5-2018

Hypophosphatemic Rickets: A Targeted Literature Review to Characterize the Multiple Causes of Phosphate Wasting Disorders and Identify Potential Disease Biomarkers

Christopher O'Mara
Dominican University of California

https://doi.org/10.33015/dominican.edu/2018.bio.04

Hypophosphatemic Rickets: A Targeted Literature Review to Characterize the Multiple Causes of Phosphate Wasting Disorders and Identify Potential Disease Biomarkers

By

Christopher Rajan O'Mara

A culminating thesis submitted to the faculty of Dominican University of California in partial fulfillment of the requirements for the degree of Master of Science in Biology

San Rafael, California

May 2018

This thesis, written under the direction of candidate's thesis advisor and approved by the thesis committee and the MS Biology program director, has been presented and accepted by the Department of Natural Sciences and Mathematics in partial fulfillment of the requirements for the degree of Master of Science in Biology at Dominican University of California. The written content presented in this work represent the work of the candidate alone.

Christopher O'Mara	_____
Candidate	05/11/2018
Maggie Louie, Ph.D.	_____
Thesis Advisor	05/11/2018
Kristylea Ojeda, Ph.D.	_____
Second Reader	05/11/2018
Meredith Protas, Ph.D.	_____
Program Director	05/11/2018

Table of Contents

List of Tables

List of Figures

Abstract

Author: Christopher Rajan O'Mara

Title: Hypophosphatemic Rickets: A Targeted Literature Review to Characterize the Multiple Causes of Phosphate Wasting Disorders and Identify Potential Disease Biomarkers

Thesis Advisor: Maggie Louie, Ph.D.

Institution: Dominican University of California

Year: 2018

Hypophosphatemic rickets is a rare, renal phosphate wasting disorder that presents various skeletal deformities. Although there are specific clinical presentations and biochemical findings used to identify hypophosphatemic rickets, there are various underlying renal phosphate wasting disorders that can lead to hypophosphatemic rickets making diagnosis extremely difficult. A targeted literature review through electronic search engines (e.g., BMC, PubMed, Google Scholar) was conducted to: 1) consolidate and summarize currently available data regarding the various renal phosphate wasting disorders, 2) identify current obstacles of successful diagnosis and treatment, and 3) propose directions for future hypophosphatemic rickets research into new biomarkers.

Acknowledgments

I would like to thank Dr. Maggie Louie, Dr. Kristylea Ojeda, Dr. Alison Skrinar, Dr. Micheal Vellard, and Dr. Phil Babij for their encouragement, support, and dedication toward helping me complete my thesis. I am also grateful for my friends, family, and colleagues that helped me reach this milestone.

Introduction

Rickets is a disorder characterized by the softening and weakening of bone due to osteomalacia, which is the deficiency of bone mineralization (Allgrove & Shaw, 2015). Poor bone mineralization often leads to skeletal deformities that include bowed legs, protruded sternum, and thickened wrists and ankles. Vitamin D deficiency is primarily attributed to rickets development due to its role in bone mineralization and maintaining calcium and phosphate homeostasis. Clinical studies in the 1930s and 1940s revealed hereditary genetic disorders as an additional source for the development of rickets (Albright, Butler, & Bloomberg, 1937; Albright & Reifenstein Jr, 1949). These discoveries led to the identification of insufficient reabsorption of phosphate by the proximal renal tubule and increased urinary phosphate excretion as the physiological cause for poor bone mineralization, which coined the term hypophosphatemic rickets.

Despite these research advancements of hypophosphatemic rickets, clinical presentations and biochemical findings used to identify hypophosphatemic rickets are similar across the various underlying phosphate wasting disorders. The focus of this targeted literature review through electronic search engines (e.g., BMC, PubMed, Google Scholar) is to: 1) consolidate and summarize currently available data regarding the various renal phosphate wasting disorders, 2) identify current obstacles of successful diagnosis and treatment, and 3) propose directions for future hypophosphatemic rickets research into new biomarkers.

Bone Development

The fully developed, adult human skeleton is comprised of 213 bones, each of which is composed of osteoblasts, osteocytes, osteoclasts, osteoids, and inorganic mineral

salts deposited within the bone matrix (Rosen, & Bouillon, 2013). Osteoblasts are responsible for bone synthesis and mineralization during initial formation by forming a closely packed sheet on the surface of the bone, from which cellular processes extend through the developing bone. Osteoblasts produce several cell products, including the enzymes such as alkaline phosphatase (ALP) and collagenase, as well as hormones like osteocalcin and collagen. These cellular products make up the organic, unmineralized component of the bone referred to as the osteoid. When the osteoblast is coated by the surrounding bone matrix it secretes, it calcifies and becomes a bone cell, also known as an osteocyte (Rosen, & Bouillon, 2013).

Osteocytes are mature bone cells located within fully formed bone. Small channels called canaliculi extend from osteocytes to other osteocytes in order to maintain bone viability by exchanging nutrients and waste products. Signaling between osteocytes is important for bone remodeling (bone metabolism), where mature bone tissue is removed from the skeleton (bone resorption) and new bone tissue is formed (ossification). Bone resorption and remodeling are activated when biomechanical forces on bone, such as fractures, are sensed by the osteocytes via the canalicular network (Noble, 2008). In the presence of a bone fracture, osteocytes function as mechanosensor cells that recruit osteoclasts and osteoblasts for the resorption of old bone and formation of new bone, respectively. Bone resorption is a complex process in which bone-specific macrophages known as osteoclasts break down bone tissue, resulting in the transfer of calcium, magnesium, phosphate and collagen into the bone's extracellular matrix (Rosen, & Bouillon, 2013). Bone resorption is critical for the formation of new bone, as resorption of old bone must precede the formation or deposition of new bone.

The formation of new bone occurs at the osteoid, which is also where bone was previously resorbed by osteoclasts. From here, osteoblasts prepare the osteoid for mineralization by synthesizing dense, crosslinked collagen to provide bone tensile strength. The continuous process of bone remodeling maintains the skeletal structure and homeostasis of calcium in the body (Driscoll, 2006). During bone resorption by osteoclasts, calcium is also released into the bloodstream and to further support continued bone growth and remodeling. Bone remodeling can be triggered to external responses, such as deformations of bone due to changes in muscular activity. For example, a long bone fracture triggers bone remodeling and new bone formation.

Role of Vitamin D

Bone formation is also impacted by the circulating levels of vitamin D, a hormone that activates the increased absorption of calcium and phosphate for bone development (Holick, 2006). Two major forms of vitamin D found in the human body are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Both forms are biologically inactive when absorbed into the body by dietary supplementation or after photobiogenesis with ultraviolet radiation via sun exposure. Both forms of vitamin D are then hydroxylated by 25-hydroxyvitamin D3 1-alpha-hydroxylase (1α-hydroxylase) in the liver and kidneys to become biologically active. Specifically, ergocalciferol is converted to 25-hydroxyergocalciferol in the liver, and then to the active form, 1,25-dihydroxyvitamin D, in the kidney (Wang, Zhu, & DeLuca, 2012). Once transformed, it binds to the vitamin D receptor (VDR), which leads to a variety of regulatory roles (Norman, 2008). Cholecalciferol is converted into calcifediol (25-hydroxycholecalciferol) in the liver, and calcifediol is further hydroxylated in the kidneys to form calcitriol (1,25-

dihydroxycholecalciferol), which is the biologically active form of vitamin D (Figure 1) (DeLuca, Holick, Schnoes, Suda, & Cousins, 1971; Gil, Plaza-Diaz, & Mesa, 2018; Norman, 2008; Silva & Furlanetto, 2018).

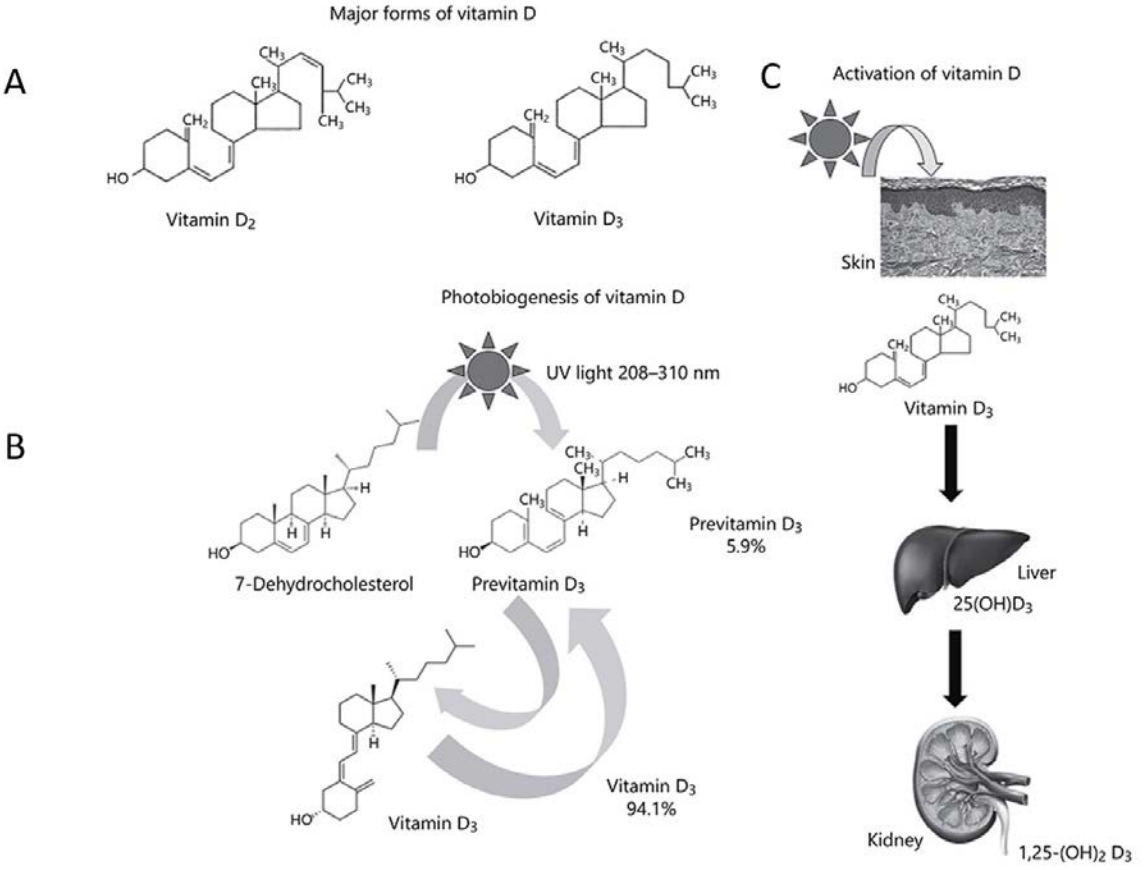


Figure 1 Depiction of vitamin D structures, process of photobiosynthesis, and process of activation (A) Two major forms of vitamin D found in the human body are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). (B) 7-dehydrocholesterol is an unsaturated sterol found in the epidermis that absorbs ultraviolet radiation via sun exposure inducing photoisomerization into previtamin D3, which undergoes spontaneous isomerization into cholecalciferol. (C) Vitamin D3 is hydroxylated in the liver by cholecalciferol 25-hydroxylase into 25(OH)D3 (calcifediol), which is further hydroxylated in the kidney into 1,25-(OH)2D3 (calcitriol) (Gil et al., 2018).

Calcitriol plays a significant role in the regulation and maintenance of serum calcium levels. Calcium is an essential nutrient for bone formation and is found as calcium hydroxyapatite in bones and teeth, where it provides structural strength (Ross et al., 2010). Although calcium can be provided by food and supplements, bone tissue serves as a major source of calcium for bone remodeling. Calcitriol binds to the VDR, and the ligand-receptor complex within the nucleus activates the transcription of calbindin-D28k, a calcium-binding protein that regulates calcium transport across the intestinal mucosa and into the blood increases (Voet & Voet, 2004). VDR is located within various cells and tissues including the osteoblasts, epithelial cells, intestine, and the kidneys. As a result of this, calcitriol is important for increasing calcium absorption in the gastrointestinal tract, increasing renal tubular absorption, and stimulating calcium release from bones (Breslau, McGuire, Zerwekh, Frenkel, & Pak, 1984).

Calcium metabolism is regulated by the parathyroid hormone (PTH), a hormone secreted by the parathyroid glands in response to low serum calcium levels. PTH binds to the parathyroid hormone 1 receptor (PTHR1) to stimulate osteoclast and osteoblast activity in bone to release calcium into the blood (Poole & Reeve, 2005). PTHR1 is expressed at high levels in the osteoblasts, and when activated, osteoblasts express Receptor Activator of Nuclear Factor kB Ligand (RANKL) (Wada, Nakashima, Hiroshi, & Penninger, 2006). RANKL is expressed in several tissues and organs such as the skeletal muscle, liver, colon, small intestine, adrenal gland, and osteoblast. RANKL binds to its cognate receptor, RANK, which is located on the osteoclast to stimulate bone resorption (Wada et al., 2006). This regulates the differentiation of precursor cells into multinucleated osteoclasts and osteoclast activation, which is associated with increased

bone resorption. Other than through the RANKL signaling, PTH also increases serum calcium levels by upregulating 1α-hydroxylase production to increase the hydroxylation of calcifediol into calcitriol in the kidney (Voet & Voet, 2004).

Aside from its role in calcium regulation, calcitriol also plays a critical role in phosphate reabsorption in the small intestine and kidneys. Phosphate is primarily combined with calcium in the form of hydroxyapatite crystals in the skeleton, and this helps provide both bone strength and rigidity (Farrow & White, 2010). Serum phosphate levels are maintained by intestinal absorption, intracellular and bone storage pool exchange, and renal tubular absorption. Approximately 65% of ingested phosphate is absorbed in the duodenum and jejunum (Prasad & Bhadauria, 2013). Under the influence of calcitriol, phosphate is transported from the small intestine into the blood where it can be used for bone deposition and remodeling. Furthermore, circulating phosphate is transported into cells via type II and type III sodium-phosphate cotransporters in order to complete cellular functions such as deoxyribonucleic acid (DNA) synthesis and intracellular signaling (Bergwitz & Jüppner, 2010). Phosphate levels are also regulated by the kidneys with phosphate being reabsorbed from urine in the renal proximal tubules via type II and type III sodium-phosphate cotransporters with excess phosphate excreted in the urine; however, the majority of the phosphate is retained within the bone for bone deposition during periods of growth (Rosen, & Bouillon, 2013).

