Molecular Diagnoses of X-Linked and Other Genetic Hypophosphatemias: Results From a Sponsored Genetic Testing Program

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ABSTRACT

X-linked hypophosphatemia (XLH), a dominant disorder caused by pathogenic variants in the PHEX gene, affects both sexes of all ages and results in elevated serum fibroblast growth factor 23 (FGF23) and below-normal serum phosphate. In XLH, rickets, osteomalacia, short stature, and lower limb deformity may be present with muscle pain and/or weakness/fatigue, bone pain, joint pain/stiffness, hearing difficulty, enthesopathy, osteoarthritis, and dental abscesses. Invitae and Ultragenyx collaborated to provide a no-charge sponsored testing program using a 13-gene next-generation sequencing panel to confirm clinical XLH or aid diagnosis of suspected XLH/other genetic hypophosphatemia. Individuals aged ≥6 months with clinical XLH or suspected genetic hypophosphatemia were eligible. Of 831 unrelated individuals tested between February 2019 and June 2020 in this cross-sectional study, 519 (62.5%) individuals had a pathogenic or likely pathogenic variant in PHEX (PHEX-positive). Among the 312 PHEX-negative individuals, 38 received molecular diagnoses in other genes, including ALPL, CYP27B1, ENPP1, and FGF23; the remaining 274 did not have a molecular diagnosis. Among 319 patients with a provider-reported clinical diagnosis of XLH, 88.7% (n = 283) had a reportable PHEX variant; 81.5% (n = 260) were PHEX-positive. The most common variant among PHEX-positive individuals was an allele with both the gain of exons 13–15 and c.*231A>G (3′UTR variant) (n = 66/519). Importantly, over 80% of copy number variants would have been missed by traditional microarray analysis. A positive molecular diagnosis in 41 probands (4.9%; 29 PHEX positive, 12 non-PHEX positive) resulted in at least one family member receiving family testing. Additional clinical or family member information resulted in variant(s) of uncertain significance (VUS) reclassification to pathogenic/likely pathogenic (P/LP) in 48 individuals, highlighting the importance of segregation and clinical data. In one of the largest XLH genetic studies to date, 65 novel PHEX variants were identified and a high XLH diagnostic yield demonstrated broad insight into the genetic basis of XLH. © 2021 The Authors. Journal of Bone and Mineral Research published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: GENETIC RESEARCH - HUMAN ASSOCIATION STUDIES; DISORDERS OF CALCIUM/PHOSPHATE METABOLISM- OTHER; DISEASES AND DISORDERS OF/RELATED TO BONE - OTHER; THERAPEUTICS – OTHER; CELL/TISSUE SIGNALING - PARACRINE PATHWAYS – OTHER
Introduction

X-linked hypophosphatemia (XLH) is a rare X-linked dominant genetic disorder of renal phosphate wasting that affects male and female individuals of all ages,\(^\text{1}\) with an estimated prevalence of 1 in 20,000 to 25,000 individuals.\(^\text{2-5}\) XLH causes varying clinical features, including rickets, osteomalacia, short stature, lower limb deformity, muscle pain, weakness, fatigue, bone pain, joint pain or stiffness, hearing difficulty, enthesopathy, osteoarthritis, and dental abscesses.\(^\text{6}\) The disorder is caused by pathogenic variants in the \(PHEX\) gene,\(^\text{7}\) which lead to elevated (or inappropriately normal) serum fibroblast growth factor 23 (FGF23) levels and ultimately below-normal serum phosphate levels.\(^\text{8}\)

XLH is the most common form of hypophosphatemic rickets,\(^\text{1}\) distinguishable from other forms by its genetic cause, pattern of inheritance, and disease management.\(^\text{8,9}\) However, it can be difficult to differentiate between the various forms of familial hypophosphatemia because they share similar signs, symptoms, and biomarkers.\(^\text{9}\) For example, pathogenic variants in \(FGF23\) are associated with autosomal dominant hypophosphatemic rickets (ADHR) and result in a clinical manifestation that is strikingly similar to XLH, though symptoms associated with ADHR later in life may help differentiate between the two.\(^\text{10}\) Further, other genetic disorders such as hypophosphatasia (HPP) show phenotypic overlap with familial hypophosphatemia but differ in serum phosphate levels and recommendations for clinical management.\(^\text{11}\) It is therefore important to confirm the underlying molecular etiology of hypophosphatemia before initiating therapy.

In February 2019, Ultragenyx Pharmaceutical and Invitae initiated the sponsored Hypophosphatemia genetic testing program, providing no-charge genetic testing to patients to confirm a clinical XLH diagnosis or to aid diagnosis of suspected XLH or other genetic hypophosphatemia. This study was designed to describe outcomes from the Hypophosphatemia sponsored program, including diagnostic yield, genetic landscape of detected \(PHEX\) variants, and clinical characteristics of all individuals in the cohort. Although the results of genetic testing typically focus on the proband, this program also supported cascade testing in identified at-risk relatives, which resulted in additional diagnoses and greatly assisted in variant reclassification.

