

Molecular and Biochemical Aspects of Hypophosphatemic Rickets: An Updated Review

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Abstract

Rickets is associated with aberrant mineralization in growth plates leading to the deformity of bone structure. The two main types of rickets are phosphopenic and calcipenic rickets. Phosphopenic rickets is found in a variety of sorts; the most common is the X-linked dominant hypophosphatemic rickets (HR) caused by a mutation in *PHEX* gene. The *FGF23*, *DMP1*, and *PHEX* are among the most important genes in the etiology of HR disorder. The interaction of these genes is essential for proper bone mineralization. However, the underlying mechanism is not comprehensively known. For data collection, we searched the most recent articles in Science Direct, PubMed and Google Scholar using hypophosphatemia, mutation, *PHEX* and *FGF23* as keywords. The search results revealed that most of the articles have mainly focused on various types of mutations causing hypophosphatemia in different populations. However, there was a lack of enough studies elucidating the interaction of genes involved in this disorder. This review mainly focuses on the various types of phosphopenic rickets and genetic mutations of various agents crucial for bone mineralization and how these mutations exert their effects on biochemical agents like vitamin D and parathyroid hormone (PTH). It also reviews the available treatment and molecular techniques for managing this disorder.

Keywords: Rickets, Mutation, Phosphate, Genetic

Introduction

Bone hypophosphatemia is a rare form of hypophosphatemic rickets (HR) that can be seen in either autosomal dominant or sporadic forms. Patients are often short in stature with skewed legs (arc shape). Additionally, they may exhibit features of osteomalacia but with very slight or no variations in growth plates.¹ In contrast, Rickets leads to abnormalities of growth plates and can be seen in long bones. Deficiencies in calcium, phosphorus as well as vitamin D lead to rickets.² The most common symptoms of rickets in children are retardation and bone malformation. Calcipenic rickets, which is also referred to as hypocalcemic and phosphopenic or

hypophosphatemic are 2 types of rickets.³ Hypocalcemic rickets is caused by a lack of calcium, the most common cause of this condition is a lack of vitamin D. Vitamin D is vital for normal calcium absorption. HR occurs in situations in which lack of phosphate concentration results in a dearth of phosphate movements into the cells.⁴ Genetics plays an important role in HR. Mutations and aberrant function in genes such as phosphate regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*), dentin matrix acidic phosphoprotein 1 (*DMP1*), Fibroblast growth factor 23 (*FGF23*) and solute carrier family 34A3 (*SLC34A3*), may result in abnormalities in reabsorption of phosphate

and calcium, absorption of vitamin D, and deformity in bone mineralization.^{2,5,6} This review has tried to focus on clinical, biochemical and molecular features of HR. Table 1 and Box 1 represent features and types of calcipenic and phosphopenic rickets respectively.

Methods

In order to find related research regarding the different aspect of hypophosphatemia, we searched 3 main databases including PubMed, Science Direct and Google Scholar using “hypophosphatemia, mutation and vitamin D” keywords.

1. Inherited Diseases of Phosphate Metabolism

1.1. X-Linked Hypophosphatemic Rickets

X-linked hypophosphatemic rickets (XLH) is the most common type of rickets in the world with the frequency of 1:20 000 live births.⁷ This disorder was first described by Albright et al.⁸ In adults, symptoms include osteomalacia, pain in joints and even dental disease. In the intensive form of hypophosphatemia, defects in mineralization of the bones can be observed, and patients with no family

history of XLH mostly have leg deformities. In this disease, females are more affected than males.^{9,10} Phosphatemia in blood, along with low level of vitamin 1,25(OH)₂D and normal serum calcium levels associated with increased alkaline phosphatase activity, accompanied with normal or mild elevation of parathyroid hormone (PTH) levels can be used to differentiate XLH patients from other sorts of hypophosphatemia. Based on the latest research, inactivating mutation of the *PHEX* leads to XLH and increases the FGF23 levels.^{11,12} This increase in FGF23 levels is due to the reduction in cleavage process which inactivates FGF23. As a result, elevated levels of FGF23 eliminate phosphate reabsorption through Klotho/FGFR receptor complex.¹³ Some researchers have illustrated that PHEX is responsible for FGF23 decay.⁹ However, other researchers could not prove this theory.^{14,15} The mouse model is mostly used by researchers for investigating different aspects of HR. However, there are some differences in human and mouse regarding gene expression and physiological traits. This compelled a group of Chinese researchers to generate a rabbit model via CRISPR/Cas9 system. This model mimics the symptoms of XLH similar to that of affected individuals. Consequently, this model can pave the way for more preclinical studies of XLH.¹⁶