Calcitriol and phosphate homeostasis is also regulated by fibroblast growth factor 23 (FGF23), a protein primarily produced by osteocytes, osteoblasts, and osteoclasts (Guo & Yuan, 2014). The major roles of FGF23 are: 1) to inhibit renal tubular phosphate reabsorption by decreasing Cyp27b1-mediated formation, and 2) suppress circulating

calcitriol levels by stimulating Cyp24a1-mediated catabolism of calcitriol (Martin, David, & Quarles, 2012). Cyp27b1 and Cyp24a1 are the renal enzymes responsible for calcitriol synthesis and calcitriol degradation into calcitroic acid, respectively (Martin et al., 2012). As a result of reduced calcitriol levels, phosphate absorption from the intestines into the blood is decreased.

Renal phosphate reabsorption is primarily regulated by feedback loops that involve PTH, FGF23, calcitriol, bone and kidney. Regulating phosphate balance in the body involves three feedback loops (Figure 2): 1) parathyroid gland produces PTH to stimulate phosphate excretion and calcitriol synthesis in kidney; 2) PTH stimulates FGF23 and phosphate release following an increase in bone remodeling, where FGF23 inhibits PTH but phosphate stimulates PTH production; 3) FGF23 in the kidney stimulates urinary phosphate excretion and inhibits calcitriol by decreasing 1α-hydroxylase and increasing 24-hydroxylase activities, both of which are located in the kidney and bone. The role of 1α-hydroxylase is to catalyze the hydroxylation of calcifediol to calcitriol, while the role of 24-hydroxylase is to catalyze hydroxylation reactions leading to the degradation of calcitriol (Rosen, & Bouillon, 2013). Ultimately, this leads to reduced serum phosphate and PTH-stimulated calcitriol production, which stimulates FGF23 production by bone cells (Lopez et al., 2011).

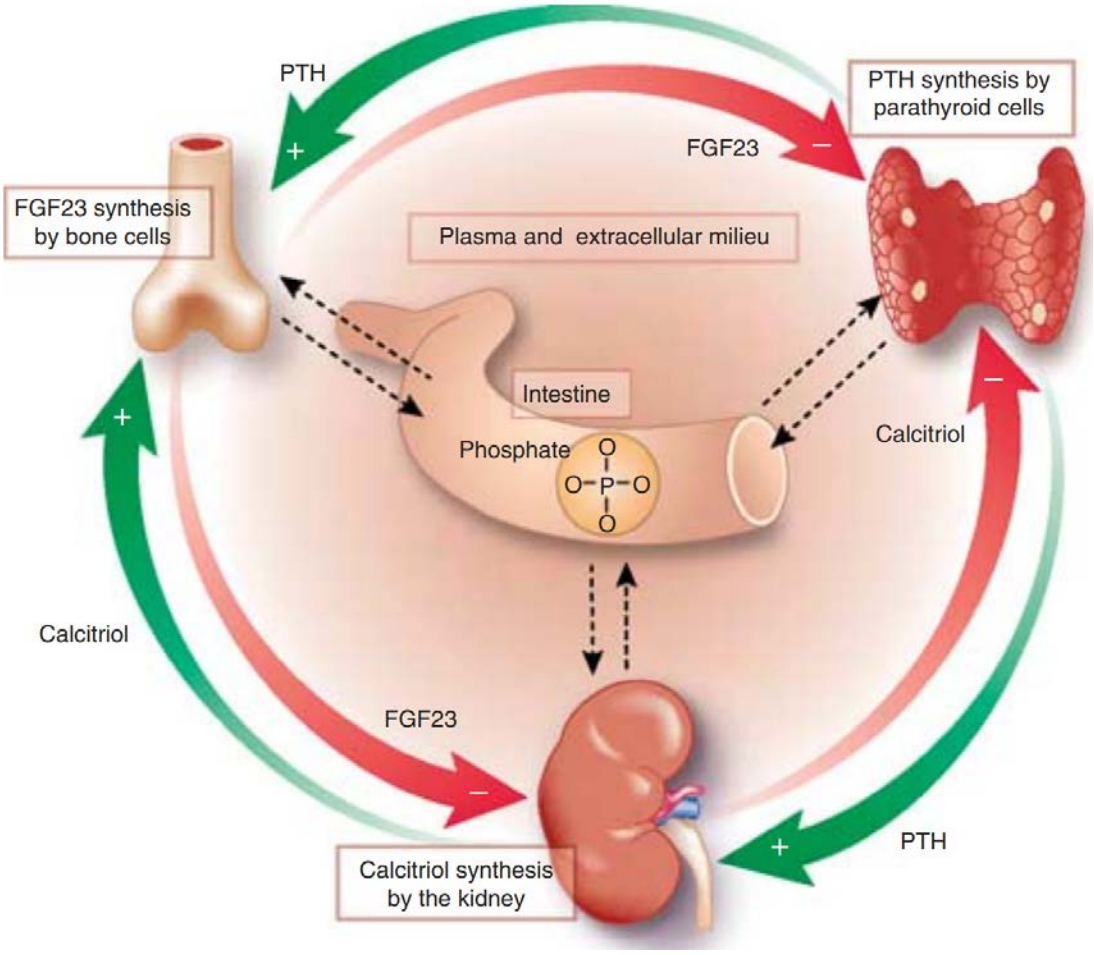


Figure 2 Depiction of the three feedback loops regulating phosphate homeostasis. Parathyroid glands produce PTH, which stimulates phosphate excretion and calcitriol synthesis in kidneys. Calcitriol in turn lowers phosphate levels and inhibits PTH production in parathyroid glands. PTH also stimulates FGF23 production in bone cells and phosphate release following increased bone remodeling. In turn, FGF23 inhibits PTH, however phosphate stimulates PTH production. FGF23 in the kidneys stimulates urinary phosphate excretion and inhibits calcitriol, helping reduce serum phosphate (Torres & De Brauwere, 2011).

In summary, phosphate is absorbed from that which is provided by dietary intake in the gut, stored in bones, and excreted via urine by the kidneys. Calcitriol stimulates phosphate absorption from dietary intake. FGF23 increases renal phosphate excretion,

suppresses synthesis of calcitriol, and can decrease PTH. PTH increases renal phosphate clearance and stimulates synthesis of calcitriol (Torres & De Brauwere, 2011).

Rickets

Chronic deficiency in vitamin D or phosphate can result in the development of severe skeletal manifestations, with the most prominent being rickets (Rosen, & Bouillon, 2013). Rickets is a bone condition characterized by weak or soft bones, bone fractures, bone pain, stunted growth, skeletal deformities, and, most notably, bowed legs. Rickets is more commonly attributed to vitamin D deficiency caused by inadequate sun exposure or dietary intake of vitamin D (Table 1) (Holick, 2006). Figure 3 highlights the downstream physiological effects of how a vitamin D and calcium deficiency can lead to skeletal defects (Holick, 2006).

Table 1 List of sources causing the clinical manifestation of rickets

Decreased intestinal absorption	**Increased urinary excretion**
Vitamin D deficiency or resistance	Renal phosphate wasting disorders
Nutritional deficiency	Primary and secondary hyperparathyroidism
Deficient sun exposure	Diabetic ketoacidosis
Deficient dietary intake	Calcitonin
Malabsorption	Diuretics
Celiac disease	Glucocorticoids
Crohn’s disease	Bicarbonate
Gastrectomy	
Pancreatitis	
Chronic diarrhea	
Chronic liver disease	
Chronic renal disease	
Vitamin D receptor defects	
Nutritional deficiencies	
Alcoholism	
Anorexia	
Starvation	

Rosen, & Bouillon, 2013

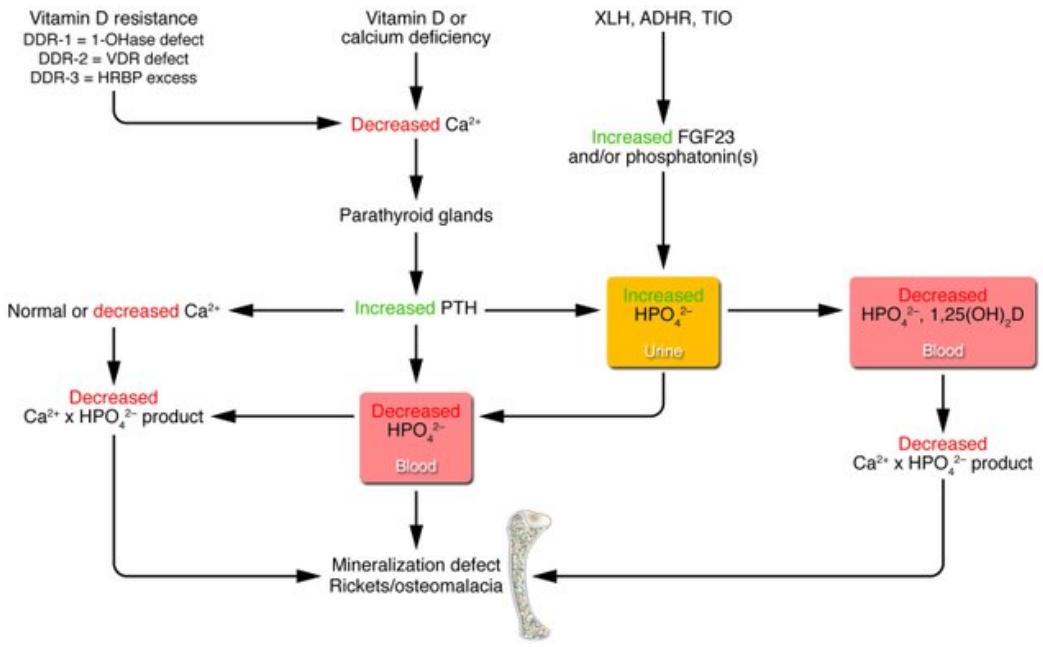


Figure 3 Downstream Physiological Effects of Metabolite Deficiency. Vitamin D or calcium deficiency leads to decreased serum calcium levels, which results in increased PTH levels. Increased PTH leads to calcium reabsorption in the kidney to correct abnormally low serum calcium levels. Additionally, PTH increases urinary phosphate excretion, resulting in a decrease in serum phosphate levels. Decreased calcium-phosphate levels cause defective bone mineralization, which leads to rickets and osteomalacia (Holick, 2006).

As mentioned earlier, decreased levels of vitamin D or calcium can result in lower serum calcium levels, resulting in increased PTH levels. Increased PTH levels subsequently promote calcium reabsorption in the kidney in an effort to correct the abnormally low serum calcium levels. Furthermore, PTH will also increase urinary phosphate excretion, resulting in decreased serum phosphate levels. These actions together cause a decrease in the calcium-phosphate levels which contribute to defective bone mineralization and eventually leads to rickets and osteomalacia (Holick, 2006).

There are three types of rickets: nutritional rickets, vitamin D-dependent rickets (VDDR), and vitamin D-resistant rickets (VDRR) (Rosen, & Bouillon, 2013). Nutritional rickets results from either inadequate sunlight exposure or inadequate dietary intake of vitamin D, calcium, or phosphate (Nield, Mahajan, Joshi, & Kamat, 2006). Nutritional rickets is most prevalent between the ages of three and 18 months (Salimpour, 1975). Additional factors shown to play an important role in the pathogenesis of nutritional rickets at this age range include solely breast-feeding, maternal vitamin D deficiency, living in temperate climates, lack of sunlight exposure, and darkly skin pigmentation (Atiq, Suria, Nizami, & Ahmed, 1998; Bassir et al., 2001). Clinical symptoms leading to the diagnosis of nutritional rickets include short stature, abnormal walking gait, and tetany, which are seizures or involuntary muscle contractions caused by increased action potential frequency of muscle cells due to hypocalcemia (Weisberg, Scanlon, Li, & Cogswell, 2004). Treatment of nutritional rickets includes increased sun exposure, correction of vitamin D insufficiency in diet, and in some cases dietary calcitriol, calcium, and phosphate supplements.

There are two forms of VDDR caused by genetic defects in the vitamin D pathway: vitamin D-dependent rickets type I (VDDR-I) and vitamin D-dependent rickets type II (VDDR-II). VDDR-I is a result from a defect in the Cyp27b1 gene, which encodes the production of 1α-hydroxylase (Carpenter, T.O., 1997). This defect causes the downregulation of Cyp27b1, resulting in low calcitriol levels. Additional characteristics of VDDR-I are normal calcifediol levels, elevated PTH levels, and low serum calcium (hypocalcemia). VDDR-II is caused by mutations in the VDR gene, which alter and prevent the VDR protein from interacting with calcitriol. By preventing the activation of

VDR through direct interaction with calcitriol, VDR is unable to bind to regulatory regions of target genes, where it acts to nucleate the formation of large protein complexes that are essential for directed changes in transcription (Pike & Meyer, 2010). As a result, VDR cannot regulate gene activity, often resulting in elevated calcitriol levels (Takeda, Yamamoto, Taketani, & Miyamoto, 1997).

Symptoms of both VDDR forms begin to manifest between the ages of six months and 24 months, and these symptoms include leg bowing, osteomalacia, muscle weakness, bone pain, delayed growth, bone fractures, low serum calcium (hypocalcemia), low serum phosphate (hypophosphatemia), secondary hyperparathyroidism, and widening of bone ends (metaphysis) in the knees, wrists, and ribs (Takeda, Yamamoto, Taketani, & Miyamoto, 1997). Treatment for both forms of VDDR include dietary calcitriol, calcium, and phosphate supplements (Rosen, & Bouillon, 2013).

VDRR is primarily characterized by the ineffectiveness of vitamin D treatment to correct rickets. Clinical symptoms of VDRR include short stature, leg bowing, and dental abnormalities, which can be seen within months following birth but can also develop later in adolescence and adulthood (Nield et al., 2006). Leg bowing, which is the primary clinical symptom of rickets, is further exacerbated in children during weight bearing activities, such as walking. VDRR was first described in 1937 by Fuller Albright, an endocrinologist at Massachusetts General Hospital, based on his 4-year study of a patient, who displayed an unusual presentation of rickets characterized by high urinary calcium and phosphate excretion (Levine, Kleeman, & Felsenfeld, 2009). Vitamin D treatment failed to increase the absorption of calcium and phosphate in the patient, and treatment with parathyroid (PTH) extract failed to increase calcium absorption. Further insight into

patients with similar symptoms revealed low serum phosphate concentration as a common factor, coining the term hypophosphatemic rickets.