PATIENTS AND METHODS

Study population

In this cross-sectional study, individuals referred for genetic testing through the sponsored Hypophosphatemia program following clinical assessment between February 27, 2019, and June 30, 2020, were included. Individuals were eligible if they were 6 months or older and (i) had a clinician-completed start form for a new treatment for XLH (burosumab; a human monoclonal antibody directed against FGF23), which served as an indication of a clinical diagnosis of XLH; (ii) had a previous clinical diagnosis related to hypophosphatemia; or (iii) was suspected to have genetic hypophosphatemia based on the presence of two or more clinical signs or symptoms. When hypophosphatemia was suspected, ordering providers were asked to report the following signs and symptoms on the requisition form: family history of confirmed XLH; muscle pain, weakness, and/or fatigue; lower limb deformities; fractures/pseudo-fractures; tooth abscesses and/or excessive dental caries; bone pain, joint pain, and/or joint stiffness; short stature; and gait abnormalities.

Genetic testing and variant interpretation

Individuals provided blood or saliva samples and were tested using next-generation sequencing (NGS) with a multi-gene panel consisting of 13 genes that were selected based on associations with hypophosphatemia as reported in the medical literature: \(ALPL\),\(^\text{12,18}\) \(CLCN5\),\(^\text{19-22}\) \(CYP2R1\),\(^\text{23-26}\) \(CYP27B1\),\(^\text{27-30}\) \(DPMP1\),\(^\text{31-38}\) \(ENPP1\),\(^\text{39-42}\) \(FAH\),\(^\text{43-45}\) \(FAM20C\),\(^\text{46-52}\) \(FGFR1\),\(^\text{60-63}\) \(PHEX\),\(^\text{64-68}\) \(SLC34A3\),\(^\text{69-71}\) and \(VDR\).\(^\text{72,73}\) Each gene in the NGS panel was targeted with oligonucleotide baits (Agilent Technologies, Santa Clara, CA, USA; Roche, Pleasanton, CA, USA; IDT, Coralville, IA, USA) that were designed to capture exons, the 10 bases flanking exonic sequences, and certain non-coding regions of interest (\(PHEX\) c.*231A>G). Genes were sequenced at a high-depth coverage (20× minimum, 150× average). A bioinformatics pipeline that incorporates community standard and custom algorithms was used to analyze NGS reads and identify single nucleotide variants (SNVs), small and large insertions/deletions (indels), structural variants, and exon-level copy number variants (CNVs).\(^\text{74,75}\) In particular, CNVs were detected using split-read analysis and copy number counting method that uses a statistical mixture model for sequence read counts within target regions (typically exons), and employs model-based segmentation algorithms optimized for use with sparsely distributed targets across the genome.\(^\text{76}\) This method estimates the most likely copy number for all segments, and critically for clinical use, each called segment is assigned a robust quality score indicating confidence in the copy number determination.

All identified variants were interpreted according to the joint consensus from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology using Invitae’s proprietary point-based scoring system, Sherloc.\(^\text{77,78}\) The following transcripts were used to interpret variants: \(ALPL\) \(NM\_000478.5\), \(CLCN5\) \(NM\_000084.4\), \(CYP2R1\) \(NM\_024514.4\), \(CYP27B1\) \(NM\_000785.3\), \(DPMP1\) \(NM\_004407.3\), \(ENPP1\) \(NM\_006208.2\), \(FAH\) \(NM\_001337.2\), \(FAM20C\) \(NM\_020223.3\), \(FGFR1\) \(NM\_020638.2\), \(FGFR1\) \(NM\_023110.2\), \(PHEX\) \(NM\_080877.2\), and \(VDR\) \(NM\_0017535.1\). Following interpretation, variants were classified as pathogenic (P), likely pathogenic (LP), variant(s) of uncertain significance (VUS), likely benign (LB), or benign (B). Variants classified as P, LP, and VUS were reported to the ordering clinician. P/LP variants were considered a positive molecular diagnosis. In addition, a variant was categorized as novel if it met the following three criteria: (i) had never been reported in ClinVar by a clinical testing laboratory (excluding the testing laboratory for this study, Invitae); (ii) had never been reported in published literature, and (iii) had never been observed by Invitae in individuals tested outside of the sponsored testing program.

Cascade testing

Individuals who were the first in their family to be tested through the sponsored testing program were considered probands. At clinician discretion, cascade testing could be offered to at-risk relatives of a proband with a positive result. These relatives were also eligible to be tested through the Hypophosphatemia sponsored program, and their data were analyzed separately in this study. The laboratory also recommended family testing when VUS in \(PHEX\) were identified and additional clinical information

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or segregation analysis could aid in reclassification. The outcomes of VUS reclassification were reported to ordering clinicians.

Data analysis

In all analyses, data were summarized using descriptive statistics. Demographic characteristics included age, sex, and ancestry. In reporting on the clinical signs and symptoms, calculations were based on the number of individuals for whom the sign or symptom was reported by the ordering clinician. A clinical diagnosis of hypophosphatemia was considered if either the treatment start form had been completed or the ordering physician had indicated that a clinical diagnosis had been made.

