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Mutational analysis of PHEX, FGF23 and DMP1 in a cohort of patients with hypophosphatemic rickets

Mary D Ruppe[1,3], **Patrick G Brosnan**[2,3], **Kit Sing Au**[2], **Phong X Tran**[2], **Barbara W Dominguez**[2,3], and **Hope Northrup**[2,3]

[1] Department of Medicine, University of Texas Health Science Center at Houston, Houston, TX

[2] Department of Pediatrics, University of Texas Health Science Center at Houston, Houston, TX

[3] Shriners Hospital for Children, Houston, TX

Summary

Background—X-linked hypophosphatemic rickets, autosomal dominant hypophosphatemic rickets and autosomal recessive hypophosphatemic rickets make up a group of renal phosphate wasting disorders with common clinical and biochemical characteristics. These three types of rickets are related to mutations in *PHEX*, *FGF23* and *DMP1*, respectively.

Objective—The objective of the study was to evaluate the frequency of mutations that occur in these three genes associated with hypophosphatemic rickets.

Patients and Methods—In this study, we sequenced these genes in 76 members of 46 kindreds from a large hypophosphatemic rickets cohort.

Results—Forty two individuals from 27 kindreds were found to have mutations in *PHEX*, 16 of which were novel. One subject had an *FGF23* mutation. No individuals were found to have mutations in *DMP1* consistent with the presence of recessive hypophosphatemic rickets.

Conclusions—Our data highlight the wide spectrum of genetic variation that can be seen in *PHEX*, *FGF23* and *DMP1* when screening a large cohort with hypophosphatemic rickets.

Keywords

PHEX; FGF23; DMP1 in phosphate rickets

INTRODUCTION

Patients with hypophosphatemic rickets suffer from lower extremity deformities, bone and/or joint pain, short stature and dental abnormalities. The skeletal defects are primarily caused by defects in phosphate metabolism; in particular, hypophosphatemia from renal phosphate wasting. In normal subjects, hypophosphatemia is accompanied by elevated 1,25-dihydroxyvitamin D levels but in patients with hypophophatemic rickets no such elevation is observed leading to a dual defect in phosphate and vitamin D metabolism.

First reported in 1939 [1], X-linked hypophosphatemic rickets (XLH; MIM # 307800) is the most common genetic form of hypophosphatemic rickets with an incidence of 3.9–5 per

*Correspondence to: Mary D Ruppe, University of Texas Health Science Center at Houston, 6431 Fannin St, MSB 5.106, Houston, TX 77030. Phone: 713-500-6901 Fax: 713-500-6646 mary.ruppe@uth.tmc.edu.

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100,000 [2–3]. XLH is associated with inactivating mutations in the phosphate regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*; MIM# 300550)[4]. Located at Xp22.2-p22.1, the *PHEX* gene produces a protein of unclear function that is expressed primarily in cells of bone lineage, including osteoblasts, osteocytes and odontoblasts [5–7]. To date more than two hundred different mutations of the *PHEX* gene have been described in patients with hypophosphatemic rickets (http://www.phexdb.mcgill.ca/).

A less common genetic form of hypophosphatemic rickets is autosomal dominant hypophosphatemic rickets (ADHR; MIM # 193100). While ADHR and XLH share clinical and biochemical characteristics, ADHR has incomplete penetrance, variable age of onset and may resolve later in life [8]. ADHR is defined by a mutation located at 12p13.3 in the gene that produces the fibroblast growth factor 23 protein (*FGF23*; MIM# 605380)[9–10]. Like the PHEX protein, FGF23 is predominantly expressed in cells of bone lineage, including osteocytes, osteoblasts and odontoblasts [11–12]. ADHR patients have a mutation at a proprotein convertase cleavage site that prevents proteolytic degradation and inactivation of the FGF23 protein[13–14].

Another rare genetic form of hypophosphatemic rickets is autosomal recessive hypophosphatemic rickets (ARHR; MIM # 241520). ARHR is defined by mutations in dentin matrix protein 1 (*DMP1;* MIM# 600980) located at 4q21 [15 16]. *DMP1* is one of a cluster of genes encoding a class of tooth and bone noncollagenous matrix proteins known as SIBLING (small integrin-binding ligand, N-linked glycoproteins) [17]. Loss of function mutations in *DMP1* have been reported in 7 kindreds with ARHR[15–16, 18–19].