Table 1. Comparison of Calcipenic and Phosphopenic Rickets

Features	Calcipenic	Phosphopenic
Muscle weakness	+	-
Bone pain	+	-
Serum calcium	Low/Normal	Normal
Serum phosphorus	Low	Low
Parathyroid hormone	Elevated	Normal elevated
Phosphatase	Elevated	Elevated
Osteopenia and osteitisfibrosa	+	-
Dental abscess	-	Maybe positive
Parts of body that involved	All parts of body	Lower parts of body

1.2. Autosomal Dominant Hypophosphatemic Rickets

Bianchine et al demonstrated autosomal dominant hypophosphatemic rickets (ADHR) in 1971.¹⁵ ADHR is equal among males and females; Phosphate wasting is observed mostly in urine.¹⁷ The clinical and biochemical manifestation of ADHR bears a close resemblance to that reported in XLHR patients. Moreover, it is a disorder with incomplete penetrance and different age of onset. In the female, during puberty and pregnancy, there are reports of late onset ADHR. It is now postulated that iron deficiency can cause increased production of FGF23. Consequently, this provides good evidence for ADHR penetrance during pregnancy and puberty.^{7,18} Inactivating mutation in *FGF23* leads to ADHR with low serum of Ca and Pi as well as normal levels of D₃ and 25 hydroxyvitamin.¹⁷ In ADHR, affected individuals exhibit increased activity in FGF23. The reason is that mutant form of FGF23 is resistant to proteolysis and it mainly remains as an intact protein, and this intact protein exerts the entire effects of FGF23 in cellular levels.^{13,19} A study on large kindred affected by ADHR revealed that patients in their childhood had bone pain, short stature, lower extremity deformities, dental abscess, and rickets. The patients affected by ADHR in their adulthood had symptoms like tumor-induced osteomalacia (TIO). Adults also had weakness, bone pain, fractures, and osteomalacia but they were not short in stature and they did not have any deformity in their lower extremity.²

Box 1. Classification of Rickets

1. Calcipenic rickets
 - Deficiency in vitamin D
 - Type 1 vitamin D dependent rickets
 - Type 2 vitamin D dependent rickets (vitamin D resistant rickets)
 - Nutritional vitamin D deficiency
2. Phosphopenic rickets
 - 2.1. Type of rickets
 - X-linked Dominant
 - Autosomal recessive (Type 1 & 2)
 - Tumor induced osteomalacia (TIO)
 - Osteoglophonic dysplasia (OGD)
 - Hereditary autosomal recessive (FAM20C mutation)
 - 2.2. Genetic basis
 - Autosomal dominant
 - Heredity hypophosphatemia with hypercalciuria
 - X- linked recessive (Dent disease)
 - (9,13) Translocation with hyperparathyroidism

1.3. Autosomal Recessive Hypophosphatemic Rickets

Inactivating mutation in *DMP1* gene produces another rare disease by the name of autosomal recessive HR.³ Its chromosomal location is 4q21. Evidence shows 6 mutations in *DMP1*, most of which are small deletion and missense.²⁰ There are 2 types of ARHR, type 1 and 2. Autosomal recessive HR type 2 has recently been reported. This disease happens due to inactivating mutation in ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*).^{21,22} *ENPP1* encodes inorganic pyrophosphate (PPi) needed as a physiologic suppressor of calcification.²¹ Elevated levels of FGF23 are observable in both types of ARHR. Furthermore, the clinical presentation of this disorder is found during childhood.⁷