The diagnostic yield was calculated for those with a molecular diagnosis in PHEX (ie, “PHEX positive;” one P/LP variant) and those with a molecular diagnosis in a gene other than PHEX (ie, “non-PHEX positive;” monoallelic for autosomal dominant or X-linked diseases and biallelic for autosomal recessive diseases). Of note, HPP (caused by pathogenic variants in ALPL) could be autosomal recessive or autosomal dominant. Demographics were compared based on genetic testing results: PHEX positive, non-PHEX positive, and no molecular diagnosis (ie, no P/LP variant for autosomal dominant or X-linked diseases; zero or one P/LP variant for an autosomal recessive disease).

The number of individuals with each clinician-reported sign or symptom was calculated and stratified by molecular diagnosis. The contribution of a history of XLH or signs and symptoms to a positive molecular diagnosis was also explored. For this analysis, probands were categorized into three groups: (i) those who had a family member with a confirmed XLH diagnosis or had a previous hypophosphatemia-related diagnosis of their own, and who had reported signs and symptoms; (ii) those who had a personal or family history but no reported signs or symptoms; and (iii) those who did not have a personal or family history but had reported signs or symptoms.

Diagnostic yield and demographic information were evaluated separately among family members seeking cascade testing following a proband’s positive test result in PHEX or another gene on the panel. In addition, the number of families who underwent testing and the mean number of family members tested per proband were calculated. Among family members who underwent testing through the sponsored program (for whom clinical symptoms were reported on the sponsored program’s requisition form), reported clinical features were summarized when available.

Statistical analysis

A one-way ANOVA assessed differences in age based on genetic test result (ie, PHEX-positive, non-PHEX-positive, negative). Based on the ANOVA results, a Tukey post hoc test evaluated differences in age among the three groups. Values of p ≤ 0.05 were considered statistically significant.

Results

Genetic testing results among probands

During the study period, 831 unrelated individuals (probands) underwent testing through the sponsored Hypophosphatemia program. Age at time of testing among probands ranged from 6 months to 79 years (mean ± standard deviation [SD], 26.9 ± 20.1 years) (Table 1). The majority of individuals were female (65.9%) and of self-reported White ancestry (57.0%). In total, 237 unique P/LP PHEX variants were reported in 519 individuals (62.5%), three of whom also carried a VUS in PHEX. In addition, 49 PHEX VUS were detected in 50 individuals (6.0%) (Fig. 1, Supplemental Table S1). In seven PHEX-positive individuals (1.3%), an additional P/LP variant was detected in another gene, including ALPL (n = 1), CYP27B1 (n = 3), FAH (n = 1), and SLC34A3 (n = 2). The additional P/LP findings were associated with a molecular diagnosis in one individual (ALPL) and carrier status for an autosomal recessive disorder in the other six individuals, two of whose P/LP variants were in SLC34A3, which has been previously reported to be associated with hypercalcemia in some carriers.69,79,80

Of the 312 cases (37.5%) in which no PHEX molecular diagnosis was found, 38 individuals had molecular diagnoses in four genes associated with other disorders (Fig. 1). In this group, the most common gene with a positive molecular diagnosis was ALPL (n = 31), which is associated with HPP; 27 probands had a single P/LP variant associated with autosomal dominant HPP and the remaining four carried two P/LP variants associated with autosomal recessive HPP. Among the remaining seven individuals with non-PHEX-positive molecular diagnoses, four had one P/LP variant in FGF23 (autosomal dominant hypophosphatemic rickets), two were biallelic for CYP27B1 (autosomal recessive vitamin D-dependent rickets), and one was biallelic for ENPP1 (autosomal recessive hypophosphatemic rickets type 2) (Fig. 1). In addition, nine individuals were identified as monoallelic for an autosomal recessive disorder. Of note, five individuals were monoallelic for SLC34A3, which is known to result in mild symptoms such as hypercalcia, renal stones, and nephrocalcinosis in approximately one-quarter of heterozygotes.79 In total, 39 unique P/LP variants and 88 unique VUS were identified in non-PHEX genes (Supplemental Table S2).

In comparing demographic characteristics of individuals based on molecular diagnosis (Table 1), age differed significantly between the groups (one-way ANOVA, F[2,830] = 10.43, p < 0.0001). A Tukey post hoc test revealed that mean age at time of testing was significantly lower in PHEX-positive individuals than in those with a non-PHEX positive result (p < 0.05). However, there was no difference in mean age at testing between those with no molecular diagnosis and those with a PHEX-positive or non-PHEX-positive diagnosis. Across all diagnostic groups, the majority of individuals were female (PHEX-positive 66.5%, non-PHEX-positive 71.1%, negative 64.2%) and of self-reported White ancestry, though the proportion of White individuals was highest among those with a non-PHEX molecular diagnosis (PHEX-positive 54.7%; non-PHEX-positive 81.6%; negative 58.0%).