In familial cases, hypophosphatemic rickets can be inherited in either an X-linked, autosomal dominant or autosomal recessive manner. The most common gene found to be mutated in these cases is *PHEX* leading to XLH. Frequently sporadic cases are found to have mutations in *PHEX* which are presumed to be de novo mutations [20]. Rarely, mutations are found in *FGF23* or *DMP1*. The Mendelian inheritance pattern of sporadic cases with no mutations found in the three genes is unclear. In order to extend our knowledge about the frequency of mutations that cause hypophosphatemic rickets, we studied a large cohort of patients at an orthopedics specialty hospital. Seventy-six patients were evaluated, and the sequence of their *PHEX, FGF23* and *DMP1* genes determined.

Patients and methods

Patients

Patients were included in the study based on laboratory parameters that included reduced serum phosphorus and reduced tubular resorption of phosphate corrected for glomerular filtration rate (TmP/GFR) according to age-adjusted standards. There needed to be no elevation of the serum calcitriol level or urinary calcium level to rule out hereditary hypophosphatemic rickets with hypercalciuria. Additionally, there was documentation of no glycosuria, bicarbonaturia or aminoaciduria which is seen in Fanconi syndrome. Detailed four generational pedigrees were obtained from each patient along with information on their racial background. Patients were excluded if clinical or biochemical features were not consistent with the three known variants of hypophosphatemic rickets, XLH, ADHR or ARHR.

Study approval was received from the Committee for Protection of Human Subjects (CPHS) at the University of Texas Health Science Center in Houston, Texas and the Shriners Hospital legal council in Tampa, Florida. Informed consent was obtained from all patients or their parents for mutational analysis.

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Methods

Mutational Analysis—DNA was extracted from whole blood utilizing Gentra Puregene DNA kits (Qiagen, Valencia, CA) or PaxGene DNA kits (Qiagen). All exonic sequences along with flanking intronic sequences of *PHEX*, *FGF23* and *DMP1* were amplified. PCR primers were based on the published primers for *PHEX*, *FGF23* or *DMP1* [15, 21–24]. The PCR products were sequenced utilizing the forward or reverse primers or a redesigned sequencing primer. Direct sequencing was carried out via an ABI 3100 automatic sequencer (Applied Biosystems, Carlsbad, CA) following standard protocol for BigDye™ (Applied Biosystems) terminator sequencing. Sequencing results were aligned with the genomic DNA sequence utilizing the NCBI BLAST program (http:www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi) or clustalW (http://www.ch.embnet.org/software/ClustalW.html). When alignment of multiple sequence fragments was necessary, CodonCode Aligner was used (CodonCode Corporation, Dedham, MA). Nucleotide changes in the intronic region were analyzed using a splice site prediction tool (http://www.fruitfly.org/seq_tools/splice.html) which finds possible 5′ and 3′ splice sites based on a neural network based approach. A mutation was determined to be disease-causing when the nucleotide change was either 1) a previously reported mutation in the *PHEX* database (http://www.phexdb.mcgill.ca/), 2) predicted to cause protein truncation or 3) disrupted a splice site. The controls were either a parent of the sporadic proband or unaffected members of the familial cohorts. Additionally, the bioinformatics tools PolyPhen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/) were used to determine the functional effects of all the missense mutations. These tools use physical and comparative considerations to predict the impact of amino acid substitution on the structure and function of proteins. Position Specific Independent Counts (PSIC) score [25] calculates the profile matrix with PolyPhen outcome representing the difference between profile scores of the allelic variants with larger values indicating rare or never observed substitution. The Sorting Intolerant from Tolerant (SIFT) score [26] represents the normalized probability that the amino acid change is tolerated. Scores less than 0.05 are predicted to be deleterious. Finally, the SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) was searched for previous reports of the variants.

Laboratory Evaluation—Samples for the laboratory tests were obtained during scheduled clinic visits. The serum and urine chemistries were performed on the day of the visit utilizing a Siemens Xpand Integrated Chemistry System (Siemens, Deerfield, IL). The TmP/GFR was calculated using the method of Walton and Bijvoet for adults [27] and using the method described by Stark [28] for children. Serum for the FGF23 levels was aliquoted and frozen on the day of the clinic visit. The samples were stored at −80 C until analysis. The FGF23 levels were measured utilizing a full length ELISA assay (Kainos, Japan). The assay was performed and the data analyzed according to the manufacturer’s recommendations.

