGF23, or in klotho (*KL)*, a cofactor for *FGF23* signaling result in hyperphosphatemia. Acquired syndromes of renal phosphate wasting, hypophosphatemia, and osteomalacia (tumor-associated osteomalacia) can be due to the excessive synthesis or release of phosphaturic factors (FGF23, FGF-7, MEPE, sFRP4) from mesenchymal tumors.

INTRODUCTION

About 85 % of total phosphate is deposited in bone and about 10 % in soft tissues, the remaining 2-3 % is found in serum, constituting a freely exchangeable and tightly regulated phosphate pool. Serum phosphate levels in adults are maintained in a narrow range of about 0.8 – 1.45 mmol/l (2.4 – 4.5 mg/dl).Deviations as found in patients with hypophosphatemia or hyperphosphatemia can cause severe disturbances of cellular and organ function such ATP depletion, rickets, osteomalacia, anemia, or excessive tissue calcifications, nephrolithiasis, arteriosclerosis and increased risk of cardiovascular morbidity and mortality [1-3].

Phosphate homeostasis is the product of intestinal uptake of phosphate (in the range of 0.9 – 1 g/day) and reabsorption of phosphate from urine (in the range of 60-80 %). Both intestinal uptake and renal reabsorption are mediated by sodium-dependent phosphate cotransporters (Na^+/P_i-cotransporters) belonging to the *SLC20* and *SLC34* gene families of solute carriers [4-5]. Their activity and expression is tightly regulated by a large variety of hormones and metabolic factors adjusting uptake and excretion to short and long-term body requirements. Inborn errors are caused by mutations in phosphate transporters and in several genes that encode factors directly and indirectly involved in their regulation. The identification of these genes and elucidation of their functions have greatly contributed to our present understanding how phosphate homeostasis is achieved. This review will give a brief overview over physiological function and regulation of renal phosphate transporters. For more extensive reviews the reader is referred to a number of recent reviews and book chapters [5-9]. The second part of this review will discuss mutations in several genes that directly or indirectly participate in (renal) control of phosphate balance.

General aspects of renal phosphate handling

About 80 % of filtered phosphate is reabsorbed from urine under normal dietary phosphate intake by the earlier convoluted parts of the proximal tubule of mostly juxtamedullary nephrons [4]. Phosphate absorption is mediated by sodium-dependent cotransporters for inorganic phosphate (Pi) located in the brush border membrane.

Three members of the type II Na^+/dependent phosphate cotransporter family have been identified and classified in the *SLC34* solute carrier gene family [4]. *SLC34A1* (*NAPI-IIa*) is predominantly expressed in kidney mediating about 70 – 80 % of overall phosphate reabsorption as suggested by a *Slc34a1* deficient mouse model [10]. Another member of this family, SLC34A3 (NAPI-IIc), is also almost exclusively found in kidney [11]. *SLC34A2* (*NAPI-IIb*) is mostly expressed in small intestine and in a number of other organs including testis, lung, liver, and lactating mammary glands [12].

Also sodium-dependent phosphate transporters from the SLC20 family are expressed in the kidney. PIT-2 (SLC20A2) is localized in the brush border membrane of the proximal tubule whereas the exact site of expression of *PIT-1* in kidney is unclear [13]. *PIT-2* is widely expressed in most tissues. Its exact role in renal phosphate reabsorption has not been clarified but simultaneous genetic deletion of *NaPi-IIa* and *NaPi-IIc* in mice reduced renal phosphate reabsorption by more than 90 % suggesting that its contribution to overall phosphate reabsorption may be minor [14]. PIT-2 is regulated by dietary phosphate intake, acid-base status, and PTH [15-16]. Mutations in *PIT-2* have been described in patients with idiopathic basal ganglia calcification [17].

SLC34A1 (NaPi-IIa) and SLC34A3 (NaPi-IIc)

The human *SLC34A1* gene lies on chromosome 5q35 [18], is about 13 kb in length and consists of 13 exons and 12 introns [19]. Recently a model for the protein structure of NAPI-IIa was suggested based on homology modeling and detailed structure-function studies [20].

The human *SLC34A3* gene spans about 5 kb containing 13 exons and lies on chromosome 9q34 [21-22]. Human NAPI-IIc contains 599 amino acids [21-22]. *NaPi-IIc* expression in mouse is stimulated in response to low dietary phosphate intake [23]. Also dietary Pi-responsive elements (potential TFE-3 binding sites) are located at -1846 and -1822 [23].