Clinical signs and symptoms

Several similarities and differences exist in which clinical signs and symptoms were reported based on genetic test results (PHEX-positive, non-PHEX-positive, negative) (Table 1). Reduced tubular maximum reabsorption of phosphate/glomerular filtration rate (TmP/GFR) (4.6%, 2.6%, 6.6%, respectively) and gait abnormalities (25.0%, 26.3%, 20.4%, respectively) were reported at similar rates across all groups. Hypophosphatemia-related clinical diagnoses (87.3%, 39.5%, 53.6%, respectively) were most commonly reported in PHEX-positive individuals, whereas tooth abscesses and/or excessive dental caries (14.8%, 36.8%, 17.5%, respectively) and fractures and/or pseudofractures (11.9%,
44.7%, 25.5%, respectively) were more commonly reported in non-PHEX-positive individuals and short stature (36.2%, 23.7%, 66.4%, respectively) was most common in individuals without a diagnosis. Individuals with a PHEX VUS had similar clinical features reported to those who were PHEX-positive, with the exception of fewer reports of family members with a confirmed XLH diagnosis or a hypophosphatemia-related clinical diagnosis (Supplemental Table S3).

A hypophosphatemia-related clinical diagnosis was reported in nearly three-quarters of all probands (n = 615/831, 74.0%) and was strongly associated with a positive molecular diagnosis (Fig. 2A). Among the 319 individuals with a confirmed XLH diagnosis or a hypophosphatemia-related clinical diagnosis, a positive molecular diagnosis was reported in nearly three-quarters of all probands (n = 615/831, 74.0%) and was strongly associated with a positive molecular diagnosis (Fig. 2A). Among the 319 individuals with a confirmed XLH diagnosis or a hypophosphatemia-related clinical diagnosis, a positive molecular diagnosis was reported in nearly three-quarters of all probands (n = 615/831, 74.0%) and was strongly associated with a positive molecular diagnosis (Fig. 2A). Among the 319 individuals with a confirmed XLH diagnosis or a hypophosphatemia-related clinical diagnosis, a positive molecular diagnosis was reported in nearly three-quarters of all probands (n = 615/831, 74.0%) and was strongly associated with a positive molecular diagnosis (Fig. 2A).
diagnosis (had a clinician-completed treatment start form for burosomab), 283 (88.7%) had a reportable P/LP variant or VUS in PHEX and 81.5% (260/319) had a molecular diagnosis. In total, 501 probands had at least one sign or symptom reported (range, one to eight), with short stature (34.8%); bone, joint pain, and/or joint stiffness (33.9%); lower limb deformities (32.5%); and muscle pain, weakness, and/or fatigue (29.2%) being the most common (Fig. 2A). However, the signs and symptoms most commonly associated with a positive PHEX molecular diagnosis were a family member with a confirmed XLH diagnosis (73.7%), lower limb deformities (70.4%), and reduced serum phosphate (67.9%) (Fig. 2A). None of the reported symptoms were strongly associated with a positive finding in a non-PHEX gene, with the most common features including fractures or pseudofractures (11.4%) and tooth abscesses and/or excessive dental caries (10.1%). A past hypophosphatemia-related diagnosis or a family member with a confirmed XLH diagnosis was the strongest indicator of a PHEX-positive molecular diagnosis, regardless of whether symptoms were reported (Fig. 2B). Family history was not reported for the majority of individuals without a molecular diagnosis and only reported signs or symptoms associated with hypophosphatemia (Fig. 2B).

Distribution and characterization of PHEX variants
Of the 237 unique P/LP PHEX variants, SNVs represented the most common variant type (n = 89, 37.6%) followed by small deletions, duplications, and insertions (n = 59, 24.9%) (Fig. 3A). Among all detected non-unique PHEX variants (n = 585), SNVs (46.3%) and CNVs (24.3%) were the most commonly detected variant types (Fig. 3B). Among CNVs (n = 142), a minimum of 81.0% would have been missed had microarray assays been

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**Fig 2.** Diversity of reported clinical features among unrelated individuals. (A) The number of individuals with each clinician-reported sign or symptom was calculated and stratified by molecular diagnosis, with proportions based on the number of individuals with each feature noted. (B) For each clinician-reported sign or symptom, the proportions of PHEX-positive and non-PHEX-positive individuals were calculated. Proportions were calculated based on the number of individuals with the clinical sign or symptom noted (“+”) or not noted (“−”), as indicated in parentheses along the X-axis.
used, due to their small size. The most common \textit{PHEX} variants identified are reported in Table 2 (see Supplementary Table S1 for a list of all P/LP variants and VUS).

The most commonly observed variants were the gain of exons 13–15 and the 3′ UTR c.\textsuperscript{*}231A>G. As previously reported,\(^{(81)}\) these two variants were observed together in the majority of probands (\(n = 65/66\)) and the majority of family members (some of whom were tested through the program and some of whom were tested at Invitae but outside of the program) (\(n = 23/24\)), suggesting that they constitute a single allele that co-segregates. In one case, an affected proband had the gain of exons 13–15 without the c.\textsuperscript{*}231A>G variant and a family member had the c.\textsuperscript{*}231A>G variant alone; however, CNV analysis had reduced sensitivity in this case due to triple X syndrome in the family member, so the presence of the duplication event in this patient is unknown. Of the 88 individuals who had both variants, 51 were confirmed to have both on the same chromosome. Phase information for the other 37 individuals is unknown. To explore the