Hypophosphatemic Rickets

Hypophosphatemic rickets is characterized by hypophosphatemia (Cho et al., 2005). There are three primary mechanisms for which hypophosphatemia can arise: 1) redistribution of phosphate from extracellular fluids into cells, 2) decreased intestinal absorption, and 3) renal phosphate wasting as characterized by increased phosphate levels in urine (Liamis, Milionis, & Elisaf, 2010). Since the discovery of VDRR, several forms of hypophosphatemic rickets have been identified, most of which are distinguished by their patterns of inheritance and genetic cause, which are summarized in Table 2.

Table 2 List of known disorders of renal phosphate wasting diseases and their accompanying biochemical profiles

Disease	Mutation or defect	Pathogenesis	Serum phosphate	Serum calcitriol	Serum FGF23
Tumor-induced osteomalacia	Mesenchymal tumor	Ectopic, unregulated production of FGF23 and phosphatonins sFRP4, MEPE, FGF7	Low	Normal	Normal or high
X-linked hypophosphatemia	PHEX mutation	Inappropriate FGF23 synthesis from bone	Low	Normal	Normal or high
Autosomal dominant hypophosphatemic rickets	FGF23 mutation	Increased circulating intact FGF23 caused by mutations that render it resistant to cleavage	Low	Normal	Normal or high
Hereditary hypophosphatemic rickets with hypercalciuria	SLC34A3 mutation	Loss of function NaPiIIc mutations resulting in renal phosphate wasting without defect in 1,25(OH)2D3 synthesis	Low	High	Low or normal
Autosomal recessive hypophosphatemic rickets Type 1	DMP1 mutation	Loss of DMP1 causes impaired osteocyte differentiation and increased production of FGF23	Low	Low or normal	Normal
Autosomal recessive hypophosphatemic rickets Type 2	ENPP1 mutation	Increased production of FGF23	Low	Low or normal	High
Hypophosphatemic rickets with hyperparathyroidism	αKlotho translocation	Increased KLOTHO, FGF23, and downstream FGF23 signaling	Low	Unknown	High
Fibrous dysplasia	GNAS mutation	Increased FGF23 production from the dysplastic bone	Low	Low or normal	High
Linear nevus sebaceous syndrome	Excess FGF23 production	Increased FGF23 production from the dysplastic bone and from the nevi	Low	Unknown	High
Osteoglophonic dysplasia	FGFR1 mutation	Increased FGF23 production from the dysplastic bone	Low	Low	Normal or high
Hypophosphatemic nephrolithiasis/osteoporosis Type 1	SLC34A1 mutation	Renal phosphate wasting without a defect in 1,25(OH)2D3 synthesis	Low or normal	High	Normal or high
Hypophosphatemic nephrolithiasis/osteoporosis Type 2	SLC9A3R1 mutation	Renal phosphate wasting through potentiation of PTH-mediated cAMP production	Low	Unknown	Unknown
Fanconi renotubular syndrome Type 2	SLC34A1 mutation	Renal phosphate wasting without a defect in 1,25(OH)2D3 synthesis	Low	Low, normal, or high	Unknown
Tumoral calcinosis	KL mutation	Unknown	High	Normal or high	Low
Hyperphosphatemia syndrome e	FGF23 or GALNT3 mutation	Unknown	High	Normal or high	High

Rosen, & Bouillon, 2013

The list of currently known renal phosphate wasting disorders is significant because roughly 50% of these disorders share a similar biochemical profile of low serum phosphate, low-to-normal serum calcitriol, and normal-to-high serum FGF23, despite their various genetic defects. Of the disorders listed in Table 2, this review will discuss further the more common forms of renal phosphate wasting disorders that lead to hypophosphatemic rickets: tumor-induced osteomalacia, X-linked hypophosphatemia, and autosomal dominant hypophosphatemic rickets.

Tumor-induced osteomalacia (TIO), or oncogenic osteomalacia, is an acquired paraneoplastic syndrome of renal phosphate wasting (Rosen, & Bouillon, 2013). The most commonly associated tumors that cause TIO are a benign mesenchymal or mixed connective tissue tumor (Zadik & Nitzan, 2012). These tumors occur equally in both soft tissue and bone, and they are characterized by an admixture of spindle cells, osteoclast-like giant cells, prominent blood vessels, cartilage-like matrix, and metaplastic bone. These tumors ectopically express and secrete FGF23, which as mentioned earlier promotes urinary phosphate excretion (De Beur et al., 2002). TIO symptoms include bone pain, muscle weakness, gait abnormalities, hypophosphatemia, multiple bone fractures, height loss, and generalized debilitated status (Hautmann, Hautmann, Kolbl, Herr, & Fleck, 2015).

Figure 4 highlights the clinical features of TIO, which include height loss, excessive outward curvature of the spine (kyphosis), and pectus carinatum (pigeon chest) (Chong, Molinolo, Chen, & Collins, 2011). Additional symptoms that require either clinical or radiographic identification are bone pain, muscle weakness, and multiple bone fractures (Jan de Beur, S M, 2005). Although TIO is not considered fatal, the physical

abnormalities significantly impact the quality of life of patients. Furthermore, the length of time from onset of symptoms until diagnosis is often prolonged due to a lack of knowledge regarding disease existence.

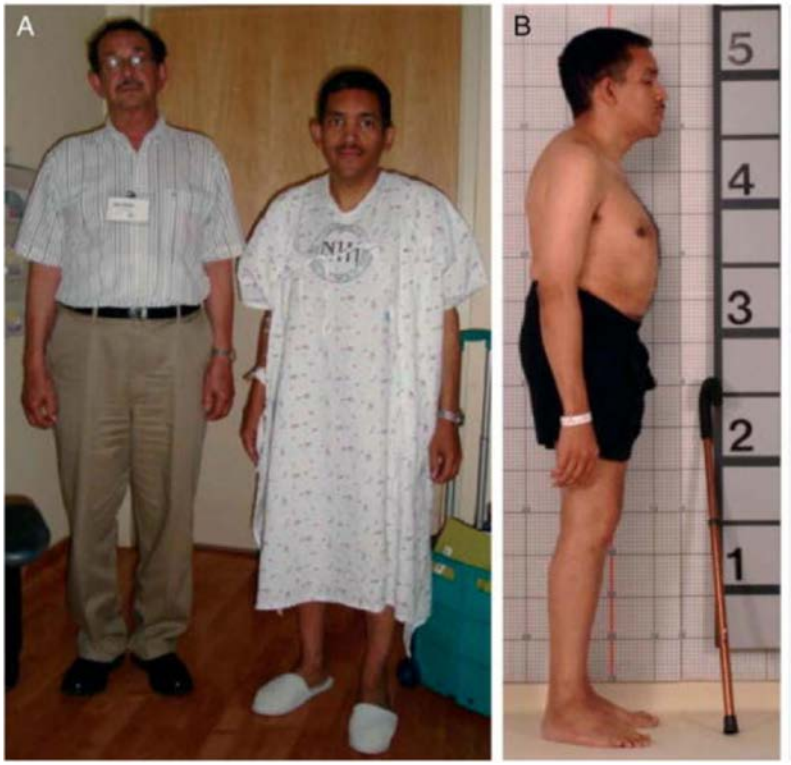


Figure 4 Clinical effects of advanced tumor-induced osteomalacia (TIO). The gowned patient in picture A is standing next to his father who does not have TIO. The patient was reported to be previously taller than his father. Picture B demonstrates kyphosis and pectus carinatum (pigeon chest), which is a result from multiple compression fractures due to osteomalacia. Pictures of the father and patient are reproduced with their permission (Chong et al., 2011).

For TIO, the detection and resection of the primary tumor are curative and can often lead to rapid resolution of the patient's symptoms (Chong et al., 2011). However, these tumors are often small, slow growing, and found in various locations throughout the body, such as sinuses, long bones, distal extremities, and the groin, making it difficult to detect and diagnose. A complete physical examination is required to assess palpable

tumor masses in subcutaneous tissues (Rosen, & Bouillon, 2013). In some cases, excision may not be possible and this largely depends on the size and location of the tumors.

While TIO symptoms rapidly resolve if the causal tumors can be resected, there are cases in which tumor resection is not feasible or recurrence of the tumor occurs after resection (Chong et al., 2011). In patients for non-excisable tumors, the current standard of care is oral phosphate and calcitriol replacement. The goal of this oral phosphate is to replace ongoing renal phosphate loss, and the calcitriol supplements replace insufficient renal production of calcitriol to enhance renal and gastrointestinal phosphate reabsorption. Treatment efficacy is limited as it does not treat the underlying cause of the disease and its benefits must be balanced with monitoring for potential risks, which include elevated serum calcium levels (hypercalcemia), the deposition of calcium oxalate and calcium phosphate in the kidney (nephrocalcinosis), and the formation of kidney stones (nephrolithiasis) (Zadik & Nitzan, 2012).

Another prominent form of hypophosphatemic rickets is X-linked hypophosphatemia (XLH), which is the most common renal phosphate wasting disorder affecting approximately one in 20,000 individuals (T. O. Carpenter, Imel, Holm, Jan de Beur, & Insogna, 2011). It is an X-linked dominant form of rickets or osteomalacia and leads to bone deformity resulting in short stature and bowed legs due to increased levels of osteoid. XLH is caused by a mutation in the phosphate-regulating endopeptidase (PHEX) gene. PHEX is a member of the M13 family of neutral endopeptidases that is expressed by osteoblasts and osteocytes. PHEX is expressed in late embryonic development as skeletal mineralization begins (Ruchon et al., 1998). Table 3 illustrates the research findings leading to the discovery of the PHEX mutation.

Table 3 Timeline of XLH pathophysiology discoveries

Date	Finding	Reference
Discovery, clinical presentation, and treatment of rickets		
1645	First published description of rickets	O'Riordan & Bijvoet, 2014
1728	Publication on the effectiveness of shark liver ointment treatmet	Johnstone, Smellie, Balfour, Balfour, & Robertson, 1779
1890	Identification of correlation between increased geographical distribution of rickets and locations with decreased sunlight	Chesney, 2012
1917	Initial use of ultraviolet light treatment	Wacker & Holick, 2013
1922	Publication suggesting the existence of a vitamin which promotes calcium deposition	McCollum et al., 2002
1937	First description of vitamin D resistant rickets	Choudhury, Jebasingh, Ranabir, & Singh, 2013
Genetics		
1958	Established as X-linked disorder	Francis, F., et al., 1995.
1959	Existence phosphate-regulating hormone (eventually found to be FGF23) causes acquired rickets in a child cured by excision of a bone-derived tumor	Prader, A., et al., 1959.
1972	Inborn error of phosphate transport	White, K.E., et al., 2000.
1976	Hyp mouse model of XLH discovered	Eicher, E.M., et al., 1976.
1995	XLH as a result of mutation in PHEX gene	Francis, F., et al., 1995.
2000	Mutation in FGF23 at a pro-protein convertase consensus site is cause of ADHR	White, K.E., et al., 2000.

XLH as a result of a circulating factor		
1974	Transplantation of normal kidney into patient with XLH did not reverse the phosphaturia	Morgan, J.M., et al., 1974.
1989	Parabiosis of Hyp and normal mice: normal mice phosphaturic	Meyer, R.A., et al., 1989.
1992	Cross-transplantation studies: Hyp mouse defect not intrinsic to kidney	Nesbitt, H., et al., 1992.
1996	Serum from Hyp mouse inhibits phosphate uptake by mouse proximal tubule cells in culture	Nesbitt, H., et al., 1992.
Abnormal Vitamin D metabolism		
1982	Serum calcitriol levels inappropriately normal in XLH	Lyles, K.W., et al., 1982.
2003	1α hydroxylase activity blunted in Hyp mouse in response to a decrease in dietary phosphate or to PTH	Fujiwara, I., et al., 2003.
Role of FGF23 in hypophosphatemia		
2002	Infusion of recombinant FGF23 or overexpression of FGF23 results in enhanced renal phosphate excretion and hypophosphatemia	Tenenhouse, H.S., 1999.
2003	FGF23-neutralizing antibodies normalize serum phosphate and calcitriol in Hyp mouse	Aono, Y., et al., 2003.
2003	FGF23 levels are elevated in many patients with XLH	Jonsson, K.B., et al., 2003.
2004	Targeted ablation of the FGF23 gene leads to hypophosphaturia and hyperphosphatemia	Sitara, D., et al., 2004.

2004	FGF23 inhibits renal proximal tubule phosphate reabsorption via suppression of the NaPi type IIa transporter activity	Shimada, T., et al., 2004.
2004	FGF23 administration lowers calcitriol levels, suppresses 1α hydroxylase activity, and stimulates 24-hydroxylase activity	Shimada, T., et al., 2004.
2006	FGF23 levels are elevated in Hyp mouse	Liu, S., et al., 2006.
2006	Klotho regulates FGF23 signaling	Kuro-o, M., 2006; Kurosu, H., et al., 2006.

The discovery of the hypophosphatemia (Hyp) mouse model, which has a large portion of the PHEX gene deleted, was of great importance for advancing XLH research because the mutant mice displayed similar XLH clinical symptoms including hypophosphatemia, bone changes resembling rickets, dwarfism, and increased excretion of phosphate anion by low net tubular reabsorption (Eicher, Southard, Scriver, & Glorieux, 1976). The Hyp mouse model has also aided the advancement of methods for identifying and treating the underlying cause of XLH, such as the role of FGF23 as a diagnostic marker and treatment target. Table 3 highlights that further research is needed to identify a treatment for the underlying cause of XLH.

Clinical manifestations of XLH typically vary in severity, however most pediatric patients show leg deformities and bowing. Progressive bowing, more specifically antero-medial rotational tibiae torsion, and short stature are the typical skeletal outcome in untreated children. Medical therapy in the form of oral phosphate and active vitamin D treatment can improve these symptoms; however, symptoms often are not completely resolved with currently available therapies.