The transport functions of SLC34A transporters and their structure-function relationship have been extensively studied elucidating important features such as cotransport mode, electrogenicity or voltage and pH-dependency [4].

Collectively these data demonstrate that NAPI-IIa transports three sodium ions together with one divalent phosphate ion (HPO_4^{2-}) per transport cycle. This transport mode is electrogenic [24-25]. The transporter homodimerizes but the functional unit is a monomer [26-27]. The type IIc cotransporter transports only 2 sodium ions per phosphate and is electroneutral indicating that divalent phosphate is its preferred substrate [28].

Regulation of renal phosphate reabsorption

A variety of factors affects renal phosphate handling as listed in table 1 [4, 29]. Alterations in the number of phosphate transporter molecules in the brush border membrane represent the main mechanism leading to changes in urinary phosphate excretion [29]. The cellular and molecular mechanisms by which NaPi-IIa and NaPi-IIc are regulated have been extensively studied for PTH and changes in dietary phosphate intake (for review: [4-5]).

PTH rapidly induces phosphaturia within minutes by decreasing apical phosphate transport activity. Activation of PLC by apical PTH receptors leads to protein kinase C dependent stimulation of ERK1/2 kinases and internalization of NaPi-IIa [29-31]. NaPi-IIa, apical PTH receptors, and PLCβ1 are organized in a macromolecular complex via the PDZ-scaffold protein NHERF1 (NHE regulating factor 1) [32-34]. In contrast, basolateral PTH receptors are linked to adenylate cyclase, protein kinase A (PKA) and ERK1/2 [31]. NaPi-IIa is not phosphorylated but dissociates from NHERF1 which is phosphorylated in response to PTH [35-

36]. A similar mechanism has been proposed for FGF23- and dopamine-induced endocytosis of NaPi-IIa [4].

The regulation of renal phosphate reabsorption by FGF23 is mediated by several mechanisms: FGF23 reduces circulating levels of active 1,25$(OH)_2$ vitamin D_3 by reducing expression of the activating enzyme CYP27B1 and by increasing expression the inactivating enzyme CYP24A1 [37]. Lower active vitamin D_3 reduces the stimulation of renal and intestinal phosphate (re)absorption. Moreover, renal phosphate reabsorption is directly reduced by FGF23 through downregulation of NaPiIIa and NaPiIIc. Two alternative but not exclusive pathways have been proposed. FGF23 binds to FGF1c receptors in the distal convoluted tubule, the main site of klotho expression in kidney, leads to ERK1/2 phosphorylation and the distal convoluted tubule then signals to the proximal tubule to internalize NaPi-IIa and NaPi-IIc [38]. Alternatively, FGF23 may bind to FGF23 receptors in the proximal tubule and lead to phosphorylation of NHERF1 via Sgk1 [39]. Also, a direct regulation of NaPiIIa activity by klotho without FGF23 has been demonstrated where klotho causes cleavage and inactivation of NaPi-IIa in the brush border membrane and subsequent internalization [40].

An acute reduction in NaPi-IIa transporters is mediated by the internalization from the apical brush border membrane via the same route as receptor-mediated endocytosis, requiring megalin and involving clathrin-coated vesicles and early endosomes [41-42]. Consequently, NaPi-IIa is routed to lysosomes for degradation [43-44]. The regulation of NaPi-IIc has been studied in less detail: in response or high phosphate NaPi-IIc downregulation occurs but is slower than for NaPi-IIa [16, 45].

Renal phosphate reabsorption closely matches dietary phosphate intake being high during dietary phosphate restriction and decreasing with high phosphate intake [11, 46-48]. In animals lacking PTH and/or 1,25 $(OH)_2$-vitamin D_3 the adaptive response of NaPi-IIa and NaPi-IIc is partially preserved indicating that other factors may be responsible for sensing and mediating changes in dietary phosphate intake [49-51].