![Fig 3. Distribution of \textit{PHEX} P/LP variants observed in probands. (A) Distribution of variant types among the 237 unique P/LP variants. (B) Recurrence of \textit{PHEX} P/LP variants by type observed across all individuals with a positive \textit{PHEX} molecular diagnosis (\(n = 585\)). Note that individuals (\(n = 65\)) with the exon 13–15 duplication and c.\textsuperscript{*}231G>A 3′ UTR variants in cis were counted in both the CNV and SNV categories. CNVs were defined as >100 base pairs. Small deletions, duplications, and insertions were defined as events involving <100 base pairs. CNV = copy number variant; P/LP = pathogenic/likely pathogenic; SNV = single nucleotide variant.](image)

| \textit{PHEX} variant | Effect | Interpretation | Number of patients |
|-----------------------|--------|----------------|-------------------|
| c.1405-?_1645+?dup    | Gain (Exons 13–15) | Likely pathogenic | 66* |
| c.\textsuperscript{231}A>G | Noncoding | Likely pathogenic | 65 |
| c.1601C>T             | p.Pro534Leu | Pathogenic | 19 |
| c.2104C>T             | p.Arg702* | Pathogenic | 16 |
| c.1735G>A             | p.Gly579Arg | Pathogenic | 15 |
| c.1645+1G>A           | Splice donor | Pathogenic | 14 |
| c.871C>T              | p.Arg291* | Pathogenic | 13 |
| c.2239C>T             | p.Arg747* | Pathogenic | 9 |
| c.1080-?_1302+?del    | Deletion (Exons 10–11) | Pathogenic | 8 |
| c.1699C>T             | p.Arg567* | Pathogenic | 8 |
| c.-562-?_118+?del     | Deletion (Exon 1) | Pathogenic | 6 |
| c.1645C>T             | p.Arg549* | Pathogenic | 6 |
| c.2071-?_3357+?del    | Deletion (Exons 21–22) | Pathogenic | 6 |
| c.304G>A              | p.Gly102Arg | Pathogenic | 6 |
| c.562-?_187+?del      | Deletion (Exons 1–2) | Pathogenic | 5 |
| c.1405-?_3357+?del    | Deletion (Exons 13–22) | Pathogenic | 4 |
| c.1483-1G>C           | Splice acceptor | Pathogenic | 4 |
| c.1646-?_1700+?del    | Deletion (Exon 16) | Pathogenic | 4 |
| c.1700+1G>A           | Splice donor | Pathogenic | 4 |
| c.1848del             | p.Lys616Asnfs*3 | Pathogenic | 4 |
| c.2028_2032dup        | p.Phe678Serfs*11 | Pathogenic | 4 |
| c.2237G>A             | p.Cys746Tyr | Pathogenic | 4 |

\(\text{LP} = \text{likely pathogenic}; \text{P} = \text{pathogenic.}\)

\(^*\text{65 of the 66 individuals with the exons 13–15 gain also had the c.\textsuperscript{*}231A>G non-coding change in the 3′ UTR.}\)
spectrum of phenotypes associated with these two variants, the frequency of each symptom was calculated among probands with both variants. Among the 65 probands with both a gain of exons 13–15 and the c.*231A>G variant, a previous hypophosphatemia-related clinical diagnosis (86.2%) was the most commonly reported clinical feature, followed by lower limb deformities (43.1%) and bone, joint pain, and/or joint stiffness (38.5%) (Table 3). These variants were observed together in individuals of each of White, Hispanic, and Black ancestries.

Among the 237 unique P/LP variants, 65 (27.4%) were novel by our reported criteria and are reported for the first time here (Supplementary Table S1). These variants were observed in 87 probands. The most common novel variants were the deletion of exon 1 (n = 6), p.Phe678Serfs*11 (c.2028_2032dup, n = 4), the deletion of exons 15–20 (n = 3), the deletion of exons 16–20 (n = 3), and p.Met300* (c.898del, n = 3). The remaining novel PHEX P/LP variants were observed in one or two individuals. The majority of these individuals (87.4%) had a previous hypophosphatemia-related diagnosis. The most common reported clinical features were short stature (33.3%), reduced serum phosphate (31.0%), lower limb deformities (24.1%), and gait abnormalities (21.8%). Five additional novel PHEX variants were classified as VUS.

Cascade testing among family members

A positive molecular diagnosis in 41 probands (4.9%; 29 PHEX positive, 12 non-PHEX positive) resulted in at least one family member receiving family testing. In total, 103 family members received genetic testing (mean, 2.5 family members per proband; range, 1 to 26).

Cascade testing for PHEX was pursued in 70 relatives from 29 families (mean, 2.4 family members per proband; range, 1 to 26). Among the 70 relatives who underwent cascade testing for PHEX (mean age, 32.4 ± 19.4 years), 40 (57.1%) received a positive molecular diagnosis in PHEX. One proband, who was found to have a gain of exons 13–15 and the c.*231A>G variant in PHEX, had 26 relatives subsequently tested. Among these relatives, symptoms varied according to clinical information available for most of them (n = 24). The two most common reported symptoms in this family were tooth abscesses and/or excessive dental caries (n = 14, 58.3%) and bone pain, joint pain, and/or joint stiffness (n = 12, 50.0%). Additional symptoms were reported in other family members, representing a spectrum of phenotypes.