1.4. Hereditary Hypophosphatemic Rickets With Hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is a form of rickets that is not caused by FGF23 mutations.^{7,23} Inactivating mutation in solute carrier family 34 (sodium phosphate) members 1 and 3 (*SLC34A3*, *SLC34A1*) lead to HHRH. Researchers have reported 21 mutations within *SLC34A3* gene, of which 46.61% were missense [http://www.hgmd.org].²⁴ Deletion in the exon 9 and 7 have also been reported in *SLC34A3*. Other types of mutations include silent and donor splice site mutations, and most of the mutations were associated with loss of function in *SLC34A3* protein.²³ In the 21 kindred studies, 43% of described *SLC34A3* mutations were homozygous while 38% and 19% consisted of compound heterozygous mutations and heterozygous mutations, respectively.²⁵ *SLC34A1-3* encodes sodium-phosphate cotransporters type 2 (*SLC34a1*; *Napi-2a/SLC34a2*; *Napi-2b/SLC34a3*; *Napi-2c*). Individuals carrying the mutation in *Napi-2a* or *Napi-2c* genes display clinical features of HHRH. These mutations are responsible for defective phosphate transport in renal tubular cells. As a result, they contribute to diminished phosphate reabsorption in patients with HHRH.^{7,26,27} The inheritance pattern of this disorder is autosomal recessive and biallelic mutation is essential for clinical manifestation. However, familial studies revealed that single mutations could also cause some clinical symptoms of HHRH.^{23,28} Bone pain, muscle weakness, and pseudofractures are common symptoms of HHRH. In comparison with other types of rickets, patients affected by HHRH exhibit an increase in serum 1,25(OH)₂D.^{23,24} Therefore, this biochemical feature can be used as a differential diagnosis of HHRH from other types of hypophosphatemia. Moreover, elevated levels of 1,25(OH)₂D₃ are accompanied by hypercalciuria and suppression of PTH secretion.²⁷ Hypercalciuria, which is present in these patients, increases the risk of nephrolithiasis.^{24,26} It is interesting to note that *Napi-2a* cannot compensate for *Napi-2c*, while both cotransporters act as a modulator of phosphate reabsorption. The reason for this discrepancy is the transient expression of these

2 factors during development. For instance, *Napi-2c* is expressed more significantly during weaning in the rat.^{27,29}

1.5. Tumor-Induced Osteomalacia

TIO, also known as oncogenic hypophosphatemic osteomalacia, is a rare disorder (paraneoplastic) of renal phosphate wasting.³⁰ Clinical symptoms such as muscle weakness, bone pain, and fatigue are reported within these patients. This disease is also dependent on the age of exposure, and radiology pictures of patients with TIO are also similar to ADHR patients.³¹ In comparison with X-linked HR and autosomal dominant HR, in TIO, calcitriol levels are more often less than XLH and ADHR. Based on recent evidence, 24 of 29 tumors were arranged as phosphaturic mesenchymal tumors. FGF23 is expressed dramatically in these tumors, and this over expression of FGF23 leads to phosphate wasting.³⁰ de Beur et al showed that the FGF23 is not a unique gene that becomes highly expressed.³² Surgical removal of the entire tumor fully resolves the symptoms.³³

1.6. X-linked Recessive Hypophosphatemic Rickets (Dent Disease)

Dent disease is the name given to a group of x-linked disorders which consist of x-linked recessive nephrolithiasis, x-linked recessive HR, and idiopathic proteinuria. It is a late-onset disorder with clinical manifestation in early adulthood. Its clinical symptoms are as follow: failure in proximal renal tubular reabsorption, nephrocalcinosis and hypercalciuria. Mutation in *CLCN5* gene located on the short arm of X chromosome (xp11, 22), is responsible for developing this disease. This gene encodes 746 amino-acids *CLC5* chloride channel which has a role in albumin as well as low molecular weight proteins reabsorption. Locus heterogeneity has been suggested in *CLCN5* gene. Additionally, another mutation in *OCRL* gene (xq26.1) has been described recently. However, in some cases, none of the 2 mutations are present in patients.³⁴

1.7. Hypophosphatemic Rickets and Hyperparathyroidism

A balanced translocation with a breakpoint adjacent to the α -Klotho gene [t (9, 13) (q21, 13; q13, 1)] leads to a type of HR with hyperparathyroidism, hypercalcemia and inappropriately normal level of 1,25(OH)₂D₃. The significant consequences of this translocation are elevated levels of FGF23 and PTH (hyperparathyroidism). The reason for this increase in FGF23 levels is the α -Klotho role in FGF23 signaling and FGF23 binding to its receptor.^{35,36}