PRIMARY INHERITED DEFECTS IN RENAL PHOSPHATE HANDLING

SLC34A1 (NAPI-IIa): hypophosphatemic nephrolithiasis/osteoporosis-1 (NPHLOP1) and Fanconi renotubular syndrome-2 (FRTS2)

Two reports addressed patients with mutations in the *SLC34A1*/*NAPI-IIa* gene. In the first report, two patients with urolithiasis or bone demineralization and persistent idiopathic hypophosphatemia with lower maximal renal phosphate reabsorption (TmP/GFR) were presented [52]. Single nucleotide changes resulting in missense mutations (A48F and V147M) were detected. These mutations were found only on one allele per patient suggesting a dominant effect [52]. Expression of the mutant *NAPI-IIa* transporters in oocytes was showed reduced phosphate transport activity, a dominant negative effect on wildtype

NAPI-IIa cotransporters, and reduced affinity for phosphate [52]. However, these data were controversially discussed. *SLC34A1* mutations were not confirmed in other kindreds with similar symptoms, relatives of patients had not been genetically investigated, and the expression assay showed only low expression even for the wildtype transporter rendering a kinetic analysis very difficult. Moreover, expression of the reported mutant *NAPI-IIa* cotransporters in Xenopus oocytes and the renal OK cell line did not show any evidence for altered subcellular localization, levels of expression, or reduced substrate affinity [53], but showed a lower transport activity.. Yet, this decrease cannot fully explain the massive phosphaturia observed. Therefore, it was suggested that the nucleotide changes may represent only polymorphisms. A similar conclusion was derived from screening a large numbers of pedigrees of calcium stone formers with low TmP/GFR [54]. Even though a number of mutations including an N-terminal deletion of 7 amino acids (91del7) in the *NAPI-IIa* gene were found, no correlation with the clinical phenotype could be established. Two of the mutations had lower phosphate transport activity after expression in Xenopus oocytes most likely due to decreased membrane abundance. However, the reduction in transport rates was mild and importantly, only one allele was found to be mutated in stone forming patients. The interpretation that these mutations may represent rather relatively common polymorphisms was further supported by the fact that these gene alterations were found also in several subjects with normal renal phosphate excretion. Thus, polymorphisms in the *NAPI-IIa* gene may be relatively common and their relevance for renal phosphate handling may require further clarification.

A second report described siblings from a consanginous Arab Israeli family with hypophosphatemic rickets, multiple fractures and stunted growth [55]. The presence of a Fanconi-like syndrome was suggested, but hallmarks of a generalized proximal tubule dysfunction are not evident from data reported. The patients suffered from hypercalciuria due to elevated serum $1,25(OH)_2D_3$ levels. Hypophosphatemia was refractory to vitamin D therapy. Genetic analysis detected a homozygous in-frame duplication of 21 base pairs leading to insertion of seven additional amino acids at position G154-V160. Heterozygous carriers of the mutation were clinically normal suggesting that the mutation did not exert a dominant negative effect. Expression of the mutant protein in Xenopus oocytes abolished all phosphate-induced currents whereas expression in renal OK cells indicated failure of the mutant protein to reach the plasma membrane.

Thus, homozygous mutations in *SLC34A1* may be responsible for renal phosphate wasting but whether this is only the case for this specific mutation and may be due to toxic effects of the mutant protein for proximal tubule functions or whether mutations in this phosphate transporter can provide evidence for the biological and clinical importance in human kidney function remains to be established.

***SLC34A3* (NAPI-IIc): hereditary hypophosphatemic rickets with hypercalciuria (HHRH)**

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)(OMIM #241530) is caused by mutations in the *SLC34A3* gene encoding for *NAPI-IIc* [21-22]. The disease was first described in a Bedouin family [56] and is characterized by renal phosphate wasting causing hypophosphatemia and secondary rickets, with elevated serum alkaline phosphatase. Serum levels of 1,25-(OH_2)-vitamin D_3 are high, PTH and FGF23 are low, resulting in enhanced intestinal calcium absorption and subsequent renal excretion (hypercalciuria) promoting nephrolithiasis or nephrocalcinosis in some patients [21-22, 56-57]. Long-term phosphate supplementation appears to reverse the clinical and biochemical abnormalities in HHRH.

The *SLC34A3* gene is localized on chromosome 9q34. Observed mutations in HHRH include frame shifts in the open reading frame with subsequent translation of sequences unrelated to NaPiIIc and premature termination, missense mutations, intronic deletions, and nucleotide changes in a putative splice site [21-22]. Inheritance follows an autosomal recessive pattern [21-22]. Some compound heterozygous patients carrying two different missense mutations were also identified [21]. However, carriers with only one allele mutated show normal phosphate balance and excretion but borderline or elevated renal calcium excretion [21-22].