Autosomal dominant hypophosphatemic rickets (ADHR) is a rare form of hypophosphatemic rickets caused by FGF23 mutations (Econs, McEnery, Lennon, & Speer, 1997). Although clinical and biochemical findings are similar to those seen in XLH during childhood, there are instances of delayed onset of clinical symptoms and, in rare cases, resolution of phosphate wasting (Imel et al., 2011). The differences between the two disorders are more noticeable in adulthood because adults with ADHR are less likely to display lower extremity deformities, which may be a result of proper growth plate fusion prior to the onset of renal phosphate wasting. Similar to XLH, ADHR treatment includes oral phosphate and calcitriol.

Clinical Presentation of Hypophosphatemic Rickets

Patients with hypophosphatemic rickets are typically born with low-to-normal or normal levels of serum phosphate, normal bone length, and no skeletal abnormalities on radiographs (Rosen, & Bouillon, 2013). However, the serum phosphate levels decrease over time leading to rickets. In addition to hypophosphatemia, affected children also have low-to-normal serum calcitriol concentrations, elevated ALP levels, normal serum calcium, normal PTH, and normal calcifediol (Carpenter et al., 2011).

Clinical manifestations of hypophosphatemic rickets vary in severity, but most children display poor growth and lower extremity bowing deformities (Rosen, & Bouillon, 2013). Once infants begin to bear weight on their legs, the compromised skeletal tissue succumbs to bowing genu valgum (knocked knees). The combination of height loss caused by the bowing of the legs and the growth plate defects can lead to a permanent loss of growth potential despite the fact that children with hypophosphatemic rickets experience a normal pubertal growth spurt (Carpenter et al., 2011). The growth

plate, also known as the epiphyseal plate or physis, is the area of growing tissue near the ends of the long bones in children and adolescents. Once growth is completed, typically in adolescence, the growth plates close and are replaced by solid bone. Once growth plates close, the hypophosphatemia and lower extremity deformities remain throughout adulthood. These structural deformities can lead to gait abnormalities, osteoarthritis, joint pain, stiffness, tendon and ligament calcification (enthesopathy), increased bone fragility, and dental abscesses (Carpenter et al., 2011; Reid et al., 1989).

Treatment of Hypophosphatemic Rickets

The primary goals of hypophosphatemic rickets treatment for children are to correct or minimize rickets, osteomalacia, radiographic abnormalities, and skeletal deformities, while in adults, the goals are to reduce pain and osteomalacia as well as improve fracture healing (Carpenter et al., 2011). The differences in treatment goals between children and adults is due to the fact that treatment during growth, specifically prior to growth plates closure, can partially correct leg deformities and lower the number of expected surgeries in adulthood along with optimizing adult height (Carpenter et al., 2011).

The current standard of care treatment for pediatric hypophosphatemic rickets patients consists of oral phosphate and high-dose calcitriol (Carpenter et al., 2011). Treatment begins with lower doses to avoid gastrointestinal side effects, and doses are eventually titrated to a weight-based dose of calcitriol at 20 to 30 ng/kg/day along with phosphate at 20 to 40 mg/kg/day administered in three to five times a day (Carpenter et al., 2011). Due to the lack of standardization for hypophosphatemic rickets treatment, it is at the clinician's discretion to administer higher doses of phosphate for up to a year. The

high-dose phosphate treatment consists of calcitriol at 50 to 70 ng/kg/day along with phosphate at 20 to 40 mg/kg/day (Carpenter et al., 2011). In adulthood, patients may be treated with lower calcitriol and phosphate doses; however, it is currently unclear if patients require long-term treatment or whether the treatment can be discontinued. Most hypophosphatemic rickets studies do not report weight-based calcitriol and phosphate treatment and when dosing is reported, the values can vary widely from 10-80 ng/kg/day of calcitriol and 30-180 mg/kg/day for phosphate, suggesting uncertainty of optional doses (Carpenter et al., 2011). Since oral phosphate and calcitriol therapy is weight-based, not reporting weight-based treatments across institutions inhibits the development of a standardized treatment regimen.

Because over treatment of phosphate can lead to hyperphosphatemia, hypercalcemia, hypercalciuria, hyperparathryodisim, and nephrocalcinosis, laboratory monitoring at three-month intervals is necessary to measure serum phosphate, serum calcium, serum creatinine, urinary calcium, urinary creatinine, and overall renal function in order to properly adjust treatment and avoid any complications (Carpenter et al., 2011). Treatment endpoints to measure therapeutic response include measuring height, the degree of skeletal deformity, and epiphyseal healing by radiographic imaging.

When successful, standard of care treatment can lead to improved growth, though still not normal, and moderate resolution of rickets as seen in radiographs. In order to successfully evaluate rickets severity, radiographic assessment by X-ray or CT scan of the distal femoral tibial sites should be taken regularly in order to ensure epiphyseal correction; however, currently there is no standardization of X-ray frequency (T. Carpenter, 1997). X-rays are also used to assess the healing of rickets and evaluate

skeletal deformities for surgical management. In adults, standard of care treatment is initiated in response to spontaneous fractures, skeletal pain, and osteomalacia. Treatment of these symptoms may eventually lead to symptom resolution; however, this approach can take several months before fractures fully heal and skeletal pain subsides. In some cases, surgical intervention may be required to correct skeletal deformities. The goals of hypophosphatemic rickets surgery include straightening or partial correction of bowed legs and gait improvement (Carpenter et al., 2011). Forms of surgery such as bone stapling and bone straightening by 8-plates can achieve leg straightening; however, the procedures are complicated by delayed bone healing in patients with hypophosphatemic rickets.

Diagnosis of Hypophosphatemic Rickets

A combination of clinical and biochemical measurements is used to correctly identify and treat hypophosphatemic rickets (Carpenter et al., 2011). As illustrated in Table 2, biochemical findings are, at times, indistinguishable from other various phosphate wasting diseases. Diagnosis of renal phosphate wasting is typically measured by calculating the percent of tubular reabsorption of phosphate (TRP) and the renal tubular reabsorption of phosphate (TMP/GFR) by fasting urine sample collection (Carpenter, 1997). TRP is the percent of filtered phosphate reabsorbed by renal tubules. It is calculated only in the fasting state with the following formula (Eq. 1):

Eq 1: 100 x [1- ((urine phosphate/urine creatinine) x (serum creatinine/serum phosphate))]

A normal TRP percentage is between 85% and 95% when phosphate levels are normal (Chong et al., 2011). A reduced TRP in the presence of hypophosphatemia indicates renal

defect in phosphate reabsorption. TMP/GFR is the renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (GFR), which describes the flow rate of filtered fluid through the kidney (Payne, 1998a). It is calculated with the following formula (Eq. 2):

Eq 2: TMP/GFR = phosphate plasma concentration – (urine phosphate concentration x creatinine plasma concentration / urine creatinine concentration)

The ratio measures the maximum renal tubular phosphate reabsorption in mass per unit volume of glomerular filtrate. It is independent on the rate of phosphate flow into the extracellular space from the gut, cells and bones, and the glomerular filtration rate, both of which affect plasma phosphate concentration (Payne, 1998b). Age-based, normal TMP/GFR measured in mmol/L are: 1.55 to 2.97 for newborns; 1.07 to 2.23 for 1 month to 2 years old; 1.10 to 1.88 for 2 to 12 years old; 0.93 to 1.71 for 12 to 16 years old; and 0.88 to 1.26 for 16 years old and older (Payne, 1998b).

Of the types of hypophosphatemic rickets, distinguishing between them can be difficult because serum chemistries are identical in TIO, XLH, and ADHR as seen in Table 2. For all three diseases, serum phosphate levels are low, serum calcitriol levels are normal, and serum FGF23 levels are normal or high. The biochemical diagnosis of specific forms of hypophosphatemic rickets in infants can be difficult since phosphate levels are usually higher in infants than older children due to skeletal development and can be mischaracterized as normal phosphate levels (Carpenter et al., 2011). As a result, genetic testing for PHEX and FGF23 mutations can help correctly diagnosis the renal phosphate wasting disorder as XLH or ADHR, respectively. In most cases, XLH first presents in early childhood, while ADHR can present in either childhood or adulthood

which may help identify the cause of renal phosphate wasting (Econs & McEnery, 1997). However, biochemical findings alone are not sufficient to correctly diagnose hypophosphatemic rickets. As a result, biochemical measurements are typically complemented with a detailed review of the patient's and patient's family history, including a review of their growth chart (Carpenter et al., 2011). The focus of the family history review is to identify family members with short stature and bowed legs.

In instances when hypophosphatemic rickets is correctly diagnosed and treatment begins, disease severity and treatment efficacy are measured by X-ray radiographic imaging (Carpenter et al., 2011). However, there is currently no adopted, standardized process for collecting and reviewing repeat X-rays. Furthermore, repeat X-ray imaging in patients leads to increased exposure to ionizing radiation and since pediatric patients are more sensitive to radiation than adults, increasing the patient's cancer risk, hypohphosphatemic rickets pediatric patients are less likely to have repeat X-rays performed (Carpenter et al., 2011).

Early diagnosis of hypophosphatemic rickets in infants is challenging when it is based solely on clinical features and biochemical findings (Pavone et al., 2015). Mutational analysis of the PHEX and FGF23 genes are available and can help; however, studies have shown that mutations can only be found in 50-70% of affected individuals (Ichikawa et al., 2007). Furthermore, mutational testing is expensive and often not covered by health insurance companies. Therefore, the goal of this literature review is to evaluate the evidence whether other methods can be used to supplement and improve disease diagnosis and treatment efficacy for patients with hypophosphatemic rickets.

Findings

Although there is a wealth of information on the disorders that cause hypophosphatemic rickets, challenges regarding the diagnosis and treatment of these disorders remain. The purpose of this targeted literature review is to determine if sufficient evidence exists to warrant the investigation or consideration of alternative biomarkers as clinical measurement tools to improve diagnosis of hypophosphatemic rickets.

Osteopontin (OPN)

Osteopontin (OPN), identified in 1985, is a non-structural protein found in the extracellular matrix (Franzén & Heinegård, 1985). It is a member of the small integrin-binding ligand, N-linked glycoprotein (SIBLING) protein family. OPN is expressed by several cell types and tissues, including osteoblasts, osteocytes, fibroblasts, skeletal muscle, and the kidney (Chen et al., 2014; Xie, Singh, Siwik, Joyner, & Singh, 2003; Zanotti et al., 2011; Z. X. Zhang et al., 2010). A primary function of OPN is regulating bone mineralization. As a member of the SIBLING protein family, OPN can bind directly to specific hydroxyapatite crystals to inhibit bone mineralization. The formation of hydroxyapatite crystals is important for bone mineralization and makes up to 50% of the volume and 70% of the weight of human bone and provide the compressive strength of bone (Palmer, Newcomb, Kaltz, Spoerke, & Stupp, 2008).

A clinical study in 2017 identified seven XLH patients with inactivating PHEX mutations and measured the expression levels of OPN at sites of defective bone mineralization. Results indicated that these XLH patients had increased levels of OPN in the bone matrix, along with an enlarged, hypomineralized, and defective pattern of

skeletal mineralization (Boukpessi et al., 2016). These findings suggest that increased levels of OPN expression near osteocytes correlates with defective bone mineralization sites in XLH patients, and that OPN expression can be used as a measurable biomarker in XLH clinical trials. Since the other known genetic biomarkers, FGF23 and PHEX, can only be found in 50-70% of individuals with XLH as noted previously, measuring OPN expression can be used as an additional genetic biomarker to help determine the specific cause of renal phosphate wasting (Boukpessi et al., 2016).

In support of this, another study comparing OPN-deficient (OPN -/-) mice with wild type (OPN +/+) control mice demonstrated that OPN -/- mouse bones are more mineralized than their age, background, and sex-matched OPN +/+ counterpart animals (Pollack, Linnemeyer, & Gill, 1994). Whereas XLH patients displayed bone hypomineralization due to increased OPN expression in the aforementioned clinical study, OPN deficient mice display bone hypermineralization. Moreover, mineral maturity, as defined by mineral hydroxyapatite crystal size and perfection, was significantly increased throughout all observed anatomic regions of OPN-/- animals. The tibia and femur sections from 12-week-old and 16-week-old mice revealed that OPN -/- mice displayed fragile bone due to hypermineralization in the more mature areas of the central cortical bone (Pollack, Linnemeyer, & Gill, 1994). Since bone differences that exist between OPN -/- and OPN +/+ mice cannot be detected by conventional imaging used for humans, fourier transform infrared microspectroscopy (FT-IRM) and infrared imaging (FT-IRI) are used to characterize bone mineral in both mouse models (Boskey, Spevak, Paschalis, Doty, & McKee, 2002). FT-IRM is a physical-chemical technique that

provides data on molecular structure at approximately 10 μm spatial resolution (Mendelsohn, Paschalis, & Boskey, 1999).

Figure 5 is an image obtained by FT-IRI that shows the increased maturity of bone crystals in the absence of OPN. The red areas in Figure 5 indicate higher levels of phosphate ion (a component of hydroxyapatite crystals), which correlate with increased crystal size and perfection (Boskey et al., 2002). The observed changes in bone mineral are consistent with the observed *in vitro* effects of OPN, in which a purified OPN solution has been shown to be an effective inhibitor of hydroxyapatite crystal formation and growth (Hunter, Kyle, & Goldberg, 1994).