1.8. Osteoglophonic Dysplasia

Osteoglophonic dysplasia (OGD) is a rare hypophosphatemic disorder with activating germline mutation in *FGFR1* (fibroblast growth factor receptor1). As a result of this mutation, increase in FGF23 level is noticeable. Therefore, it is assumed that activating mutation

of *FGFR1* is the main culprit of hypophosphatemia and elevated FGF23 in patients with OGD.³⁷

1.9. FGF23-Related Hypophosphatemia With Mutation in *FAM20C*

A non-lethal osteosclerotic bone dysplasia has been described with FGF23-related hypophosphatemia. This disorder is a severe skeletal dysplasia which leads to death at birth. However, non-lethal cases have also been reported. This syndrome is a hereditary autosomal recessive disorder caused by homozygous or compound heterozygous mutation in *FAM20C* gene. The protein encoded by this gene is a phosphorylase kinase and acts as a biomineralizing factor. *FAM20C* phosphorylates many secreted proteins such as DMP1 and MEPE.^{38,39} Moreover, FGF23 protein is phosphorylated by *FAM20C* on serin180. This phosphorylation process enhances proteolytic cleavage of FGF23, leading to FGF23 degradation. Inactivating mutation in *FAM20C* is associated with FGF23-related hypophosphatemia.^{38,40,41}

2. Molecular Function and Genetic Mutations of Various Agents in Hypophosphatemic Rickets

2.1. FGF23 Functions and Mutations

FGF23 gene is assigned to 12q13.3 region.⁴² The protein encoded by this gene is expressed in osteocytes, osteoblast, and odontoblasts and has a 25% to 35% similarity to fibroblast growth factor.³³ Intact FGF23 protein is assumed to be the active hormone capable of inducing hypophosphatemia. Mutation in this gene eliminates its cleavage between Arg179 and Ser180 by subtilisin-like proprotein convertase (SPCs). N and C fragments of FGF23 protein either act as an inhibitor at high concentration or are devoid of any activity.⁴³ FGF23 eliminates phosphate reabsorption through down-regulation of type 2a and 2c sodium-phosphate cotransporters. Moreover, it promotes the expression of *CYP24A1* while *CYP27B1* expression is suppressed. The protein produced by *CYP24A1* gene is responsible for 1,25OHD₃ degradation whereas the *CYP27B1* gene enhances the production of 1,25OH₂D₃, leading to more phosphate reabsorption from the intestine.^{44,45} There is a possibility that both DMP1 and PHEX negatively regulate FGF23. However, the underlying mechanism is unknown.^{43,46} In one study, lack of PHEX, as well as DMP1 in differentiated bone marrow stromal cells, contributed to the activation of FGFR pathway.³⁷ The *FGF23* mutation occurs within RXXR motif, and it is prevalent among patients with ADHR.⁴⁷ So far, 11 mutations have been reported, most of which are missense type (<http://www.hgmd.org>).²

2.1.1. FGF23 Receptors (FGFRs)

FGF23 exerts its effects at the cellular level through FGF receptors. In the absence of its coreceptor, FGF23 binds

to its receptor with low affinity. α -Klotho is a coreceptor of FGF23 which contributes to FGF23 binding to its receptor with high affinity. There are various types of FGF receptors, namely FGFR1, 2, 3 and 4. Each of these receptors is encoded by specific genes. FGFRs are comprised of three different domains including intracellular tyrosine kinase domain, a transmembrane domain and three extracellular immunoglobulin domains.⁴⁸ Pharmacological inhibition of FGFRs leads to the suppression of FGF23/Klotho signaling and succeeding elevation of 1,25OH₂D₃ and phosphate.^{37,49} As a result, elevated levels of vitamin D (hypervitaminosis D) cause a high level of FGF23 in serum while FGFR inhibitors block transcription of FGF23 in bone which is a dominant process responsible for impinging on systemic levels of FGF23 in patients.⁴⁹ Overall, therapies targeting the FGFRs are considered to be the most promising approaches for handling hypophosphatemic condition mediated by FGF23. Inhibition of FGFR by NVP-BGJ398 as one of FGFR inhibitors disrupts FGF23 signaling.⁵⁰