Cascade testing for non-PHEX genes was pursued for 33 individuals from 12 families (mean, 2.8 family members per proband; range, 1 to 6). All but one proband had received a molecular diagnosis in ALPL; the remaining proband had received a molecular diagnosis in FGF23. Among the 33 relatives who underwent cascade testing for non-PHEX genes (mean age, 28.7 ± 20.5 years), 19 (57.6%) received a positive molecular diagnosis.

VUS resolution

Family testing or clinical information (eg, family or personal history, laboratory data) was requested to help with reclassification of 26 VUS. Thirteen of these VUS were subsequently reclassified as P or LP due to this additional information, impacting 48 individuals (Table 4). In addition, three VUS were reclassified to P during the study period due either to the variant being observed in individuals tested outside of the program or to additional literature, impacting nine individuals tested through the program.

Discussion

In this study, we report the results from a sponsored genetic testing program in individuals who are known or suspected to have a genetic hypophosphatemia. In just over 1 year, the sponsored Hypophosphatemia program provided access to genetic testing for 831 probands—the largest cohort to date of individuals with a molecular diagnosis in PHEX. Although XLH has been estimated to affect 1 in 20,000 to 25,000 individuals, based on data presented here from 831 probands and their family members, this prevalence may be underestimated.

The ability to test a large number of individuals also allows a fuller view of the genetic landscape of this condition. An analysis of the distribution of P/LP PHEX variants observed in this study identified 237 unique variants, 65 of which were novel. Of all P/LP PHEX variants observed (n = 585, including non-unique variants), a large proportion were CNV events (23.6%, n = 138). Due to the nature of the NGS assay, the full size of deletion and duplication events that include either the first or the last exon of PHEX cannot be determined, as these events likely extend beyond the reportable range of the gene. However, 81% of the PHEX P/LP CNV events detected were subgenic (ie, smaller than a full gene deletion with both PHEX intronic boundaries determined) and would be missed by traditional microarray analysis due to their small size. This finding highlights the importance of CNV testing with single-exon resolution (eg, NGS, multiplex ligation-dependent probe amplification) for patients with a suspected diagnosis of XLH. Though NGS panels can detect a wide range of changes at the DNA sequence level, other assays can detect a different set of abnormalities such as cytogenetic changes (balanced translocations, large inversions, etc.) or mitochondrial genome variants. These assays include array comparative genomic hybridization (CGH), traditional G-banded karyotyping, RNA analysis, long-range PCR, or mitochondrial DNA sequencing. Limited studies have demonstrated the use of such testing methods for hereditary hypophosphatemia. Selecting the appropriate genetic test will depend on the patient’s clinical presentation, prior genetic testing results, and family history.

Table 3. Symptoms and Other Clinical Information Reported for Probands with Gain of Exons 13–15 and 3’UTR c.*231A>G Variants

| Clinical information                                      | Number (%) (n = 65) |
|----------------------------------------------------------|---------------------|
| Previous diagnosis related to                            | 56 (86.2)           |
| Hypophosphatemia                                         |                     |
| Lower limb deformities                                   | 28 (43.1)           |
| Short stature                                            | 23 (35.4)           |
| Family member of a confirmed XLH patient                | 22 (33.9)           |
| Gait abnormalities                                       | 16 (24.6)           |
| Bone, joint pain, and/or joint stiffness                 | 25 (38.5)           |
| Muscle pain, weakness, and/or fatigue                    | 18 (27.7)           |
| Tooth abscesses and/or excessive dental caries           | 13 (20.0)           |
| Fractures/pseudofractures                                | 5 (7.7)             |
| Reduced serum phosphate                                 | 27 (41.5)           |
| Reduced TmP/GFR (<LLN)                                  | 2 (3.1)             |

LLN = lower limit of normal; TmP/GFR = ratio of tubular maximum reabsorption rate of phosphate to glomerular filtration rate; XLH = X-linked hypophosphatemia.
The most commonly observed event among individuals with a positive \textit{PHEX} molecular diagnosis was the combined presence of a gain of exons 13–15 and the 3'UTR \texttt{c.231A>G} variant, seen in 65 probands and 23 family members tested either through the program at Invitae or outside of the program. Previous literature reports that identified the \texttt{c.231A>G} variant as causative for XLH in six unrelated probands did not include copy number detection of \textit{PHEX}.\cite{85,86} Although the exact position of the gain of exons 13–15 in our cohort cannot be determined with the NGS assay used in this study, a large-scale analysis has found that the majority of subgenic gains are in tandem.\cite{87} If this particular gain is in tandem, it would be predicted to create a frameshift because the duplicated exons are out of frame, suggesting that the causative variant may be the gain of exons 13–15 in these patients and not the \texttt{c.231A>G} variant. Supporting this hypothesis, we did find one proband who had the gain without the \texttt{c.231A>G} variant, suggesting that the duplication can contribute to disease on its own. However, further functional studies...