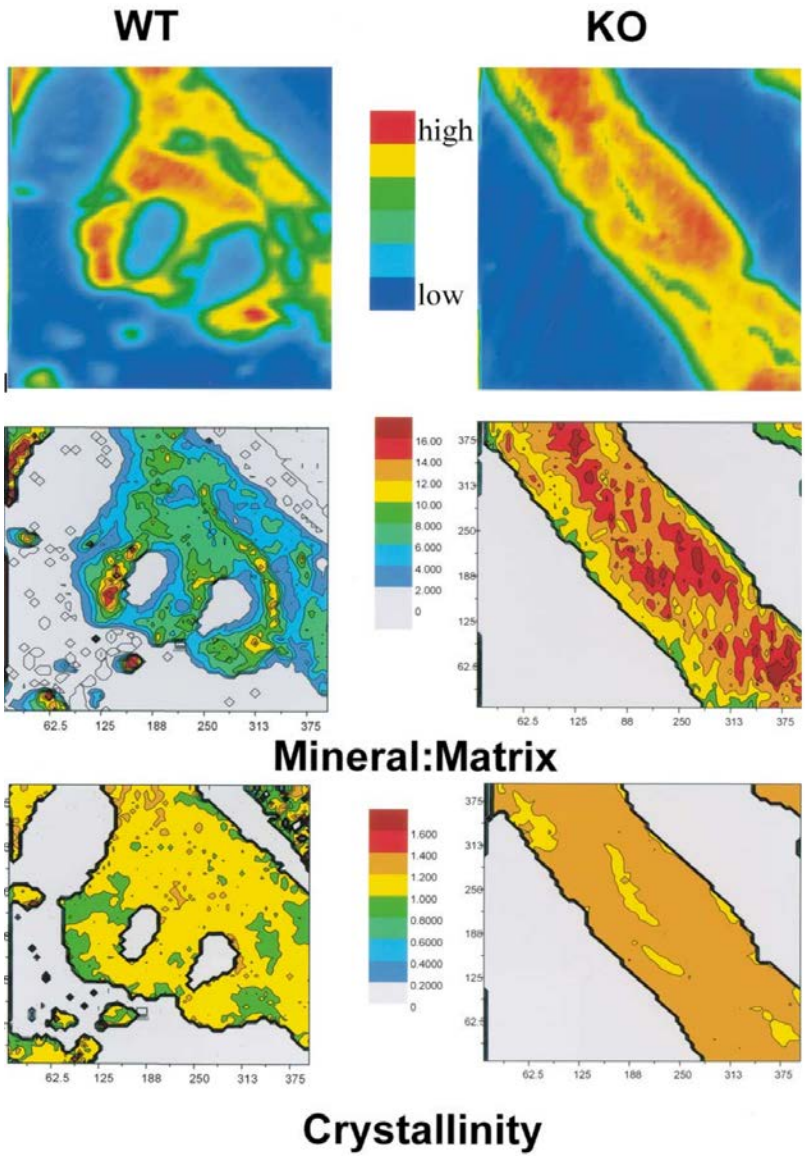


Figure 5 Mineralization and Crystallinity of Osteoponin-Deficient Mice. FT-IRI of mineral-to-matrix ratio in long bones of a OPN-deficient mouse (KO; OPN-/-) and an age-matched and background-matched wild type mouse (WT; OPN+/+). These are typical images of mineral-to-matrix ratio in the cortices of the knockout (KO) mouse and wild type (WT) mouse at 16 weeks of age. The red areas indicate higher levels of phosphate ion, which correlate with increased hydroxyapatite crystal size and perfection. The bone mineral changes observed are consistent with *in vitro* effects of OPN indicating that OPN solution purified from bone is an effective inhibitor of hydroxyapatite crystal formation and growth. The top image displays the raw data expressed as the integrated area of phosphate bands. The middle image displays the mineral-to-matrix ratio. The bottom image displays the crystallinity intensity ratio (Boskey et al., 2002).

A disadvantage to the current standard of care treatment for hypophosphatemic rickets is acute kidney injury as a result of nephrocalcinosis, a disorder in which there is increased calcium deposited in the kidneys (Carpenter et al., 2011). As previously mentioned, one of the goals of oral phosphate and calcitriol treatment is to normalize serum phosphate levels, which requires careful monitoring to avoid phosphate overdose, hypercalciuria with nephrocalcinosis, and renal insufficiency (Taylor, Sherman, & Norman, 1995). A clinical study revealed that 80% of hypophosphatemic rickets patients receiving standard of care treatment exhibited nephrocalcinosis as detected by ultrasonography (Kooh, Binet, & Daneman, 1994). Although OPN is produced by osteoblasts and is one of the most abundant non-collagenous bone proteins, high levels of OPN can be seen in the kidneys, specifically in the loop of Henle and the distal convoluted tubules during nephritis or inflammation of kidneys (M Giachelli et al., 1994; Sodek, Ganss, & McKee, 2000). While the role of OPN in cellular processes within the kidneys are not fully understood, research has shown that increased OPN expression by tubular epithelial cells promotes the development of renal ischemia reperfusion injury (IRI), which is characterized by reduced renal blood flow (Zhang et al., 2010). In IRI, natural killer cells, a type of cytotoxic lymphocyte critical to the innate immune system, migrate to the kidney and induce tubular epithelial cells apoptosis. Although the mechanism of natural killer cell migration and activation during IRI is still unknown, a study identified the following: 1) Tubular epithelial cells express high levels of OPN *in vivo*; and 2) OPN-deficient mice have reduced infiltration of natural killer cells and less kidney tissue damage when compared to wild-type mice (Zhang et al., 2010). Taken together, these results suggest that inhibiting OPN expression may preserve kidney

function. A potential advantage of targeting and inhibiting osteopontin over the current standard of care treatment for hypophosphatemic rickets would then be the potential decrease in acute kidney damage.

Currently there is one OPN inhibitor (brefelamide) which was used to inhibit OPN expression and function in A549 human lung carcinoma cells (J. Zhang et al., 2016). As seen in Figure 6, A549 cells treated with increasing concentrations of brefelamide displayed lower OPN expression levels in comparison to the untreated control group (Zhang et al., 2016). Although brefelamide has not been tested in the clinical setting for bone mineralization disorders, it may represent a promising therapeutic modality for hypophosphatemic rickets and warrants further testing.

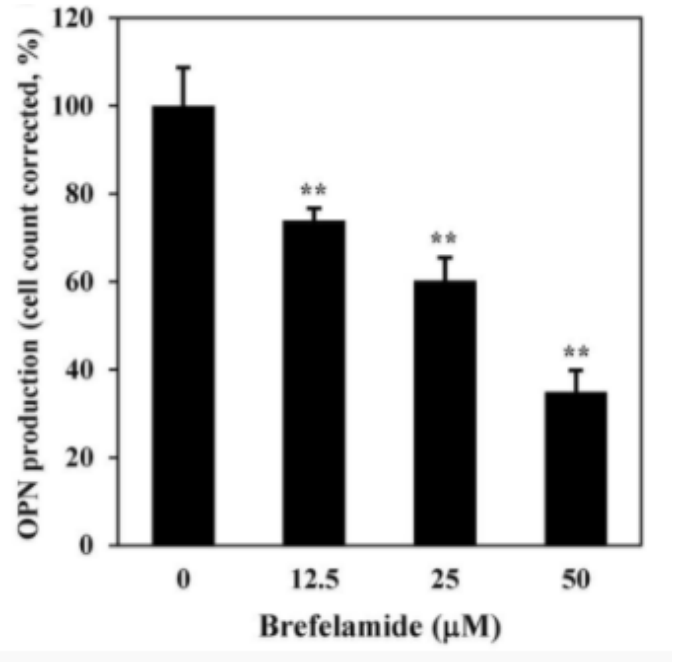


Figure 6 Osteopontin Concentrations with Brefelamide Treatment. A549 cells were cultured in the absence or presence of various brefelamide concentrations. After 48 hours of culture, supernatant aliquots were analyzed by ELISA for OPN production. Cell counts were simultaneously measured with Cell Proliferation Reagent WST-1 for normalization. Normalized OPN secretion from the control group was set as 100%, and those from other brefelamide-treated groups were expressed as relative percentages. Results were presented

as the means ± standard deviations of three independent triplicate transfections. ** indicates P<1.01 versus the vehicle control (Zhang et al., 2016).

The majority of current clinical trials treating patients with hypophosphatemic rickets do not analyze OPN expression. However, common endpoints for current hypophosphatemic rickets clinical trials do capture FGF23 and PHEX as biochemical biomarkers. A recommendation for future hypophophatemic rickets clinical trials and natural history studies is to include osteopontin analysis, which would provide additional data whether OPN can serve as a potential diagnostic biomarker and allow scientists and physicians to understand whether a correlation exist between OPN and renal phosphate levels and the severity of defective bone mineralization.

Magnetic Resonance Imaging (MRI)

Diagnosis of hypophosphatemic rickets can prove difficult in the absence of a clinician with rare disease experience or a family member with a genetically confirmed diagnosis of a renal phosphate wasting disorder (Carpenter et al., 2011). In these instances, radiographic imaging of rickets can help identify and diagnose hypophosphatemic. X-rays and CAT (or CT) scans are forms of radiographic imaging that can reveal bone deformities and measure disease severity and treatment efficacy.

Figure 7 shows the differences between radiographic bone features between a patient with XLH and one with no disease. In individuals without disease, the physis appears as a flat disc between the epiphysis and metaphysis, while the normal zone of provisional calcification (ZPC) is visualized as a continuous, thin band (Lempicki et al., 2017). In the XLH patient, the ZPC is undefined and the physis is widened.

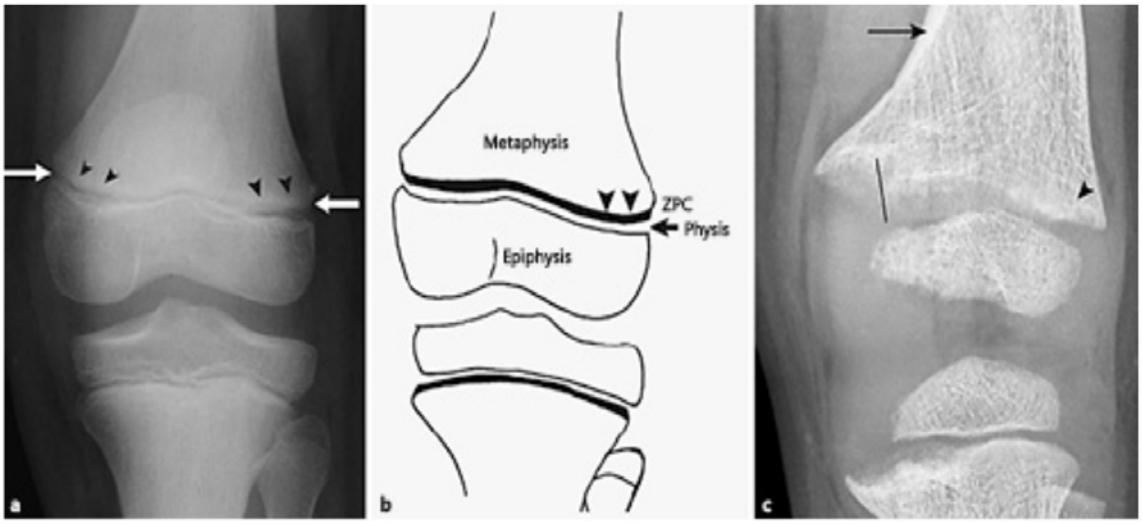


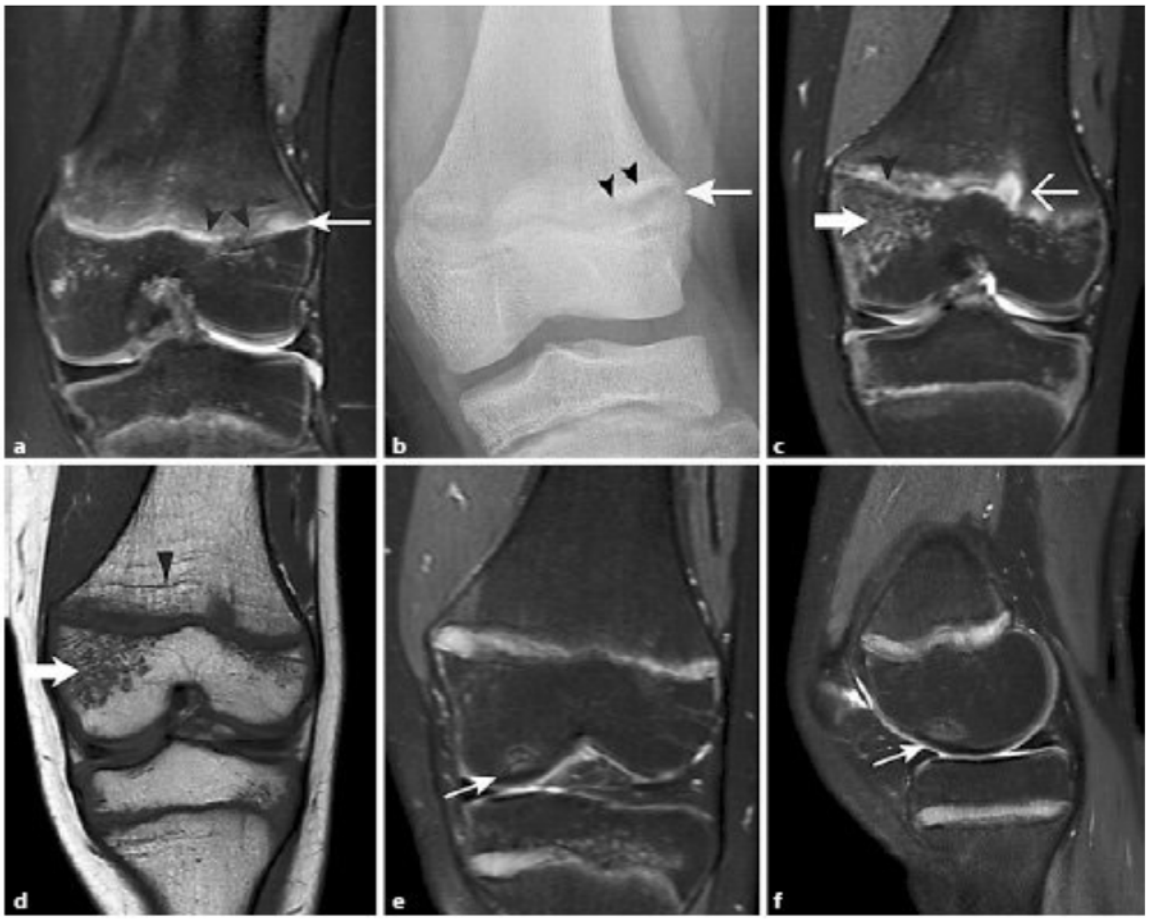
Figure 7 XLH and normal radiographic patterns. **(a)** and **(b)** indicate routine radiograph of a healthy 9-year-old boy showing normal pattern of the left knee on a frontal plain radiograph **(a)** and a drawing **(b)**. The physis (white arrows) appears as a radiolucent flat disc between the epiphysis and the metaphysis. The normal ZPC (arrowheads) is a continuous, regular, thin (<1 mm thick) radiodense band. **(c)** Frontal radiograph of a boy aged 3 years and 5 months treated for XLH. The ZPC (arrowhead) is ill defined, and the physis (solid, vertical line) is widened. Note the preserved cortical bone (black arrow) and the coarse trabecular bone (Lempicki et al., 2017).