Results

From 1998–2009, patients from 70 kindreds with renal phosphate wasting disorders have been evaluated at the Shriners Hospital for Children in Houston. Of these, seven individuals representing 7 kindreds were diagnosed with other condition associated with renal phosphate wasting and hypophosphatemic rickets. These subjects had linear nevus sebaceous syndrome (2 individuals) and McCune Albright Syndrome (5 individuals). Of the remaining 63 kindreds, members of 46 kindreds were available for the study.

Based on the obtained pedigrees, a familial inheritance pattern was established in 20 of the 46 kindreds examined; the remaining 26 kindreds contained sporadic cases, assumed to be a

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new genetic event which could be consistent with any form of hypophosphatemic rickets. Ethnically, 21 kindreds were Hispanic (46%), 22 non-Hispanic Whites (48%), 2 African American (4%) and 1 Asian American (2%). Included in the mutational analysis were 76 patients (26 male and 50 female) from the 46 kindreds and 18 unaffected family members (8 male and 10 female).

PHEX mutational analysis

We identified 27 *PHEX* mutations in 27 kindreds (12 familial and 15 sporadic); no *PHEX* mutations were found in the other 19 kindreds (Table 1 lists each mutation and Table 2 summarizes their racial and inheritance characteristics). Of the 27 *PHEX* mutations, 7 were missense mutations (26%), 6 were nonsense mutations (22.2%), 5 were splice site mutations (18.5%), 5 were deletions (18.5%) and 4 were insertions (14.8%). Additionally, there was one synonymous change seen (Table 3). Six of the 7 missense mutations were analyzed using the SIFT and PolyPhen. Five of the 6 that were analyzed were found to be damaging (Table 3). The seventh missense mutation was not analyzed as it disrupted a stop codon (see below) and it is not possible to analyze this type of change with the software.

Sixteen of the 27 *PHEX* mutations have not previously been reported; one novel mutation was particularly interesting. A sporadic mutation in an affected child from kindred 35 was a missense mutation in which the stop codon for the last exon (exon 22) in the *PHEX* gene was mutated. Because another in-frame stop codon is found downstream, this mutation should result in the production of the full length PHEX protein with an additional 10 amino acid fragment (LWLGRWFMAS).

A probably damaging missense mutation in exon 19 (c.1936 G>A, p.646 D>N) was found in the proband of kindred 19 who at the time of initial presentation was thought to be the only affected member of the cohort. There were four members of the kindred tested. The proband was a female who was heterozygous for the mutation. Prior to treatment at the age of 3, she displayed hypophosphatemia (0.6 mmol/L; normal range for age 1.45–1.78 mmol/L), decreased TmP/GFR (0.68–0.74 mmol/L; normal range for age [28] 1.3–1.5 mmol/L) and severe lower extremity bowing requiring hemiepiphysiodesis. On treatment FGF23 levels were elevated (100–120 pg/mL, normal range 10–50 pg/mL). A subsequent off treatment FGF23 level at the age of 14 remained elevated at 80 pg/mL with a simultaneous serum phosphate level 0.6 mmol/L (normal range for age [28] 0.87–1.45 mmol/L). The brother of the proband was hemizygous for the mutation and had normal serum phosphate (1.5 mmol/L) when he was initially evaluated at the age of 4. He has maintained his serum phosphorus at a normal level through the age of ten (1.45–1.60 mmol/L; normal range for age 1.45–1.78 mmol/L) with the exception of a single measurement of 1.10 mmol/L. During this time, he has had persistently low TmP/GFR (0.88–1.12 mmol/L; normal range for age normal range for age [28] 1.3–1.5 mmol/L), high normal to slightly elevated FGF23 (37–54 pg/mL), no lower extremity bowing, extreme short stature (<3% for height) and frontal bossing. The mother of the proband was heterozygous for the mutation, and had normal height, no evidence of lower extremity bowing, low normal serum phosphate 0.90 mmol/L; normal range for age 0.8 mmol/L– 1.55 mmol/L), low TmP/GFR 0.88 mmol/L (normal range for age 1.12–1.76 mmol/L) and slightly high FGF23 (57 pg/mL). The father was unaffected and had a normal genotype.

FGF23 mutational analysis

We identified one disease causing *FGF23* mutation. The proband was a female with sporadic inheritance who was diagnosed with hypophosphatemia during an evaluation for lower extremity bowing at one year of age. Her mutation was located at c.535C>T, p.

179R>W which is a previously reported mutation at the consensus sequence for the proprotein convertase cleavage site.