Since *NAPI-IIc* mutations are associated with a severe renal phosphate leak, *NAPI-IIc* may have a more important role in human kidney function than in rodents. NAPI-IIc function in man remains important during adulthood in contrast to rodents where NaPi-IIc may play an important role only during growth [11].

Accordingly, two mouse models of *Slc34a3* deficiency show only mild hypercalcuria without hyperphosphaturia or even no symptoms in the case of a kidney-specific gene deletion [58-59].

SLC9A3R1 (NHERF1): Na^+/H^+ exchanger regulating factor 1: hypophosphatemic nephrolithiasis/osteoporosis-2 (NPHLOP2)

NHERF1 mutations were detected in eight patients with mildly reduced TmP/GFR, slightly lower serum phosphate levels, nephrolithiasis in four out of eight patients, lower bone mineral density in one patient, normal to low PTH and calcium values and $1,25(OH)_2D_3$ just above the normal range in all patients. Four NHERF1 mutations (E68A, L110V, R153Q, E225K) were found. All patients were heterozygous [60-61]. The mechanisms by which mutant NHERF1 may cause phosphaturia may be site-specific. In the case of the L110V, R153Q and E225K mutations, PTH and cAMP dependent inhibition of phosphate transport may be increased, whereas the E68A mutation may reduce the stability of the NaPiIIa transporter in the plasma membrane leading in all cases to lower transport activities [60-61].

DEFECTS IN RENAL PHOSPHATE HANDLING SECONDARY TO EXTRARENAL INHERITED DEFECTS

Fibroblast Growth Factor 23 (FGF23)

FGF23 is primarily expressed in osteocytes [62-64]. Synthesis and release of FGF23 from bone are increased during hyperphosphatemia or high intake of phosphate [9, 65]. The major functions of FGF23 are the inhibition of renal phosphate reabsorption by downregulation of *NAPI-IIa* and *NAPI-IIc* expression and activity and by reducing the synthesis of active vitamin D_3 through lowering expression of the renal *CYP27B1* (1,25-alpha hydroxylase) and increasing *CYP24A1* (1,24,25 hydroxylase). Lower concentrations of vitamin D_3 decrease intestinal phosphate absorption. In addition, FGF23 suppresses PTH secretion. The effects of FGF23 on phosphate homeostasis are mediated by FGFR1c and FGFR4 receptors and require klotho as an obligatory co-ligand converting the low affinity receptors into high affinity FGF23 receptors [9, 66].

Synthesis of FGF23 in osteocytes is regulated a number of factors including including PTH, 1,25 vitamin D_3 and a cascade of proteins consisting of PHEX, 7B2/PC2, BMP1 and DMP1. PTH and 1,25 vitamin D_3 transcriptionaly stimulate FGF23 production by mechanisms involving PKA/Wnt and the VDR receptor, respectively [67-68]. Activation of this cascade increases proteolytic processing of DMP1 into N- and C-terminal fragments, from which the latest one is proposed to blunt the transcription of FGF23 (for review see: [69]).

FGF23 is degraded by C-terminal cleavage most likely by subtilisin-like proprotein convertases [70]. FGF23 possesses a recognition site for cleavage by subtilisin-like proprotein convertases consisting of a RXXR motif at position 176. A recent report suggested that the PC2 propotein convertase, complexed with its chaperon 7B2, may mediate this process [71].

FGF23 activating mutations: Autosomal dominant hypophosphatemic rickets (ADHR)

Autosomal dominant hypophosphatemic rickets (OMIM # 193100) is characterized by rickets, hypophosphatemia, hyperphosphaturia, fatigue, bone pain, lower bone deformities in face of inappropriately low or normal vitamin D_3 levels. ADHR is caused by mutations destroying the RXXR cleavage motifmentioned above, causing excessive levels of intact FGF23 [72]. Similarly, injection of mice withwildtype *Fgf23* or *Fgf23*^{R179Q} caused ADHR like symptoms in mice. Mutant *Fgf23* caused hypophosphatemia, hyperphosphaturia, reduced intestinal phosphate absorption and suppressed serum vitamin D_3 levels. In serum, high levels of mutant *Fgf23* were detected whereas wildtype *Fgf23* was degraded [73]. Transgenic mice for *Fgf23*^{R176Q} display typical ADHR symptoms and show signs of secondary hyperparathyroidism [74]. Thus, ADHR is caused by mutations in a motif important for cleavage and degradation of FGF23 resulting in excessive FGF23 signaling.