Both forms of radiographic imaging expose patients to radiation that can lead to tissue damage and increase the patient's risk to cancer (Carpenter et al., 2011). This is especially true in children who are more sensitive to radiation than adults, especially considering that radiographs are often performed on a regular basis for patients with hypophosphatemic rickets (Lempicki et al., 2017). MRI is a form of imaging that uses strong magnetic fields and radio waves to produce detailed images of the body. Unlike X-rays and CT scans, MRI scanners use powerful magnets to align water protons into one direction. A combination of distributed and received radio signals are used to capture an image of various body parts, including bones and joints. Since MRI scanners do not involve exposing the body to radiation, like X-ray and CT scanners, MRI provides a safer

means of imaging for individuals more vulnerable to radiation exposure, such as children with hypophosphatemic rickets.

A clinical study was performed in pediatric patients using MRI to describe features in hypophosphatemic rickets, specifically XLH, and identify correlations between these features and XLH severity. Disease severity was based on both clinical and laboratory markers. Twenty-seven pediatric patients with PHEX-confirmed XLH had their distal femur imaged by MRI, which was then correlated to height measurement, leg bowing, dental abscesses, and ALP concentration. The distal femur was chosen for imaging because clinical features of XLH are most prominent at the epiphysis. In order to be eligible for participation, patients were required to: 1) be under the age of 18 years; 2) be monitored at the same clinic (French Reference Center for Rare Disorders of Calcium and Phosphate Metabolism); 3) present with hypophosphatemic rickets due to XLH with a confirmed PHEX mutation; 4) currently receiving standard of care treatment; 5) consent to an MRI scan of the distal left femur (or, if not possible, the distal right femur); and 6) not require sedation for the MRI scan. Twenty-four of the 27 patients had a frontal radiograph of the distal left femur within 1 year of their MRI(Lempicki et al., 2017). The frontal radiographs were part of the participants' standard follow up for XLH.

Results seen in Figure 8 display the comparison of X-ray and MRI radiographs in patients with XLH. X-ray and MRI examples of patients with XLH (a 13-year-old and 11-year-old with XLH) show ill-defined ZPC and physis lines in the X-rays, whereas the MRI images enables precise analysis of the region due to presence of image hyperintensity (Lempicki et al., 2017).



(Figure 8 Radiographic and MRI patterns of XLH patients. **a)** MRI image and **(b)** radiograph of a 13-year-old boy treated for XLH. Both ZPC (arrowheads) and physis (arrows) appear less defined on the radiograph compared to the MRI. **(c)** MRI findings of an 11-year-old boy treated for XLH with an enlarged physis (thin arrow) and discontinuous and irregular ZPC (arrowhead). **(d)** In the same 11-year-old boy, note the abnormal epiphyseal abnormalities (thick arrows) and metaphyseal Harris lines (arrowhead), which are lines of increased bone density caused by bone-growth arrest. **(e)** and **(f)** indicate medial femoral osteochondritis (arrows) in an asymptomatic 13-year-old girl with XLH who displays round-shaped abnormal hyperintensity in the subchondral area (Lempicki et al., 2017).

Hyperintensity is a term used to describe regions of a MRI that appear lighter in color than the surrounding tissues, as most MRIs are black and white with shades of gray. If an abnormality is bright (white), it is referred to as hyperintense. If an abnormality is dark, it is described as hypointense. These images indicate that the MRI is a more precise tool, due to its better resolution and 3-dimensional acquisition, in comparison to the

standard radiography used for evaluating physeal widening. The results of the study demonstrated that 78% of MRI images displaying abnormal calcification of the distal femur correlated with abnormally elevated ALP levels, which is a well-established biochemical marker of XLH disease activity (Lempicki et al., 2017). However, because the primary objective of the study was to describe MRI findings in XLH, a comparison of ALP levels with X-rays were not performed.

These findings conclude that MRI of the distal femur provides precise rickets patterns that are correlated with ALP, an established biochemical marker of the XLH, while avoiding radiation exposure by X-ray or CT and providing a new qualitative marker for measuring disease severity. However, MRI is not easily accessible at clinics that see patients with hypophosphatemic rickets due to the cost of the imaging equipment and trained personnel required. According to Healthcare Bluebook, the cost of an MRI of the knee can range between $1000 and $4000 as opposed to an X-ray of the knee, which can range from $80 to $400. Furthermore, MRI image acquisition takes approximately 30 minutes. As a result, sedation is often required in children, which may prevent its routine use throughout childhood follow-ups.

Anti-FGF23 Targeted Therapy

Despite the benefits of oral calcitriol and phosphate as the standard of care treatment for hypophosphatemic rickets, it offers limited treatment efficacy, is inconvenient to administer for the patient, and requires regular monitoring by a physician for potential toxicities in patients (Carpenter et al., 2011). Furthermore, administration of oral calcitriol and phosphate can be associated with several adverse events such as hypercalcemia, hypercalciuria, and nephrocalcinosis (Fukumoto, 2018). As previously

mentioned, XLH is caused by inactivating mutations in the PHEX gene. In the absence of PHEX, the release of FGF23 by osteocytes is increased, which leads to increased renal phosphate excretion, decreased calcitriol production, and decreased intestinal absorption of calcium and phosphate (Carpenter et al., 2011). Because of its role in the development of hypophosphatemic rickets, FGF23 has been identified as a therapeutic target for patients with hypophosphatemic rickets.

Pre-clinical evidence has demonstrated that increased FGF23 activity is the underlying pathogenesis of FGF23-related hypophosphatemic rickets (Yamazaki et al., 2008). In an effort to confirm the effects of anti-FGF23 treatment in the pre-clinical setting, two mouse anti-FGF23 antibodies (FN1 and FC1) that recognize the N- and C-terminal regions of FGF23, respectively, were developed. Two groups of 12-week-old male BALB/c mice were treated with either FN1 or FC1. Seven-week-old male Sprague-Dawley rats treated with phosphate buffered saline were used as the control group. Blood serum was taken from each group at various time points from 1 hour-post treatment and 36 hours post-treatment. FN1 and FC1 administration caused marked increases in serum phosphate and calcitriol levels in the BALB/c mice compared to the Sprague-Dawley rats (Figure 9) (Yamazaki et al., 2008).

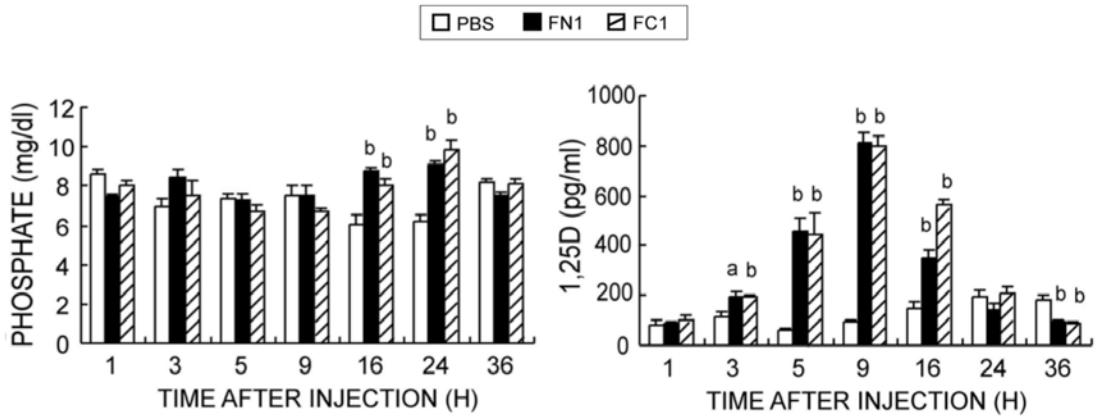


Figure 9 Neutralizing activity of FN1 or FC1 *in vivo.* Time course of changes in serum parameters after a single administration of FN1, FC1, or phosphate buffered solution (PBS). Mice were killed at every time point for each treatment group to collect blood samples. The results represent means ± SE (N = 5). (b) $p < 0.01$ vs PBS-treated group at each time point (Yamazaki et al., 2008).

The FN1 and FC1 antibodies were also tested in the Hyp mouse model, which showed increased serum phosphate and calcitriol levels (Aono et al., 2009). In a single dose preclinical study, 4 mg/kg (2 mg/kg of FN1 and 2 mg/kg of FC1) of anti-FGF23 antibodies were administered subcutaneously into 6 to 8 weeks-old male Hyp mice and normal, wildtype mice. Another group of Hyp and wildtype mice were treated with 16 mg/kg (8 mg/kg of FN1 and 8 mg/kg of FC1) of anti-FGF23 antibodies. As a control group, Hyp mice were treated with 16 mg/kg of an isotype-matched control antibody, thrombopoietin (TPO). Serum phosphate and calcitriol levels were measured over a 14-day period. Hyp mice treated with 4 mg/kg of anti-FGF23 antibody had serum phosphate and calcitriol levels of 7.6 ± 0.4 mg/dl and 1007 ± 19 pg/ml, respectively. Hyp mice treated with 16 mg/kg of anti-FGF23 antibody had serum phosphate and calcitriol levels of 11.6 ± 0.9 mg/dl and 901 ± 25 pg/ml, respectively. Normal mice treated with anti-FGF23 antibodies had serum phosphate and calcitriol levels of 7.6 ± 0.3 mg/dl and 139 ±

14 pg/ml, respectively. Hyp mice treated with TPO had serum phosphate and calcitriol levels of 5.1 ± 02 mg/dl and 137 ± 8, respectively (Aono et al., 2009). Therefore, results of the pre-clinical study indicate that a single subcutaneous injection of anti-FGF23 antibodies succeeded in increasing serum phosphate and calcitriol levels in Hyp mice.

Repeat injections of anti-FGF23 antibodies were also performed to observe the potential therapeutic benefit as defined by improved skeletal development in anti-FGF23 antibody-treated Hyp mice. Hyp mice began treatment (4 or 16 mg/kg) at four weeks of age, which continued weekly for one month for a total of five doses (Figure 10). Compared to the untreated control mice, the treated Hyp mice displayed improved elongation of the femoral and tibial bones. Additionally, anti-FGF23 antibody treatment alleviate the enlarged epiphyses, which is typically observed in Hyp mice bones. Anti-FGF23 antibody treatment improved skeletal development in Hyp mice, and these results suggest that FGF23 inhibition may improve biochemical and clinical abnormalities of patients with FGF23-related hypophosphatemic rickets (Aono et al., 2009).

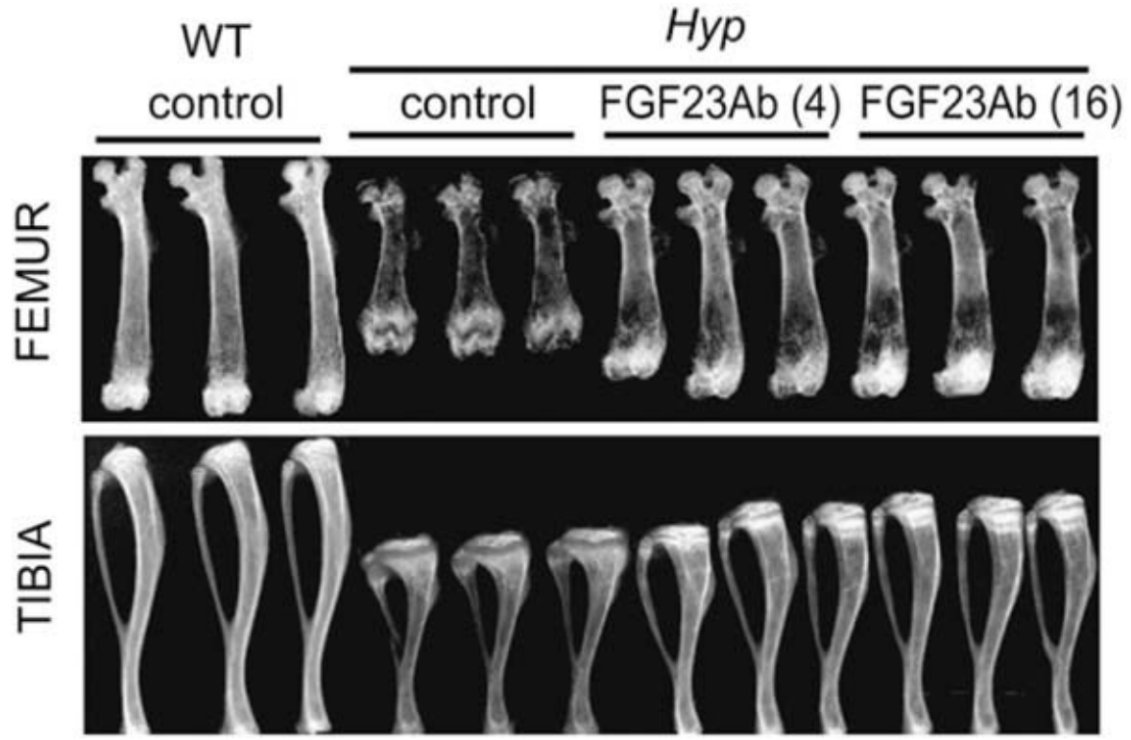


Figure 10 Radiographs of Mice After Fibroblast Growth Factor 23 Treatment. Beneficial effects of repeated injections of FGF23 antibodies (FGF23Ab) on long bones in Hyp mice. Human anti-FGF23 antibodies were subcutaneously administered to two Hyp mouse model groups (4 mg/kg or 16 mg/kg) at 4 weeks of age (day 0), followed by four subsequent injections on days 7, 14, 21, and 28. Representative radiographs of femurs and tibias from three separate mice isolated after 31 days following treatment (Aono et al., 2009).

KRN23 is an investigational human monoclonal immunoglobulin 1 antibody targeted against FGF23 and is currently being studied in clinical trials (Aono et al., 2009). A phase 2 open-label, dose-escalation trial of adults with XLH was conducted to measure if monthly KRN23 treatment would safely improve serum phosphate levels. The purpose of a phase 2 trial is to measure the efficacy and side effects of an investigational drug. An open-label trial is a clinical trial in which both the researchers and participants know which treatment is being administered.