An additional *FGF23* variant was identified outside the consensus sequences and was thus assumed to be non-disease causing. The variant consisted of a single amino acid replacement (c.716C>T, p.239T>M), a change evaluated by SIFT and PolyPhen as benign (Table 3).

Four of the 6 families with the benign *FGF23* variants also were found to have *PHEX* mutations.

DMP1 mutational analysis

None of the 46 kindreds were found to have a homozygous *DMP1* mutation or a compound heterozygous mutation consistent with the development of ARHR.

Seven heterozygous variants in *DMP1* were found, all located in exon 6 (Table 3). Four of the variants were synonymous and three were missense. Two of the nonsynonymous changes were evaluated by SIFT and PolyPhen as possibly damaging (c.205A>T, p.69S>C and c.475C>A, p.159Q>K).

The first possibly damaging missense mutation was found in one family, who was also found to have a *PHEX* mutation. The second possibly damaging missense mutation was found in 7 families, 4 of which also had *PHEX* mutations. This suggests that these genetic variants are not responsible for the disease.

Discussion

We performed a genetic analysis of the *PHEX*, *FGF23* and *DMP1* genes in a large cohort of patients with hypophophatemic rickets. More than half of the kindreds had mutations in *PHEX*, the gene associated with XLH. One kindred had a mutation in *FGF23*, the gene associated with ADHR. No mutations were found in *DMP1*, the gene for ARHR.

The *PHEX* gene encodes a protein of unknown function but with homologies to members of the M13 family of metallopeptidases [29]. The structure of the protein consists of a short amino terminal cytoplasmic domain, a single transmembrane domain and a large extracellular domain containing a zinc binding motif and conserved cysteine residues. Characterizing mutations in the *PHEX* gene and their phenotypes may reveal functionally important regions of the gene product.

Sixteen novel mutations (27 total mutations) were found in the *PHEX* gene. The PHEX database and other reports have found mutations predominantly in the 3′ end of the gene [20,30]. However, the mutations in our cohort were distributed throughout the length of gene with 18 exonic regions and 5 intronic areas represented (out of 22 exons).

One mutation was of particular interest. A missense mutation (c.2249 A>T, p. 750 X>L) causing the stop codon for the last exon to be mutated, resulted in the production of the full length PHEX protein with an additional 10 amino acid fragment. The fact that the proband for this mutation suffered from hypophosphatemic rickets emphasizes the importance of the 3′ end of the gene for maintaining normal function and is consistent with the preponderance of 3′ end mutations in the PHEX database.

Additionally, one kindred highlights the large variability in disease presentation that can occur within a single family. In kindred 19, the *PHEX* mutation was a probably damaging missense mutation in exon 19 (c. 1936 G>A, p. 646 D>N). The proband, the proband’s

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brother and the proband's mother had the mutation and had XLH as evidenced by renal phosphate wasting and slightly elevated FGF23 levels. However, only the proband had marked hypophosphatemia and significant bone related clinical features although the proband's brother had short stature and mild frontal bossing. This suggests that other genetic, metabolic or development factors must mediate the effect of this *PHEX* mutation and illustrates the importance of completing a thorough evaluation of apparently non-affected family members in order to accurately determine future familial genetic risk. While we did not identify genetic variants in the other two genes examined (*FGF23* or *DMP1*) for this kindred, there may be other interacting genes involved. It has been established that the phenotypic presentation of XLH can vary greatly, even within a single kindred [31]. Also, the link between mutations in *PHEX* and the pathophysiology of XLH is not understood. Furthermore, it is widely accepted that XLH has complete penetrance. Contrary to this accepted view, a recent report describes discordance for clinical XLH disease in monozygotic twin girls with a family history of X-linked inheritance and no detectable *PHEX* or *FGF23* mutations [32]. This supports the idea that other factors work synergistically with *PHEX* mutations to determine the pathophysiology of hypophosphatemia.

Much rarer than XLH, ADHR is associated with mutations in *FGF23*. FGF23 is a 251 amino acid protein which influences renal phosphate wasting through two main mechanisms. FGF23 influences the internalization of the renal tubule sodium phosphate co-transporter- 2a, a proximal tubule protein that reabsorbs phosphate that has been filtered by the glomerulus. FGF23 also down regulates the one alpha hydroxylase enzyme in the kidney, a cytochrome P450 enzyme that aids the hydroxylation of 25 OH vitamin D to 1,25 $(OH)_2$ vitamin D [33–34]. We describe one sporadic patient with an *FGF23* mutation. As with other disease causing *FGF23* mutations, it was found in the consensus sequence for the proprotein convertase cleavage site.