Loss of function FGF23 mutations: familial tumoral calcinosis

Familial tumoral calcinosis (FTC, OMIM # 211900) is the mirror image of phosphate wasting diseases such as ADHR, TIO, and XLH. FTC is characterized by hyperphosphatemia, reduced renal phosphate excretion, ectopic calcifications, and inappropriately normal or elevated 1,25 (OH_2) vitamin D_3

levels. In a subset of FTC patients a homozygous 211A-G transition in the *FGF23* gene was identified, resulting in the substitution at an evolutionarily conserved serine to glycine (S71G) [75]. This mutation may cause loss of function [75]. Patients have abnormally low FGF23 plasma concentrations. Mutant FGF23 protein expressed in vitro, was not secreted and retained intracellularly [75].

Fibrous Dysplasia (McCune-Albright syndrome)

Fibrous dysplasia (FD, OMIM #174800) is caused by non-inherited genetic somatic activating missense mutations in the α-subunit of the stimulatory G protein, G_s [76-77]. FD is characterized by abnormalities in bone (monostotic or polyostotic fibrous dysplasia), in skin (pigmentation), and in the endocrine system (thyrotoxicosis, pituitary gigantism, and Cushing syndrome). The severity of disease and particularly the association with skin and endocrine symptoms shows a wide variability. Renal phosphate wasting is detected in about 50 % of patients [78]. FGF23 levels are elevated and caused by a large mass of FGF23 producing cells in fibrous bone lesions. Abnormal high FGF23 levels are caused by an increase in FGF23 producing cells but not by abnormal production of FGF23 per se [62, 79].

Klotho: familial tumoral calcinosis and hypophosphatemic rickets with hyperparathyrodism

Mutations in the alpha-klotho (*KL*) gene can be another reason for familial tumoral calcinosis besides mutations in *GALNT3* or loss-of-function mutations in *FGF23*. A single patient with a missense mutation was identified [80]. The mutation is predicted to affect the glucosidase domain of klotho which is involved in the regulation of TRPV5 calcium channels [81]. Moreover, reduced expression and glycosylation of klotho was demonstrated impairing the ability to interact with FGF23 and the FGF1R receptor. Accordingly, the patient had hyperphosphatemia, increased vitamin D_3 and PTH levels and ectopic calcifications of vessels.

The mirror image was described in another single case of a balanced translocation between chromosome 13 and 9 at a position on chromosome 13 adjacent to the alpha klotho gene. The translocation massively increased circulating klotho levels leading to hypophosphatemia, hyperphosphaturia, hypercalcemia, elevated PTH and FGF23, and low vitamin D_3 [82]. The elevated FGF23 levels may be explained by a feedback loop by which circulating alpha-klotho stimulates bone FGF23 expression [83].

Dentin matrix protein 1 (DMP1): Autosomal recessive hypophosphatemic rickets (ARHR)

Patients with mutations in *DMP1* suffer from hypophosphatemia due to hyperphosphaturia with inappropriately normal levels of 1,25-$(OH)_2$ vitamin D_3 [84-85]. DMP1 belongs to the large SIBLING family (small integrin-binding ligand, N-linked glycoproteins) of extracellular matrix proteins and is mainly coexpressed with FGF23 in bone. The similar phenotype of patients with X-linked hypophosphatemia (XLH) and autosomal recessive hypophosphatemia with rickets (ARHR) suggested a link to FGF23. Indeed, in patients with loss of intact DMP1, FGF23 levels in serum were normal or elevated [84-85]. DMP1 (in particular the C-terminal fragment) blocks the transcription of FGF23; therefore, its absence may result in the release of this negative control leading to higher FGF23 production. The hypophosphatemia in DMP1 deficient patients causes severe rickets in children or osteomalacia in adults [84-85] which is characterized by abnormal amounts of osteoid indicating defective mineralization and maturation of bone [85]. Similarly a mouse model deficient for DMP1 shows hypophosphatemia, hyperphosphaturia, rickets, retarded skeletal growth with abnormal mineralization, disturbed lacunocanicular organization, and defective osteoblast to osteocyte differentiation [85].