Twenty-eight adults with XLH participated in a 4-month dose-escalation study with the option to continue into a 12-month extension study. The initial dose was 0.05 mg/kg and would be increased to 0.1, 0.3, then 0.6 mg/kg over the course of the 4-month study. KRN23 doses were increased if the participant's serum phosphate level were below the normal range and deemed safe by the treating investigator. If appropriate, the KRN23 dose would be increased to 1.0 mg/kg during the 12-month extension study based on serum phosphate levels. KRN23 was injected subcutaneously once every 28 days. The primary objective of the clinical trial was evaluated by measuring the proportion of subjects who achieved maximum serum phosphate levels within the normal range (>2.5 to ≤4.5 mg/dL) (Imel et al., 2015). Serum phosphate ranges were defined as: less than normal (≤2.5 mg/dL), lower half of the normal range (>2.5 to ≤3.5 mg/dL), upper half of the normal range (>3.5 to ≤4.5 mg/dL), and above the normal range (>4.5 mg/dL) (Imel et al., 2015).

Out of the 28 participants who received KRN23 treatment during the dose-escalation study, 26 subjects received all four doses. Out of these 26 participants, 22 participants entered the extension study and 19 received all 16 doses of KRN23. Two subjects withdrew during dose escalation, and three withdrew during the extension for unspecified reasons. After each dose of KRN23 during the dose-escalation study, the mean serum phosphate peaked by day 7, decreasing to a trough on day 28 (Figure 11). After each of the four escalating doses, peak serum phosphate was within the normal range in 14.8, 37.0, 74.1, and 88.5% of subjects, respectively (Imel et al., 2015).

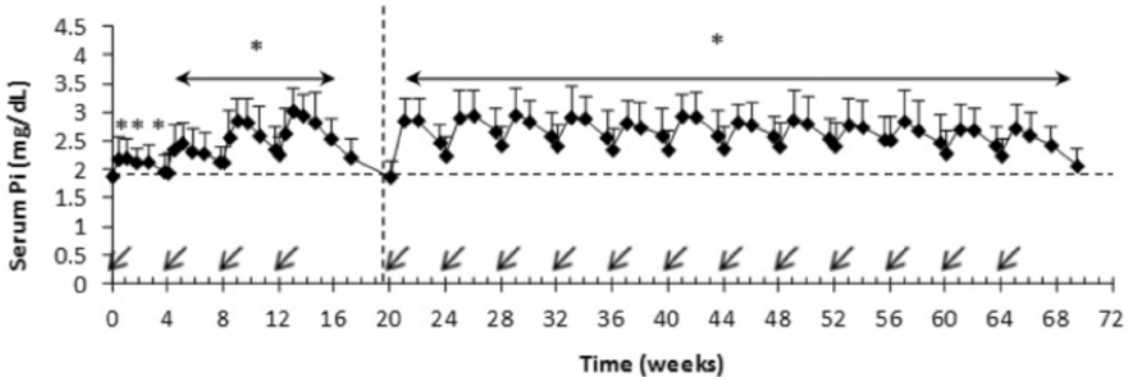


Figure 11 Effect of KRN23 on Serum Phosphate in Adults with XLH. Effect of KRN23 on serum phosphate (Pi) in adults with XLH. KRN23 administration (arrow) occurred every 28 days. Data are presented as mean ± SD. *, *P* values remaining <0.05 were considered significantly different from the baseline values, which were recorded prior to beginning KRN23 treatment. An asterisk over a horizontal line with arrowheads indicates that all values shown under that line were different from baseline ($P < 0.05$). The horizontal broken lines indicate baseline level. The vertical broken lines separate the 4-month dose-escalation study from the 12-month extension study; n = 28 for the dose-escalation study; n = 22 for the extension study (Imel et al., 2015).

Results from the dose-escalation study indicate that serum phosphate levels increased after each successive dose with the peak level reaching 3.0 ± 0.4 mg/dL after the fourth dose. During the 12-month extension study, 88.5% of the extension study participants reached a peak serum phosphate within the normal rangeAdditionally, 42.9% of the extension study participants maintained a normal serum phosphate level through the trough levels. Furthermore, serum phosphate did not exceed normal levels in any participant (Imel et al., 2015). These studies indicate that KRN23 treatment in adults with XLH can result in improved serum phosphate. Thus, KRN23 shows a strong potential for improving an important biochemical outcome for patients with hypophosphatemic rickets. However, further studies are required to demonstrate whether KRN23 improves bone mineralization.

Discussion and Conclusion

Hypophosphatemic rickets is a rare disease that is a result of various phosphate wasting diseases, many of which have similar clinical and biochemical profiles. Coupled with low disease prevalence and a lack of understanding of the underlying disease pathogenesis for each phosphate wasting disorder, hypophosphatemic rickets continues to be difficult to diagnose and treat.

Despite the limitations of current diagnostic tools and standard of care therapy for hypophosphatemic rickets, this targeted literature review has yielded potential directions for new disease biomarkers and treatments. OPN, a protein primarily expressed in bone and kidney, is a known regulator of bone mineralization and is expressed at high levels at sites of defective bone mineralization. Although current clinical trials for hypophosphatemic rickets collect data on other known genetic biomarkers associated with hypophosphatemic rickets, such as FGF23 and PHEX, they do not measure OPN expression in the clinical setting but should be considered for future trials. The availability of brefelamide, an OPN inhibitor currently being used in preclinical trials, presents a future application for OPN as a therapy for hypophosphatemic rickets.

Additionally, MRI as a clinical tool for measuring disease severity and treatment efficacy shows a clear advantage over X-ray imaging, which is the current standard. Specifically, MRI is demonstrated to be a more precise tool in comparison to the standard radiography used for assessing rickets and poor bone mineralization severity. Furthermore, MRI scanners do not expose individuals to radiation, thereby providing a safer means of imaging for individuals more vulnerable to radiation exposure.

Lastly, findings from the KRN23 clinical trials show that anti-FGF23 therapy can improve serum phosphate levels, which is an important biochemical outcome measurement for patients with hypophosphatemic rickets. Because KRN23 currently shows biochemical improvement in adults with XLH, its application in various renal phosphate wasting disorders and pediatric populations are potential directions for future hypophosphatemic rickets research.

Disclosure of Conflict of Interest

This research did not receive any specific funding from public, commercial, or not-for-profit agencies.

References

Albright, F., Butler, A. M., & Bloomberg, E. (1937). Rickets resistant to vitamin d therapy. *American Journal of Diseases of Children Pages = {529-547}, 54*(3)10.1001/archpedi.1937.01980030073005 [doi]

Albright, F., & Reifenstein Jr, E. C. (1949). The parathyroid glands and metabolic bone disease. baltimore, 1948. *Williams & Wilkins Co, 6*, 311,195.

Allgrove, J., & Shaw, N. J. (2015). A practical approach to vitamin D deficiency and rickets. *Endocrine Development, 28*, 119. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26138839

Aono, Y., Yamazaki, Y., Yasutake, J., Kawata, T., Hasegawa, H., Urakawa, I., . . . Shimada, T. (2009). Therapeutic effects of anti-FGF23 antibodies in hypophosphatemic rickets/osteomalacia. *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research, 24*(11), 1879-1888. 10.1359/jbmr.090509 [doi]

Atiq, M., Suria, A., Nizami, S. Q., & Ahmed, I. (1998). Maternal vitamin-D deficiency in pakistan. *Acta Obstetricia Et Gynecologica Scandinavica, 77*(10), 970-973. 10.1034/j.1600-0412.1998.771004.x Retrieved from http://dx.doi.org/10.1034/j.1600-0412.1998.771004.x

Bassir, M., Laborie, S., Lapillonne, A., Claris, O., Chappuis, M. C., & Salle, B. L. (2001). Vitamin D deficiency in iranian mothers and their neonates: A pilot study. *Acta Paediatrica (Oslo, Norway : 1992), 90*(5), 577-579.

Bergwitz, C., & Jüppner, H. (2010). Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annual Review of Medicine, 61*, 91-104. 10.1146/annurev.med.051308.111339 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4777331/

Boskey, A. L., Spevak, L., Paschalis, E., Doty, S. B., & McKee, M. D. (2002). Osteopontin deficiency increases mineral content and mineral crystallinity in mouse bone. *Calcified Tissue International, 71*(2), 145-154. 10.1007/s00223-001-1121-z [doi]

Boukpessi, T., Hoac, B., Coyac, B. R., Leger, T., Garcia, C., Wicart, P., . . . McKee, M. D.Osteopontin and the dento-osseous pathobiology of X-linked hypophosphatemia. *Bone, 95*, 151-161. 10.1016/j.bone.2016.11.019 Retrieved from http://dx.doi.org/10.1016/j.bone.2016.11.019

Breslau, N. A., McGuire, J. L., Zerwekh, J. E., Frenkel, E. P., & Pak, C. C. (1984). Hypercalcemia associated with increased serum calcitriol levels in three patients with lymphoma. *Annals of Internal Medicine Pages = {1-7}, 100*(1)10.7326/0003-4819-100-1-1 [doi]

Carpenter, T. (1997). *New perspectives on the biology and treatment of X-linked hypophosphatemic rickets*//doi.org/10.1016/S0031-3955(05)70485-5 " Retrieved from http://www.sciencedirect.com/science/article/pii/S0031395505704855

Carpenter, T. O., Imel, E. A., Holm, I. A., Jan de Beur, S.,M., & Insogna, K. L. (2011). A clinician's guide to X-linked hypophosphatemia. *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research, 26*(7), 1381-1388. 10.1002/jbmr.340 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3157040/

Chen, Q., Shou, P., Zhang, L., Xu, C., Zheng, C., Han, Y., . . . Shi, Y. (2014). An osteopontin-integrin interaction plays a critical role in directing adipogenesis and osteogenesis by mesenchymal stem cells. *Stem Cells, 32*(2), 327-337. 10.1002/stem.1567 Retrieved from http://dx.doi.org/10.1002/stem.1567

Chesney, R. W. (2012). Theobald palm and his remarkable observation: How the sunshine vitamin came to be recognized. *Nutrients, 4*(1), 42-51. 10.3390/nu4010042 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3277100/

Cho, H. Y., Lee, B. H., Kang, J. H., Ha, I. S., Cheong, H. I., & Choi, Y. (2005). A clinical and molecular genetic study of hypophosphatemic rickets in children. *Pediatric Research, 58*, 329. Retrieved from http://dx.doi.org/10.1203/01.PDR.0000169983.40758.7B

Chong, W. H., Molinolo, A. A., Chen, C. C., & Collins, M. T. (2011). Tumor-induced osteomalacia. *Endocrine-Related Cancer, 18*(3), R77. 10.1530/ERC-11-0006 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3433741/

Choudhury, S., Jebasingh, K. F., Ranabir, S., & Singh, T. P. (2013). Familial vitamin D resistant rickets: End-organ resistance to 1,25-dihydroxyvitamin D. *Indian Journal of Endocrinology and Metabolism, 17*, S227. 10.4103/2230-8210.119579 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3830312/

De Beur, S. M., Finnegan, R. B., Vassiliadis, J., Cook, B., Barberio, D., Estes, S., . . . Schiavi, S. C. (2002). Tumors associated with oncogenic osteomalacia express genes important in bone and mineral metabolism. *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research, 17*(6), 1102-1110. 10.1359/jbmr.2002.17.6.1102 [doi]

DeLuca, H. F., Holick, M. F., Schnoes, H. K., Suda, T., & Cousins, R. J. (1971). Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. *Biochemistry, 10*(14), 2799-2804. 10.1021/bi00790a023 Retrieved from https://doi.org/10.1021/bi00790a023

Driscoll, P. (2006). Gray's anatomy, 39th edition. *Emergency Medicine Journal : EMJ, 23*(6), 492. 10.1136/emj.2005.027847 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2564372/

Econs, M. J., McEnery, P. T., Lennon, F., & Speer, M. C. (1997). Autosomal dominant hypophosphatemic rickets is linked to chromosome 12p13. *The Journal of Clinical Investigation, 100*(11), 2653-2657. 10.1172/JCI119809 [doi]

Econs, M.,J., & McEnery, P.,T. (1997). Autosomal dominant hypophosphatemic rickets/osteomalacia: Clinical characterization of a novel renal phosphate-wasting disorder. *The Journal of Clinical Endocrinology & Metabolism, 82*(2), 674-681. 10.1210/jcem.82.2.3765 [doi]

Eicher, E. M., Southard, J. L., Scriver, C. R., & Glorieux, F. H. (1976). Hypophosphatemia: Mouse model for human familial hypophosphatemic (vitamin D-resistant) rickets. *Proc Natl Acad Sci USA, 73*(12), 4667. Retrieved from http://www.pnas.org/content/73/12/4667.abstract

Endo, I., Fukumoto, S., Ozono, K., Namba, N., Tanaka, H., Inoue, D., . . . Matsumoto, T. (2008). Clinical usefulness of measurement of fibroblast growth factor 23 (FGF23) in hypophosphatemic patients: Proposal of diagnostic criteria using FGF23 measurement. *Bone, 42*(6), 1235-1239. 10.1016/j.bone.2008.02.014 [doi]

Farrow, E. G., & White, K. E. (2010). Recent advances in renal phosphate handling. *Nature Reviews.Nephrology, 6*(4), 207-217. 10.1038/nrneph.2010.17 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3050486/

Franzén, A., & Heinegård, D. (1985). Isolation and characterization of two sialoproteins present only in bone calcified matrix. *Biochemical Journal, 232*(3), 715-724. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1152943/

Fukumoto, S. (2018). Targeting fibroblast growth factor 23 signaling with antibodies and inhibitors, is there a rationale? *Frontiers in Endocrinology*, *9*, 48. 10.3389/fendo.2018.00048 [doi]

Gil, A., Plaza-Diaz, J., & Mesa, M. D. (2018). Vitamin D: Classic and novel actions. *Annals of Nutrition and Metabolism, 72*(2), 87-95. Retrieved from https://www.karger.com/DOI/10.1159/000486536

Guo, Y., & Yuan, Q. (2014). Fibroblast growth factor 23 and bone mineralisation. *International Journal of Oral Science, 7*(1), 8-13. 10.1038/ijos.2015.1 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4817534/