DMP1 mutations, associated with ARHR, have been described in only a handful of kindreds worldwide [15–16, 18, 35]. Because we did not observe any mutations in *DMP1*, DMP1 is unlikely to be a causative factor in the hypophosphatemic rickets observed in our cohort. We did find a large number of kindreds with sequence variants and heterozygous changes in the exon 6 of the *DMP1* gene suggesting that this area may be highly susceptible to genetic change.

In keeping with past literature, a high percentage of our patients (41%) were found to have no detectable mutation in *PHEX* [30, 36–37]. Although not performed as part of this study, it may be helpful to include multiplex ligation-dependent probe amplification analysis as part of further testing. This technique that allows multiple DNA targets to be amplified with a single primer pair and has recently been used to detect large deletions in a cohort of patients with XLH[38]. A small fraction of the patients with no *PHEX* mutations did have sequence variants in *FGF23*, and no patients were found with *DMP1* mutations, leaving 39% with no assignable genetic locus for their disease. This suggests that other non-coding areas of the sequenced genes, including regulatory elements are may be involved in hypophosphatemic rickets. Additionally, mutations in other genes that have not previously been identified may also lead to development of disease. Two groups have recently identified and sequenced a new gene involved in hypophosphatemic rickets known as the ectonucleotide pyrophosphate/phosphodiesterase gene (*ENPP1*; MIM # 173335). ENPP1 is a regulator of extracellular pyrophosphate [39–40] and has been previously been linked to the development of generalized arterial calcification of infancy (*GACI*; MIM #208000). Loss of function mutations were observed in five families with ARHR2 (MIM #613312). It would be of interest to evaluate *ENPP1* in our 11 kindreds where neither an X-linked inheritance pattern nor *PHEX*, *FGF23* or *DMP1* mutations could be established. However, because of the rarity of the *ENPP1* mutation it is unlikely that it accounts for all of our patients who suffered

from hypophosphatemia but did not have disease-causing mutation in *PHEX*, *FGF23* or *DMP1*. Therefore, it is likely that there are still other genes for this complex disorder that are waiting to be discovered.

In conclusion, we report the detailed genetic analysis of a large cohort of subjects with hypophosphatemic rickets. We found a large number of novel *PHEX* mutations in patients and a single disease causing *FGF23* mutation. This study serves to extend the spectrum of mutations that occur in hypophosphatemic rickets. In conclusion, the significant rate of kindreds that were found to lack a mutation in the three genes associated with hypophosphatemic rickets indicates that the continued investigation and discovery of causative genes may aid the further unraveling of the link between genetic mutations and pathophysiology in hypophosphatemic rickets.

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Table 1

Variants, excluding synonymous changes found in *PHEX* and *FGF23*

Gene	Kindred	Location	Genomic DNA	Type	Protein	Type	Mutation
PHEX	8	E1	58 C>T	Replacement	20 R>X	nonsense	Not novel
PHEX	37	E3	212 A>C	Replacement	79 N>T	missense	Novel
PHEX	38	E4	397 C>T	Replacement	133 Q>X	nonsense	Not novel
PHEX	18	E5	482 G>C	Replacement	161 R>P	missense	Novel
PHEX	23	E6	674 delA	Deletion	225 A>E, 238>X	frameshift, nonsense	Novel
PHEX	3	I7	849 + 1 G>T	Replacement	283 splice	Splice site mutation	Not novel
PHEX	27	E9	1042 delA	Deletion	348 K>K, 354 L>X	frameshift, nonsense	Not novel
PHEX	32	I9	1079 +1 G>A	Replacement	360 splice	Splice site mutation	Not novel
PHEX	26	E10	1163insA	Insertion	389 F>I	frameshift	Novel
PHEX	25	E6–10	662 -1175del	Deletion	del222 -392	deletion	Novel
PHEX	21	E11	1230 G>T	Replacement	411 E>X	nonsense	Novel
PHEX	41	E11	1305insC	Insertion	406 C>L, 411 E>X	frameshift, nonsense	Novel
PHEX	34	E12	1348 T>C	Replacement	450 L>P	missense	Novel
PHEX	30	I13	1483+1 G>C	Replacement	494 splice	Splice site mutation	Not novel
PHEX	29	E14	1522 C>T	Replacement	508 Q>X	nonsense	Not novel
PHEX	12	E15	1601 C>T	Replacement	534 P>L	missense	Not novel
PHEX	22	E16	1658 G>A	Replacement	553 G>E	missense	Novel
PHEX	36	E16	1699 C>T	Replacement	567 R>X	nonsense	Not novel
PHEX	17	E17	1705_6delCT	Deletion	569 L>E, 580H>X	frameshift, nonsense	Novel
PHEX	9	E17	1727_1738del	Deletion	576D V>D, del577_580	missense, deletion	Novel
PHEX	39	I17	1768 −1 G>C	Replacement	591 splice	Splice site mutation	Novel
PHEX	5	E18	1847insCAAA	Insertion	616 K>N, 621 Q>X	frameshift, nonsense	Novel
PHEX	46	I18	1899 + 1 G>T	Replacement	633 splice	Splice site mutation	Not novel
PHEX	19	E19	1936 G>A	Replacement	646 D>N	missense	Novel
PHEX	44	E20	1966insGCTT	Insertion	657 Y>C, 664 R>X	frameshift, nonsense	Novel
PHEX	10	E22	2239 C>T	Replacement	747 R>X	nonsense	Not novel
PHEX	35	E22	2249 A>T	Replacement	750 X>L	missense	Novel