\textbf{Table 4. Summary of VUS Resolution Efforts}

| Variant | Classification change | Additional clinical information provided for reclassification | Reason for reclassification | Individuals impacted (n) |
|---------|-----------------------|-------------------------------------------------------------|----------------------------|--------------------------|
| c.1109T>G (p.Met370Arg) | VUS > LP | Yes | Clinical information | 5 |
| c.1173+5G>A (Intronic) | VUS > LP | Yes | Segregation | 2 |
| c.1237G>C (p.Ala413Pro) | VUS > LP | Yes | Clinical information and segregation | 5 |
| c.1382C>G (p.Thr461Arg) | VUS > LP | Yes | Clinical information and segregation | 4 |
| c.1403A>C (p.Lys468Thr) | VUS > LP | Yes | Segregation (de novo) | 1 |
| c.1482+3A>C (Intronic) | VUS > P | Yes | Clinical information | 4 |
| c.1768+1G>A (Intronic) | VUS > P | No | Additional literature support | 3 |
| c.1936G>C (p.Asp646His) | VUS > LP | No | Found a patient with different variant at this codon | 1 |
| c.230G>A (p.Cys77Tyr) | VUS > LP | Yes | Clinical information | 2 |
| c.425G>A (p.Cys142Phe) | VUS > P | Yes | Segregation (de novo) | 1 |
| c.436+4A>G (Intronic) | VUS > P | No | Additional patients | 5 |
| c.499T>C (p.Trp167Arg) | VUS > LP | Yes | Segregation | 5 |
| c.503G>T (p.Pro168Leu) | VUS > LP | Yes | Clinical information | 2 |
| c.663G>A (Silent) | VUS > LP | Yes | Clinical information and segregation | 4 |
| Deletion (Exon 7) | VUS > LP | Yes | Clinical information | 1 |
| Gain (Exons 13–20) | VUS > LP | Yes | Segregation | 3 |
| c.1520T>C (p.Leu507Pro) | VUS (no change) | Yes | None available | NA |
| c.1702T>C (p.Ser568Pro) | VUS (no change) | Yes | None available | NA |
| c.1757T>C (p.Phe586Ser) | VUS (no change) | Yes | None available | NA |
| c.1850G>T (p.Cys617Phe) | VUS (no change) | Yes | None available | NA |
| c.1973_1984dup (p.Arg658_Ile661dup) | VUS (no change) | Yes | None available | NA |
| c.2065G>C (p.Arg669Pro) | VUS (no change) | Yes | None available | NA |
| c.2147G>C (p.Arg716Thr) | VUS (no change) | Yes | None available | NA |
| c.2198G>T (p.Cys733Phe) | VUS (no change) | Yes | None available | NA |
| c.416A>G (p.Tyr139Cys) | VUS (no change) | Yes | None available | NA |
| c.482G>C (p.Arg161Pro) | VUS (no change) | Yes | None available | NA |
| c.934_16_934-12delinsCTAC_CTAACCTAGGAT (Intronic) | VUS (no change) | Yes | None available | NA |

\texttt{LP} = likely pathogenic; \texttt{P} = pathogenic; \texttt{VUS} = variant of uncertain significance.
to understand how one or both variants confer pathogenicity are needed. Our cohort, which to date represents the largest number of individuals with both variants \(n = 88\), including 51 individuals confirmed to harbor them in cis, supports the previous observation among eight patients that these two variants segregate in affected individuals.\(^{81}\)

Recently, the c.*231A>G variant was reported to be associated with mild disease severity in a small cohort.\(^{88}\) However, within our cohort, a wide spectrum of clinical features was reported for patients with both the gain of exons 13–15 and the c.*231A>G variant. Supporting our result, another large family with these variants, tested through the program, was recently reported to have symptoms with a wide spectrum of severity, including hearing loss.\(^{89}\) These findings, together with the eligibility criteria for the sponsored testing program, do not suggest that these variants confer only mild disease.

For a subset of individuals included in this study, reported clinical features were used to characterize which features were associated with XLH and other disorders. The majority of individuals had a previous clinical diagnosis related to hypophosphatemia, with three-quarters having a confirmed molecular diagnosis through the sponsored program. Roughly 90% of patients who had a clinical diagnosis of XLH specifically, had a reportable variant (P/LP/VUS) identified in PHEX. Given the high probability of identifying a variant for patients with a confirmed XLH clinical diagnosis, at least some of the 10% of patients that did not have a detectable PHEX variant may harbor noncoding variants not detectable by current methods. We detected a small portion of individuals with molecular diagnosis for a gene other than PHEX, suggesting that another portion of the 10% of individuals without a PHEX variant may have a positive finding in another gene not included in the genetic test. A recent study demonstrated that RNA analysis is able to identify P/LP variants in deep intronic regions of the assay, such as a deep intronic pathogenic variant.\(^{89}\) These findings, together with the eligibility criteria for the sponsored testing program, do not suggest that these variants confer only mild disease.

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to clinical management that prevents or slows disease progression. In this cohort, 5.6% of probands with a molecular diagnosis in PHEX had family members pursue subsequent testing. Although such positive molecular diagnoses may have clear clinical utility for family members, additional awareness and education for clinicians should be pursued so that cascade testing is more consistently recommended when a positive molecular diagnosis is detected.