Tumor-induced osteomalacia (TIO)

Tumor-induced osteomalacia (TIO) is a rare paraneoplastic syndrome mostly associated with mesenchymal tumors releasing (a) phosphaturic factor(s). Symptoms include renal phosphate wasting causing hypophosphatemia, osteomalacia, and abnormal vitamin D metabolism [86]. Surgical removal of the tumor reverses symptoms. In contrast to syndromes of hyperparathyroidism or humoral hypercalcemia of malignancy, the plasma concentrations of calcium, PTH, and PTH related protein (PTHrp) are in the normal range. Several proteins, such as FGF23, sFRP4, FGF-7, and MEPE, have been identified that are produced and secreted from tumors from patients with TIO. Some of these proteins have been shown to regulate phosphate transport in vitro and/or in vivo.

Secreted frizzled-related protein-4 (sFRP-4) is highly upregulated in tumor tissue from patients with renal phosphate wasting [87] and inhibits phosphate transport in the renal OK cell line as well as in vivo [87-88]. However, mice lacking *sfrp4* do not show any abnormalities of systemic phosphate balance [89]. Thus, the relevance of sFRP4 for phosphate homeostasis remains to be further clarified.

Matrix extracellular phospho-glycoprotein (MEPE): oncogenic hypophosphatemia (OHO)

MEPE, a glycosylated protein of about 60 kDa, was initially cloned from tumor tissue obtained from a patient with oncogenic hypophosphatemia (OHO) [90]. Bone cells (osteoblasts, osteocytes and odontoblasts) are the major source of MEPE. MEPE like DMP1 is another member of the SIBLING family of extracellular matrix proteins involved in bone regulation. Injection of MEPE into intact mice results in hypophosphatemia, hyperphosphaturia and mild increases in circulating 1,25 $(OH)_2$ vitamin D_3 levels [91]. Moreover, implantation of MEPE producing CHO cells into nude mice caused renal phosphate wasting, whereas *MEPE* deficient mice have higher bone density [92]. The interactions between MEPE and other hormones regulating phosphate homeostasis and handling require more investigation.

PHEX: X-linked hypophosphatemic rickets (XLH)

X-linked hypophosphatemic rickets (XLH)(OMIM #307800) is the most common heritable form of rickets with a prevalence of about 1 in 20.000. The disease is characterized by hypophosphatemia due to excessive renal phosphate wasting leading to rickets, lower extremities deformity, short stature, bone and joint pain, enthesopathy, and dental abscesses. Vitamin D_3 levels are inappropriately normal or even low [93]. The disorder is inherited in a dominant manner. Positional cloning identified the *PHEX* gene (PHosphate regulating gene with homology to Endopeptidases on the X chromosome) in XLH patients [93]. Several mouse models of XLH are available: *Hyp*, *Gy*, and *Ska1* mice resembling XLH and which were later shown to have mutations in the mouse *Phex* homologue [94-96]. The X-linked *Hyp* mouse model demonstrates a defect in proximal tubular phosphate absorption, decreased expression of *NaPi-IIa* and *NaPi-IIc* [97-99]. Serum FGF23 levels are highly elevated in XLH patients [100]. Crossing of hypophosphatemic *Hyp* mice with hyperphosphatemic *Fgf23* deficient mice produced hyperphosphatemic mice that showed exactly the same phenotype as *Fgf23* null mice, indicating that both mutations affect the same system and that FGF23 may act downstream of PHEX [101]. Observations in Hyp and *FGF23* null mice indicate that PHEX and FGF23 may regulate each others expression levels and that loss of PHEX may lead to higher expression levels of FGF23 [63, 65]. Although originally proposed, PHEX seems not to mediate direct cleaving of FGF23 [63, 70]. Instead, PHEX may activate the PC2 proprotein convertase by promoting the transcription of its 7B2 chaperon [71]. Transfection of osteoblast with 7B2.PC2 promoted cleavage of FGF23 whereas depletion of 7B2 mRNA reduced FGF23 cleavage and increased its transcription. The activated FGF23 transcription seems to result from an impaired proteolytic processing of DMP1 (see above). In agreement with this model, the mRNA levels of 7B2 are reduced in bones from Hyp mice [71].