Hautmann, A. H., Hautmann, M. G., Kolbl, O., Herr, W., & Fleck, M. (2015). Tumor-induced osteomalacia: An up-to-date review. *Current Rheumatology Reports*, *17*(6), 5. 10.1007/s11926-015-0512-5 [doi]

Holick, M. F. (2006). Resurrection of vitamin D deficiency and rickets. *The Journal of Clinical Investigation, 116*(8), 2062-2072. 10.1172/JCI29449 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1523417/

Hunter, G. K., Kyle, C. L., & Goldberg, H. A. (1994). Modulation of crystal formation by bone phosphoproteins: Structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. *Biochemical Journal, 300*, 723-728. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1138226/

Ichikawa, S., Guigonis, V., Imel, E.,A., Courouble, M., Heissat, S., Henley, J.,D., . . . Econs, M.,J. (2007). Novel GALNT3 mutations causing hyperostosis-hyperphosphatemia syndrome result in low intact fibroblast growth factor 23 concentrations. *The Journal of Clinical Endocrinology & Metabolism, 92*(5), 1943-1947. 10.1210/jc.2006-1825 [doi]

Imel, E. A., Peacock, M., Gray, A. K., Padgett, L. R., Hui, S. L., & Econs, M. J. (2011). Iron modifies plasma FGF23 differently in autosomal dominant hypophosphatemic rickets and healthy humans. *The Journal of Clinical Endocrinology and Metabolism, 96*(11), 3541-3549. 10.1210/jc.2011-1239 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3205884/

Imel, E. A., Zhang, X., Ruppe, M. D., Weber, T. J., Klausner, M. A., Ito, T., . . . Carpenter, T. O. (2015). Prolonged correction of serum phosphorus in adults with X-linked hypophosphatemia using monthly doses of KRN23. *The Journal of Clinical Endocrinology and Metabolism, 100*(7), 2565-2573. 10.1210/jc.2015-1551 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4495171/

Jan de Beur, S M. (2005). Tumor-induced osteomalacia. *Jama, 294*(10), 1260-1267. 294/10/1260 [pii]

Johnstone, E., Smellie, W., Balfour, J., Balfour, a. S., & Robertson, W. (1779). *Dissertatio medica inauguralis de febre puerperali*. Edinburgi: apud Balfour et Smellie .. Retrieved from http://hdl.handle.net/2027/ucm.5329189946

Kooh, S. W., Binet, A., & Daneman, A. (1994). Nephrocalcinosis in X-linked hypophosphataemic rickets: Its relationship to treatment, kidney function, and growth. *Clinical and Investigative Medicine.Medecine Clinique Et Experimentale, 17*(2), 123-130.

Lempicki, M., Rothenbuhler, A., Merzoug, V., Franchi-Abella, S., Chaussain, C., Adamsbaum, C., & Linglart, A. (2017). Magnetic resonance imaging features as surrogate markers of X-linked hypophosphatemic rickets activity. *Hormone Research in Paediatrics, 87*(4), 244-253. 10.1159/000464142 [doi]

Levine, B. S., Kleeman, C. R., & Felsenfeld, A. J. (2009). The journey from vitamin D-resistant rickets to the regulation of renal phosphate transport. *Clinical Journal of the American Society of Nephrology : CJASN, 4*(11), 1866-1877. 10.2215/CJN.03000509 [doi]

Liamis, G., Milionis, H. J., & Elisaf, M. (2010). Medication-induced hypophosphatemia: A review. *QJM : Monthly Journal of the Association of Physicians, 103*(7), 449-459. 10.1093/qjmed/hcq039 [doi]

Lopez, I., Rodriguez-Ortiz, M. E., Almaden, Y., Guerrero, F., de Oca, A. M., Pineda, C., . . . Aguilera-Tejero, E. (2011). Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 *in vivo*. *Kidney International, 80*(5), 475-482. 10.1038/ki.2011.107 [doi]

M Giachelli, C., Pichler, R., Lombardi, D., T Denhardt, D., Alpers, C., M Schwartz, S., . . . Johnson, R. (1994). *Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis*10.1038/ki.1994.67

Martin, A., David, V., & Quarles, L. D. (2012). Regulation and function of the FGF23/klotho endocrine pathways. *Physiological Reviews, 92*(1), 131-155. 10.1152/physrev.00002.2011 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3306265/

McCollum, E. V., Pitz, W., Simmonds, N., Becker, J. E., Shipley, P. G., & Bunting, R. W. (2002). The effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. studies on experimental rickets. XXI. an experimental demonstration of the existence of a vitamin which promotes calcium deposition. 1922. the effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. *The Journal of Biological Chemistry, 277*(19), E8. 277/19/e8 [pii]

Mendelsohn, R., Paschalis, E. P., & Boskey, A. L. (1999). Infrared spectroscopy, microscopy, and microscopic imaging of mineralizing tissues: Spectra-structure correlations from human iliac crest biopsies. *Journal of Biomedical Optics, 4*(1), 14-21. 1101230 [pii]

Nield, L. S., Mahajan, P., Joshi, A., & Kamat, D. (2006). Rickets: Not a disease of the past. *American Family Physician, 74*(4), 619-626.

Noble, B. S. (2008). The osteocyte lineage. *Archives of Biochemistry and Biophysics, 473*(2), 106-111. 10.1016/j.abb.2008.04.009 [doi]

Norman, A. W. (2008). From vitamin D to hormone D: Fundamentals of the vitamin D endocrine system essential for good health. *The American Journal of Clinical Nutrition, 88*(2), 499S. 88/2/491S [pii]

O'Riordan, J.,L.H., & Bijvoet, O. L. M. (2014). Rickets before the discovery of vitamin D. *BoneKEy Rep, 3* Retrieved from http://dx.doi.org/10.1038/bonekey.2013.212

Palmer, L. C., Newcomb, C. J., Kaltz, S. R., Spoerke, E. D., & Stupp, S. I. (2008). Biomimetic systems for hydroxyapatite mineralization inspired by bone and enamel. *Chemical Reviews, 108*(11), 4754-4783. 10.1021/cr8004422 Retrieved from https://doi.org/10.1021/cr8004422

Pavone, V., Testa, G., Gioitta Iachino, S., Evola, F. R., Avondo, S., & Sessa, G. (2015). Hypophosphatemic rickets: Etiology, clinical features and treatment. *European Journal of Orthopaedic Surgery & Traumatology, 25*(2), 221-226. 10.1007/s00590-014-1496-y Retrieved from https://doi.org/10.1007/s00590-014-1496-y

Payne, R. B. (1998a). Renal tubular reabsorption of phosphate (TmP/GFR): Indications and interpretation. *Annals of Clinical Biochemistry, 35 (Pt 2)*(Pt 2), 201-206. 10.1177/000456329803500203 [doi]

Payne, R. B. (1998b). Renal tubular reabsorption of phosphate (TmP/GFR): Indications and interpretation. *Annals of Clinical Biochemistry, 35 (Pt 2)*(Pt 2), 201-206. 10.1177/000456329803500203 [doi]

Pike, J. W., & Meyer, M. B. (2010). The Vitamin D Receptor: New Paradigms for the Regulation of Gene Expression by 1,25-Dihydroxyvitamin D_3. *Endocrinology and Metabolism Clinics of North America*, *39*(2), 255–269. http://doi.org/10.1016/j.ecl.2010.02.007

Pollack, S. B., Linnemeyer, P. A., & Gill, S. (1994). Induction of osteopontin mRNA expression during activation of murine NK cells. *Journal of Leukocyte Biology, 55*(3), 398-400.

Poole, K. E., & Reeve, J. (2005). Parathyroid hormone - a bone anabolic and catabolic agent. *Current Opinion in Pharmacology, 5*(6), 612-617. S1471-4892(05)00145-1 [pii]

Prasad, N., & Bhadauria, D. (2013). Renal phosphate handling: Physiology. *Indian Journal of Endocrinology and Metabolism, 17*(4), 620-627. 10.4103/2230-8210.113752 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3743361/

Reid, I. R., Hardy, D. C., Murphy, W. A., Teitelbaum, S. L., Bergfeld, M. A., & Whyte, M. P. (1989). X-linked hypophosphatemia: A clinical, biochemical, and histopathologic assessment of morbidity in adults. *Medicine, 68*(6), 336-352.

Rosen, Clifford J & Bouillon, R & Compston, Juliet & Rosen, Vicki (2013). *Primer on the metabolic bone diseases and disorders of mineral metabolism* (8th ed). Wiley, Hoboken.

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., . . . Shapses, S. A. (2010). The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: What clinicians need to know. *The Journal of Clinical Endocrinology and Metabolism, 96*(1), 53-58. 10.1210/jc.2010-2704 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3046611/

Ruchon, A. F., Marcinkiewicz, M., Siegfried, G., Tenenhouse, H. S., DesGroseillers, L., Crine, P., & Boileau, G. (1998). Pex mRNA is localized in developing mouse osteoblasts and odontoblasts. *The Journal of Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society, 46*(4), 459-468. 10.1177/002215549804600405 [doi]

Ruppe, M. D., Zhang, X., Imel, E. A., Weber, T. J., Klausner, M. A., Ito, T., . . . Carpenter, T. O. (2016). Effect of four monthly doses of a human monoclonal anti-FGF23 antibody (KRN23) on quality of life in X-linked hypophosphatemia. *Bone Reports, 5*, 158-162.

10.1016/j.bonr.2016.05.004 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4926842/

Salimpour, R. (1975). Rickets in tehran. study of 200 cases. *Archives of Disease in Childhood, 50*(1), 63-66.

Shafeghati, Y., Momenin, N., Esfahani, T., Reyniers, E., & Wuyts, W. (2008). Vitamin D-dependent rickets type II: Report of a novel mutation in the vitamin D receptor gene. *Archives of Iranian Medicine, 11*(3), 330-334. 08113/AIM.0017 [doi]

Silva, M. C., & Furlanetto, T. W. (2018). Intestinal absorption of vitamin D: A systematic review. *Nutrition Reviews, 76*(1), 60-76. 10.1093/nutrit/nux034 [doi]

Sodek, J., Ganss, B., & McKee, M. D. (2000). Osteopontin. *Critical Reviews in Oral Biology and Medicine : An Official Publication of the American Association of Oral Biologists, 11*(3), 279-303.

Takeda, E., Yamamoto, H., Taketani, Y., & Miyamoto, K. (1997). Vitamin D-dependent rickets type I and type II. *Acta Paediatrica Japonica : Overseas Edition, 39*(4), 508-513.

Taylor, A., Sherman, N. H., & Norman, M. E. (1995). Nephrocalcinosis in X-linked hypophosphatemia: Effect of treatment versus disease. *Pediatric Nephrology (Berlin, Germany), 9*(2), 173-175.

Torres, P. A. U., & De Brauwere, D. P. (2011). *Three feedback loops precisely regulating serum phosphate concentration*//doi.org/10.1038/ki.2011.146 Retrieved from http://www.sciencedirect.com/science/article/pii/S0085253815550692

Voet, D., & Voet, J. G. (2004). *Biochemistry: Biomolecules, mechanisms of enzyme action, and metabolism. volume one* Recording for the Blind & Dyslexic.

Wacker, M., & Holick, M. F. (2013). Sunlight and vitamin D. *Dermato-Endocrinology, 5*(1), 51-108. 10.4161/derm.24494 Retrieved from https://doi.org/10.4161/derm.24494

Wada, T., Nakashima, T., Hiroshi, N., & Penninger, J. M. (2006). RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends in Molecular Medicine, 12*(1), 17-25. S1471-4914(05)00267-4 [pii]

Wang, Y., Zhu, J., & DeLuca, H. F. (2012). Where is the vitamin D receptor? *Archives of Biochemistry and Biophysics; the Many Faces of Vitamin D, 523*(1), 123-133. //doi.org/10.1016/j.abb.2012.04.001 Retrieved from http://www.sciencedirect.com/science/article/pii/S0003986112001324

Weisberg, P., Scanlon, K. S., Li, R., & Cogswell, M. E. (2004). Nutritional rickets among children in the united states: Review of cases reported between 1986 and 2003. *The American Journal of Clinical Nutrition, 80*(6 Suppl), 705S. 80/6/1697S [pii]

Xie, Z., Singh, M., Siwik, D. A., Joyner, W. L., & Singh, K. (2003). Osteopontin inhibits interleukin-1beta-stimulated increases in matrix metalloproteinase activity in adult rat cardiac fibroblasts: Role of protein kinase C-zeta. *The Journal of Biological Chemistry, 278*(49), 48546-48552. 10.1074/jbc.M302727200 [doi]

Yamazaki Y, Tamada T, Kasai N, Urakawa I, Aono Y, Hasegawa H, et al. Anti-FGF23 neutralizing antibodies show the physiological role and structural features of FGF23. J Bone Miner Res (2008) 23(9):1509–18.10.1359/jbmr.080417

Zadik, Y., & Nitzan, D. W. (2012). Tumor induced osteomalacia: A forgotten paraneoplastic syndrome? *Oral Oncology, 48*(2), 9. 10.1016/j.oraloncology.2011.09.011 [doi]

Zanotti, S., Gibertini, S., Di Blasi, C., Cappelletti, C., Bernasconi, P., Mantegazza, R., . . . Mora, M. (2011). Osteopontin is highly expressed in severely dystrophic muscle and seems to play a role in muscle regeneration and fibrosis. *Histopathology, 59*(6), 1215-1228. 10.1111/j.1365-2559.2011.04051.x [doi]

Zhang, J., Yamada, O., Kida, S., Matsushita, Y., Murase, S., Hattori, T., . . . Oshima, Y. (2016). Identification of brefelamide as a novel inhibitor of osteopontin that suppresses invasion of A549 lung cancer cells. *Oncology Reports, 36*(4), 2357-2364. 10.3892/or.2016.5006 [doi]

Zhang, Z. X., Shek, K., Wang, S., Huang, X., Lau, A., Yin, Z., . . . Jevnikar, A. M. (2010). Osteopontin expressed in tubular epithelial cells regulates NK cell-mediated kidney ischemia reperfusion injury. *Journal of Immunology (Baltimore, Md.: 1950), 185*(2), 967-973. 10.4049/jimmunol.0903245 [doi]