2.1.2. The α -Klotho as a Coreceptor

The α -Klotho has a role in aging and regulation of FGF23. As it was mentioned earlier, de novo translocation adjacent to α -Klotho causes a specific type of hypophosphatemia with hyperparathyroidism.³⁵ The membrane-bound type of α -Klotho mediates FGF23 signaling. The intracellular domain of membrane-bound Klotho is detectable in kidney and parathyroid gland, the 2 FGF23 target tissues.⁴⁸ The other 2 soluble types are SKL and CKL. However, just CKL has been identified in human.³⁶ In fact, α -Klotho inhibits *CYP27B1* by enhancing FGF23 signaling pathway. Furthermore, it reduces expression of the renal sodium-phosphate transporter (Napi-11a) and intestinal phosphate transporter (Napi-11b). The presence of α -Klotho is crucial for signal transduction of FGF23 through its receptor.⁵¹

2.1.3. FGF23/KLOTHO Complex

Mitogen-activated protein kinase (MAPK) is activated by the FGF23-Klotho-FGFR complex. Subsequently, phosphorylation of ERK1/2 is induced by MPAK. As a result of this complex formation, the Napi-2a and Napi-2C protein expression are down-regulated in kidney, and 1,25OH₂D₃ synthesis is suppressed. The consequence of 1,25OH₂D₃ suppression is diminished levels of the Napi-2b transporter in the intestine and subsequent reduction in Pi absorption. Based on recent research, Klotho is capable of impinging on the expression of Napi-2a in an independent manner.⁵¹

2.1.4. Iron Supplementation in Hypophosphatemic Rickets

Recent studies have shown that in ADHR patients, an indirect correlation exists between low iron concentration

and intact as well as c-terminal FGF23 concentration. Iron supplementation can rescue patient's phenotype and normalize the renal phosphate reabsorption. However, the mechanism is not known comprehensively. Iron deficiency can provide evidence for the variable age of onset in ADHR phenotype penetrance, and waxing and waning of symptoms observed in patients. Therefore, delayed onset of phenotype manifestation in ADHR is more likely to occur in women during puberty and immediately after pregnancy when iron deficiency is more prominent.^{18,52,53}

2.2. PHEX Functions and Mutations

Phosphate regulating gene with homologies to endopeptidase on the X chromosome (PHEX) is the most common mutated gene, and its mutations account for approximately 80% of all familial hypophosphatemia. It constitutes 22 coding exons. The protein encoded by this gene is predominantly expressed in osteoblasts, osteocytes, and odontoblasts and contributes to bone formation.^{13,28,42} Furthermore, PHEX is a member of endopeptidase family, and acts as a suppressor for bone mineralization inhibitors and degrades phosphaturic hormones like FGF23. Additionally, it regulates phosphorus activity.^{45,47,54} PHEX protein has 3 different domains namely N-terminal cytoplasmic domain which is short, the transmembrane domain and long C-terminal extracellular domain with zinc-binding sites and cysteine residues. Cysteine residues generate the secondary structure of the protein while zinc-binding sites are necessary for catalytic activity of protein.⁵⁵ Loss of function mutation in *PHEX* gene stimulates FGF23 in bone. There are different types of mutations in *PHEX* gene including nonsense, deletion, missense, insertion, and mutation in splice site. However, C-terminal mutations and mutations that result in truncated protein are more devastating. According to the previous studies, 300 mutations have been reported in the PHEX mutation database (<http://www.phexdb.mcgill.ca>). Based on this database, each of the mutations has different frequencies. For example, frameshift mutations account for 25% whereas 23% is reported for alternative splicing, other frequencies are as follow: 22% missense, 18% nonsense, 8% deletion and 4% polymorphisms.^{11,13,28,55} Mutations mostly affect the protein secondary structure. Exon 7 is the most infrequent mutant exon reported in PHEX database. On the other hand, more than 50% of mutations are reported in C-terminal around exon 18 to 22. Therefore, this region is assumed to be the most important domain for the function of PHEX and it is associated with more severe symptoms.⁵⁵ Splice site mutation like c.1080 A>G leads to the production of mRNA that lacks exon 10 and subsequent loss of zinc-binding motif as well as cysteine residues.¹¹ Moreover, substitution of glycine for arginine alters the charge of PHEX protein and results in different