Gene	Kindred	Location	Genomic DNA	Type	Protein	Type	Mutation
FGF23	47	E3	535 C>T	Replacement	179 R>W	missense	Not novel

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Table 2

The presence of *PHEX* and *FGF23* mutations by racial and inheritance characteristics

		Kindreds	PHEX mutation	FGF23 mutation
Ethnicity	non-Hispanic Whites	22	9 (41%)	1(100%)
	Hispanic	21	15 (71%)	0
	African American	2	2 (100%)	0
	Asian American	1	1 (100%)	0
Inheritance	Familial	20	12 (60%)	0
	Sporadic	26	15 (58%)	1 (100%)

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Table 3

PolyPhen and SIFT evaluation of missense variants and reporting of synonomous changes found in *PHEX*, *FGF23* and *DMP1*

Gene	Kindreds	Variant	PolyPhen	PSIC	SIFT	SIFT score	dbSNP ID
PHEX	37	c.212 A>C, p. 79 N>T	Probably Damaging	2.133	Damaging	0.01	Novel
	18	c. 482 G>C, p. 161 R>P	Benign	1.044	Tolerated	0.27	Novel
	37	c. 1118 C>T, p. 373 S>S	Synonymous	N/A	Synonymous	N/A	Novel
	34	c. 1348 T>C, p. 450 L>P	Probably Damaging	2.541	Damaging	0	Novel
	12	c. 1601 C>T, p. 534 P>L	Probably Damaging	2.768	Damaging	0.05	Novel[1]
	22	c. 1658 G>A, p. 553 G>E	Probably Damaging	2.543	Damaging	0	Novel
	19	c. 1936 G>A, p. 646 D>N	Probably Damaging	2.543	Damaging	0	Novel
FGF23	46	c. 535 C>T, p. 79 R>W	Probably Damaging	2.654	Damaging	0	rs28937882:A
	2,13,22,32, 34,39	c. 716 C>T, p. 239 T>M	Benign	0.183	Tolerated	0.25	rs7955866:T
DMP1	2,5,8,14,27, 33,42	c. 1230 G>A, p.410 E>E	Synonymous	N/A	Synonymous	N/A	rs2615497:A
	8	c. 1255 C>T, p. 419 P>S	Benign	N/A	Tolerated	0.15	Novel
	2,6,10,15,35,37,41	c. 205 A>T, p. 69 S>C	Possibly Damaging	1.773	Damaging Low confidence	0	rs10019009:T
	18,27,33,42	c. 1218 C>T, p.406 S>S	Synonymous	N/A	Synonymous	N/A	rs2615498:T
	27	c. 879 T>C, p. 293 S>S	Synonymous	N/A	Synonymous	N/A	Novel
	25	c. 475 C>A, p. 159 Q>K	Possibly Damaging	1.736	Damaging	0.03	rs79402270:A
	40	c. 753 T>A, p. 251 T>T	Synonymous	N/A	Synonymous	N/A	Novel

[1] Although the mutation that was described for kindred 12 (c.1601 C>T, p. 534 P>L) has previously been reported in XLH and appears in the PHEX database, it does not have an identifier in the dbSNP. Therefore, it is listed as "not novel" as a mutation in Table 1 but "novel" for a dbSNP ID in Table 3.