UDP-N-acetyl-α-D galactosamine:polypeptide N-acetyl-galactosaminyltransferase (GALNT3): Familial tumoral calcinosis

Homozygous loss of function mutations of the UDP-N-acetyl–α-D galactosamine:polypeptide N-acetyl-galactosaminyltransferase (*GALNT3*), a glycosyltransferase involved in mucin-type O-glycosylation, underlie also familial tumoral calcinosis (FTC, OMIM # 211900) [102-103]. However, patients carrying only one mutated allele appear to have also mild symptoms, leading to the initial description of FTC as an autosomal dominant disease [104]. Because inactivating mutations in *FGF23* also cause FTC and FGF23 has some O-glycosylation sites within the subtilisin-recognition sites, it had been speculated that GALNT3 is critical for FGF23 gylcosylation and full function. Accordingly, in vitro secretion of FGF23 from CHO cells deficient in O-glycosylation is impaired and cotransfection of GALNT3 markedly increases O-glycosylation and secretion of FGF23. Thus, GALNT3 may play an important role in FGF23 secretion by mediating its O-glycosylation [105]. This function may also explain how loss of function mutations in either FGF23 or GALNT3 can produce the same disease, FTC.

Fibroblast Growth Factor Receptor 1 (FGFR1): Osteoglophonic dysplasia

Osteoglophonic dysplasia (OMIM #166250) is caused by mutations in the fibroblast growth factor receptor 1 (FGFR1) that result in a gain-of-function and activation of the receptor [106]. Patients suffer from craniosynostosis, prominent supraorbital ridge, and depressed nasal bridge, as well as from rhizomelic dwarfism and nonossifying bone lesions. Several missense mutations in the *FGFR1* gene were identified in 4 patients. Three patients were hypophosphatemic due to massive renal phosphate wasting [106]. One patient had inappropriately high FGF23 levels, two patients had high 1,25-$(OH)_2$ vitamin D_3 levels. As two related patients carried the *Y372C FGFR1* mutation. The activity of the mutant receptor was highly increased suggesting that overactivity of the FGF1 receptor is responsible for this disease [106]. The FGFR1 receptor mediates the downregulation of NAPI-IIa and NAPI-IIc by FGF23, whereas the effects of FGF23 on vitamin D_3 metabolism may involve the FGFR4 receptor [66].

FAM20C: Raine syndrome (osteosclerotic bone dysplasia)

Raine syndrome is caused by mutations in the protein kinase FAM20C which resides in the Golgi apparatus and is secreted [107-108]. Patients develop generalized higher bone density with characteristic changes and enhancement of the ossification of the skull, cerebral calcification, and in some case hypoplastic lungs. The disease is often lethal in the first weeks after birth but patients with longer survival reaching into puberty have been described. The renal phenotype of patients with Raine syndrome has not been reported in detail. Targets phosphorylated by FAM20C are casein, osteoprotegerin (OPN), DMP1, and MEPE, members of the SIBLINGs family. In Fam20c deficient mice, hypophosphatemic rickets was observed possibly due to dysregulation of FGF23 (which is very high) due to lack of DMP1 phosphorylation [109].

CONCLUSION AND OUTLOOK

Renal and extrarenal control of systemic phosphate homeostasis requires a complicated network of regulatory factors, phosphate transporters in kidney, intestine, and bone, and intracellular protein-protein interactions. Rare human genetic disorders and mouse genetics have greatly contributed to our current understanding of (renal) phosphate homeostasis. Importantly, population based genome wide association studies identified SLC34A1 and FGF23 as important determinants of plasma phosphate levels [110]. Moreover, the highly elevated FGF23 levels in patients with various types of CKD and the potential link between FGF23 and cardiovascular disease in these patients underlines even further the biological importance of this homeostatic system for health and disease. Nevertheless, our understanding of key elements of this system is incomplete and we require deeper insights how organs and cells sense phosphate and how changes in systemic phosphate levels can trigger disease. The identification of key players through genetics may also pave the way for therapies targeting disturbed phosphate balance in both rare inherited disorders as well as in more common diseases such as CKD.

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Conflict of interest

The authors declare not conflict of interest and that the results presented in this paper have not been published previously in whole or part.

Table 1

Summary of known factors influencing renal phosphate excretion

For explanation and references see text.

Table 2

Summary of disorders affecting renal phosphate handling

For explanation and references see text.

Table 3

Factors affecting renal phosphate handling due to acquired syndromes

For explanation and references see text.