spatial conformation.⁵⁵

2.2.1. Matrix Extracellular Phosphoglycoprotein With ASARM Motif (MEPE)

The protein encoded by *MEPE* gene is a secreted calcium binding phosphoprotein. It belongs to the short integrin-binding ligand-interacting glycoproteins (SIBLING) family. C-terminal region of MEPE protein is known as the ASARM motif which is a common motif among the family of proteins called SIBLING. DMP1 is another member of this family. DMP1 and MEPE have an impact on FGF23 expression through interaction with PHEX. MEPE modulates mineralization, and it is considered as an inhibitory factor for phosphate reabsorption. Moreover, ASARM motif is a protease-resistant peptide which is capable of inhibiting mineralization. Interestingly, PHEX binds to MEPE via ASARM motif (nonproteolytic interaction). As a result, it prevents MEPE cleavage and ASARM release. Therefore, a mutation in *PHEX* gene may lead to an increased level of ASARM peptide. HR is a disease associated with MEPE.⁵⁶⁻⁶⁰

2.3. DMP1F

Dentin matrix protein1 (DMP1) belongs to a group of short integrin-binding ligand interacting glycoproteins (SIBLING) family. The protein encoded by this gene has a role in bone mineralization through elevation of Wnt/b-catenin expression and declining the Wnt/b-catenin inhibitor called DKK1 as well as FGF23 inhibition.⁶¹ Osteoblasts and osteocytes are the 2 main sites of DMP1 protein production. Loss of function mutation in DMP1 leads to FGF23 elevation.⁴² The proteolytic process seems to be essential for normal functioning of DMP1. The 37-KDa-N-terminus fragment as well as 57-KDa-C-terminus fragment is produced following proteolytic cleavage in DMP1 protein. Bone morphogenetic protein1 (BMP1) is a subtilisin-like proprotein convertase, responsible for proteolytic cleavage of DMP1 protein.⁶² Both integrin binding site (RGD motif) and ASARM motif are located on the 57-KDa-C-terminus peptide, which is highly phosphorylated. Because of this, the 57-KDa-C-terminus fragment is proposed as a functional domain of the DMP1 protein.^{58,62,63} The 57KDa fragment has roles in bone mineralization and osteocyte maturation via down-regulation of genes predominantly expressed by osteoblast (OSX and Col). It also binds to DNA and modulates gene expression.^{20,62}

2.4. ENPP1 Functions and Mutations

ENPP1 is a member of ecto-nucleotide pyrophosphatase/phosphodiesterase family. The protein encoded by this gene is a type II transmembrane glycoprotein. ENPP1 protein is in charge of modulating inorganic pyrophosphate (ppi). Inorganic pyrophosphate is considered as a crucial factor for inhibiting calcification.

Therefore, bone mineralization depends on the ratio of both phosphate and inorganic phosphate which is closely modulated by the ENPP1. When loss of function mutation occurs in ENPP1, Ca production cannot be normalized and suppressed. As a result, phosphate levels will decline. Most importantly, this type of mutation can cause an elevation in FGF23.^{22,42} It is worthwhile to mention that loss of function mutation in *ENPP1* can lead to either autosomal recessive hypophosphatemia or generalized arterial calcification in infancy (GACI).^{21, 64} It is hard to determine the potential factors for developing either ARHR2 or GACI. One assumption is the possible indirect correlation between abnormal calcification and hypophosphatemia. Other types of mutations such as missense, nonsense and premature stop codon are also reported in *ENPP1* gene.⁶⁴ HR with the loss of function in ENPP1 is sometimes accompanied by hearing loss which can be used as a diagnostic symptom.⁶⁵

3. Biochemical Alterations in Hypophosphatemic Rickets

3.1. Vitamin D Regulatory Mechanism in Bone and Kidney

The protein encoded by *CYP27B1* gene is a member of cytochrome p450 superfamily. It acts as a 1 α -hydroxylase in the kidney. *CYP27B1* converts 25OH₂D₃ to 1 α 25OH₂D₃ (active form of vitamin D). 1,25OH₂D₃ exerts its effects on bone and kidney through genomic pathway, regulating many factors acting on these sites. 1 α -hydroxylase activity is modulated by PTH, Ca²⁺, FGF23, PPi concentration. When 1,25OH₂D₃ binds to its receptor (VDR), it elevates Ca and phosphate absorption and decreases urinary Ca excretion.⁴³ The FGF23 level is also induced by *CYP27B1* and PTH while FGF23 has an inhibitory effect on PTH and *CYP27B1*. FGF23 mediates this inhibitory effect in several sites in the body such as heart and aorta.⁶⁶ 1,25OH₂D₃ stimulates FGF23 secretion by binding to its receptor called VDR (vitamin D receptor). VDR heterodimerize with RXR (retinoid x receptor) and binds to vitamin D response element (VDRE) in the promoter region of *FGF23* gene, thereby, up-regulating FGF23

expression. Moreover, 1,25OH₂D₃ up-regulates *Klotho* expression via the genomic pathway. It is proposed that vitamin D mediates this regulatory effect on FGF23 secretion through down-regulation of *DMP1* and *PHEX*.^{59,67,68} 1,25OH₂D₃ regulates both anabolic and catabolic genes. *LRP5*, *Runx2*, *TRPV6*, *Npt2c* are among the anabolic genes regulated by 1,25OH₂D₃ whereas *PTH* and *RANKL* (induced osteoclastogenesis) are catabolic genes. *LRP5* is essential for proliferation as well as the function of osteoblast, *Runx2* is a transcription factor mediating bone formation, *TRPV6* is a calcium channel crucial for calcium absorption, and mineralization of bones and *Npt2c* is a renal cotransporter.⁶⁷

3.2. Parathyroid Hormone

Like FGF23, PTH is another major hormone in charge of regulating renal phosphate handling. Through down-regulation of sodium phosphate co-transporters, both PTH and FGF3 cause a decline in phosphate reabsorption in renal proximal tubule, As a result, inducing hypophosphatemia. PTH increases 1,25(OH)₂D₃ production by inducing 1 α -hydroxylase activity. The expression and secretion of FGF23 are also stimulated due to PTH.¹ Parathyroid cells proliferation and PTH release are induced by elevated levels of phosphate and diminished levels of Ca²⁺.³⁵ Table 2 summarizes the data on the regulatory effects of PHEX, PTH, FGF23, and 1,25OH₂D₃.

4. Polymorphism Detection Using Genome-Wide Association Study

The concentration of serum phosphorus is associated with single nucleotide polymorphism (SNP) at loci located on the chromosome 1, 5, 6, 12, using genome-wide association study. In this respect, SNP (rs1697421) on chromosome 1, at locus region of 1p36.13 was significantly correlated with serum phosphorus concentration. This region is 10 kb away from genes responsible for alkaline phosphatase expression. Mutation of these genes leads to hypophosphatasia. Other top SNPs are as follow:

Table 2. PTH, 1,25OH₂D₃, FGF23 and PHEX Regulatory Effects (Increase/Decrease)

PTH		1,25OH ₂ D ₃		FGF23		PHEX	
Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease
FGF23	Napi2a Napi2c	Ca reabsorption	PTH	CYP24A1	PTH	--	FGF23/klotho function
CYP27B1	CYP24A1	Napi2b	--	--	CYP27B1	--	ASARM peptide
Serum Ca	Renal phosphate reabsorption	Intestinal phosphate reabsorption	--	--	Napi2a Napi2c Napi2b	--	--
--	--	FGF23 Klotho	--	--	Renal phosphate reabsorption	--	--

Abbreviations: PTH, parathyroid hormone; FGF23, fibroblast growth factor 23; PHEX, phosphate regulating gene with homologies to endopeptidase on the X chromosome; NaPi2, type II sodium-phosphate cotransporters; ASARM, acidic serine aspartate-rich MEPE-associated motif; CYP27B1, cytochrome P450 family 27 subfamily B member 1.