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Table 1

Factors affecting phosphate reabsorption in the proximal tubule

Factors that decrease Pi reabsorption	**Factors that increase Pi reabsorption**
Acidosis	Alkalosis
High dietary Pi intake	Low dietary Pi intake
Parathyroid hormone/ cAMP	Parathyroidectomy
Atrial Natriuretic Peptide/ cGMP	Thyroid hormone
Glucocorticoids	$1\alpha,25(OH)_2D_3$
Dopamine	Growth hormone(s)
Fibroblast growth factor 23	Insulin-like growth factor
MEPE	
Frizzled related protein 4	

Table 2

Genes/ diseases causing altered renal phosphate handling

Gene	Chromosome/ OMIM	Disease name	Major symptoms	Inheritance	Tissue distribution of protein
SLC34A1 (NaPiIIa)	5q35 OMIM#612286 OMIM#613388	hypophosphatemic nephrolithiasis/osteoporosis-1 (NPHLOP1) Fanconi renotubular syndrome-2 (FRTS2)	Hypophosphatemia, urolithiasis, osteoporosis ? Fanconi-like syndrome	Autosomal dominant Autosomal recessive	Kidney (proximal tubule)
SLC34A3 (NaPiIIc)	9q34 OMIM#241530	Hereditary hypophosphatemic rickets with hypercalciuria (HHRH)	Hypophosphatemia, hyperphosphaturia, rickets, Hypercalciuria Normal-low FGF23, high 1,25-D_3	Autosomal recessive	Kidney (proximal tubule)
SLC9A3R1 (NHERF1)	17q25.1 OMIM#612287	hypophosphatemic nephrolithiasis/osteoporosis-2 (NPHLOP2)	Hypophosphatemia Hyperphosphaturia Normal-low PTH Mildy elevated 1,25-D_3 Nephrolithiasis	Autosomal dominant	Kidney, intestine
FGF23	12p13.3 OMIM#193100	- Autosomal dominant hypophosphatemic rickets (ADHR) - Familial tumoral calcinosis	Hyperphosphaturia Hypophosphatemia Rickets/osteomalacia Normal 1,25-D_3 High FGF23	Autosomal dominant	Mainly bone

	OMIM#211900	(FTC)			
Klotho	13q13.1 OMIM#211900	Familial tumoral calcinosis Hypophosphatemic rickets with hyperparathyroidism	Hyperphosphatemia hypercalcemia Heterotopic calcifications high intact FGF23 high PTH Hypophosphatemia Hyperphosphaturia High FGF23 High PTH	Autosomal recessive Translocation, dominant	Kidney, parathyroid gland, brain
PHEX	Xp22.2-p22.1 OMIM#307800	X-linked hypophosphatemia (XLH)	Hyperphosphaturia Hypophosphatemia Rickets/osteomalacia Normal vit. D High FGF23	X-linked	Mainly bone
GALNT3	2q24-q31 OMIM#211900	Familial tumoral calcinosis (FTC)	Hyperphosphatemia heterotopic calcification	Autosomal recessive	Pancreas, testis
GNAS1	20q13.2 OMIM#174800	Fibrous dysplasia (McCune-Albright syndrome)	Hyperphosphaturia, high FGF-23 levels, polyostotic fibrous dysplasia, pigmentation, thyrotoxicosis, Cushing	Autosomal dominant, mosaicism	ubiquitous
FAM20c	7p22.3 OMIM#259775	Raine syndrome Osteosclerotic bone dysplasia	Increased bone density, periostal bone growth Skull deformation	Autosomal recessive	Bone, kidney,mammary gland, and others

FGFR1	8p11.2-p11.1 OMIM#166250	Osteoglophonic dysplasia	Hypophosphatemia Hyperphosphaturia Craniosynostosis	Autosomal dominant ?	Ubiquitous, bone, kidney
DMP1	4q21 OMIM#241520	Autosomal recessive hypophosphatemic rickets (ARHR) Autosomal recessive hypophosphatemia (ARHP)	Hypophosphatemia Hyperphosphaturia Rickets, osteomalacia	Autosomal recessive	Ubiquitous, Bone, heart, kidney

Table 3: Acquired forms of renal phosphate wasting

Gene/factor	**Chromosome/OMIM**	**Disease**	**Symptoms**	**Occurrence**	**Tissue distribution**
sFRP4	7p14-p13 OMIM606570	Tumor induced osteomalacia (TIO)	Hypophosphatemia Hyperphosphaturia Osteomalacia	Spontaneous ?	Tumor bone
FGF7	15q15-q21.1 OMIM148180	Tumor induced osteomalacia (TIO)	Hypophosphatemia Hyperphosphaturia Osteomalacia	Spontaneous ?	Tumor
MEPE	4q21.1 OMIM605912	Tumor induced osteomalacia (TIO)	Hypophosphatemia Hyperphosphaturia Osteomalacia	Spontaneous ?	Tumor, bone