SNP (rs453639) at the intronic region of *ENPP3* (the ectonucleotide pyrophosphatase/phosphodiesterase 3) gene on chromosome 6p23.1 adjacent to *ENPP1* gene. SNP (rs4.74995) at locus region of 5p35.3, which encodes RGS14 (G protein regulator) adjacent to *SLC34A1*, and SNP (rs2970818) at locus region of 12p13.32, which is the upstream of *FGF23*. These SNPs are located in the regions that are close to genes which have a pivotal role in phosphorous metabolism.⁶⁹

5. Molecular Tests

For a better understanding of the types of the HR at the first step, it can be useful to check the HR's genes and direct sequencing. Polymerase chain reaction (PCR) at the first step of our research on HR may be preferable and easier, amplifying all of the exon coding regions of all genes related to HR is recommended, and mutations in each gene can be observed in sequence analysis. Other molecular methods such as qPCR, long-range PCR, DNA microarray, and MLPA are available but the most cost efficient is conventional PCR and direct sequencing. In HR, a mutation in *PHEX* is the most common cause of developing hypophosphatemia. Therefore, its mutation analysis must be the first measure of diagnosis. In 57% up to 78% of affected individuals with XLH, sequence analysis of *PHEX* coding region as well as its intronic border has succeeded in detecting *PHEX* mutations. Furthermore, using exon sequencing along with MLPA analysis leads to the more precise diagnosis of XLH.¹³ Mutation analysis in ADHR mostly focused on mutations that occur in R176 and R179 residues of *FGF23*. Mutations in these residues are the main culprit of ADHR. In the case of ARHR, PCR reaction is used to amplify the coding region of *DMP1* gene. Subsequently, PCR products are sequenced for mutation detection. For the study of protein, researchers use a powerful method called proteomics, which is, in fact, a long process but yields very precise results. Although laboratory kits are accurate enough, we should remember the sensitivity and specificity of kits, and that all of our results are not exactly accurate because false positive or negative results sometimes occur, which renders the results unacceptable. Thus, it is better to use a minimum of 2 methods in suspicious cases. Moreover, aside from using the molecular technique for HR diagnosis, genetic counseling can also provide helpful information. For instance, the inheritance pattern of male-to-male transmission, eliminates the possibility of XLH, while the presence of affected parent obviates the possibility of ARHR.¹⁹

6. Treatments Available for Hypophosphatemic Rickets

Phenotype heterogeneity is a common feature observed among patients with similar genotype. This heterogeneity is possibly due to late diagnosis and treatment of HR. This means that early diagnosis and treatment implications

can alter the clinical outcome of various types of HR. Additionally, this amount of heterogeneity in patients with HR emphasizes the role of other regulators in phosphate hemostasis.^{13,56} In children, the goal of treatment is accurate tooth and bone mineralization and sufficient longitudinal growth whereas, in infant, immediate diagnosis of HR prevents the development of rickets.⁷⁰ In HHRH, phosphate supplementation alone can be beneficial for the amelioration of patient's condition, and there is no need for vitamin D add-on therapy.^{25,71,72} However, in other types of HR, the combination of phosphate and vitamin D supplementation is required for effective treatment.¹⁹ The implication of anti-*FGF23* antibody, namely KRN23 has a considerable outcome in *PHEX* mutant mouse. This IgG1 monoclonal antibody inhibits the activity of *FGF23*, leading to elevated levels of Pi and $1,25\text{OH}_2\text{D}_3$. This treatment is more likely to improve XLH symptoms with less adverse effects in comparison with daily treatment of phosphate and vitamin D.⁷³

Conclusion

Patients suspected of HR, must be diagnosed carefully. After carrying out biochemical and radiologic tests and obtaining family history, molecular tests can be valuable in determining the exact type of rickets. These patients need lifetime care and treatment in addition to supplementation of phosphate. Genetic counseling centers all over the world can play an important role in the prevention of these kinds of diseases by informing expectant parents of their child's risk. Besides, more investigations should be carried out in order to elucidate the possible interaction of *FGF23*, *DMP1*, and *PHEX*, 3 major mutated genes in different types of HR.

Ethical Approval

Not applicable.

Competing Interests

Authors declare that they have no competing interests.

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