Through this sponsored testing program, 50 VUS in PHEX were detected in 53 individuals. In 26 cases, Invitae sought additional clinical information from the proband or family testing for segregation analysis in an effort to reclassify VUS as P/LP or (likely) benign. Sufficient information was available to reclassify 13 VUS as P/LP, ultimately impacting 39 individuals who had originally received a VUS result. There are many approaches to reclassifying VUS, each providing different types of evidence to aid in reinterpreting the variant. In this study, seeking additional clinical information or requesting family testing for segregation analysis were leveraged for VUS resolution. The outcomes reported here show how effective these two methods are and highlight the importance of providing detailed clinical information to the testing laboratory for variant interpretation. Analyzing messenger RNA isoforms is another method, developed to aid in VUS reclassification when a variant is suspected to disrupt a splice site. RNA analysis of an individual’s sample may inform whether the variant alters gene expression, though it would be required for the gene of interest to be expressed in the sample provided to the testing laboratory. Functional studies in model systems and cell lines that investigate protein levels, interactions, reactivity, or other functions in relation to the VUS may also provide evidence for VUS reclassification, though these studies have their own limitations. As a VUS does not have enough evidence to suggest that the variant is pathogenic (or benign), this result may leave clinicians and patients in a state of uncertainty, possibly excluding patients from medical treatments that require a positive molecular diagnosis. Thus, it is critical for genetic testing laboratories to continually revisit VUS and utilize all available information for reclassification.

An important benefit to a sponsored testing program is increased access to testing and care. One critical element of access is the recent decreases in costs associated with genetic testing and the advances in technology that allow for more detailed investigation of the genome in a single assay. Genetic testing has been recommended as a means to confirm a clinical diagnosis of XLH and enabling screening of at-risk relatives, though it is not required for a diagnosis. In spite of this, a positive molecular genetic test result is often an eligibility requirement for enrolling in clinical trials or receiving targeted therapies. Collaborative efforts among clinicians, pharmaceutical companies, and genetic testing laboratories can aid in increasing access to genetic testing, trials, or treatments that may improve clinical outcomes and in robustly characterizing the genetic spectrum of disease. As more collaborations occur to provide genetic testing results, the full benefits, and possible disadvantages, will become increasingly apparent.

In considering the results from this study, analysis of the study design reveals that interpretation of clinical features associated with XLH may be limited. As part of the eligibility criteria, clinicians were required to report if an individual had a confirmed or suspected XLH diagnosis. Further, clinical signs and symptoms were reported only for individuals with suspected diagnoses (ie, those displaying at least two signs or symptoms). Thus, in addition to a limited number of individuals with reported clinical information, there may have been variability in reporting from clinician to clinician. In addition, multigene panels query a specific set of genes and do not allow for identifying causative variants in other genes, including novel genes not currently linked to genetic hypophosphatemia. However, the panel utilized in this cohort was designed to capture the most common genes associated with hypophosphatemia and since the end of this study period and data cut, four additional genes (CTNS, GNAS, OCRL, and SLC34A1) have been added to the panel.

Taken together, the results from this study show the benefits of sponsored testing programs for identifying and/or confirming diagnoses for rare diseases, as evidenced by the high diagnostic yield and novel insights into the genetic landscape contributing to XLH. Additionally, we were able to leverage the results of the program to identify novel variants and understand more about phenotypic variability and provider ordering preferences. As more individuals undergo testing, future analysis of the sponsored Hypophosphatemia program will continue to be an invaluable resource in understanding PHEX-related hypophosphatemia.

**Disclosures**

Eric T. Rush and Norma E. Guerra have received consulting and speaking fees from Ultragenyx Pharmaceutical Inc. Kathryn Dahir has received consulting and speaking fees from Ultragrenyx Pharmaceutical Inc and is an X-Linked Hypophosphatemia Disease Monitoring Program (XLH-DMP) investigator. Britt Johnson, Swaroop Aradhya, Daniel Beltran, Sara L. Bristow, Ana Morales, and Rebecca Truty are shareholders and employees of Invitae. Scott Eisenbeis, Stan Krolczyk, Nicole Miller, Prameela Ramesan, and Soodabeh Sarafrazi are employees of Ultragenyx Pharmaceutical Inc. SE, SK, NM, and PR, are shareholders of Ultragenyx Pharmaceuticals, Inc.

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**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request. All variants reported in this article have been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/).
References

1. Pavone V, Testa G, Giotta Iachino S, Evola FR, Avondo S, Sessa G. Hypophosphatemic rickets: etiology, clinical features and treatment. Eur J Orthop Surg Traumatol. 2015;25(2):221-226.

2. Beck-Nielsen SS, Brock-Jacobsen B, Gram J, Brixen K, Jensen TK. Incidence and prevalence of nutritional and hereditary rickets in southern Denmark. Eur J Endocrinol. 2009;160(3):491-497.

3. Endo I, Fukumoto S, Ozono K, et al. Nationwide survey of fibroblast growth factor 23 (FGF23)-related hypophosphatetic diseases in Japan: prevalence, biochemical data and treatment. Endocr J. 2015;62(9):811-816.

4. Raafayez S, Johansson S, Raeder H, Bjerknes R. Hereditary hypophosphatemia in Norway: a retrospective population-based study of genotypes, phenotypes, and treatment complications. Eur J Endocrinol. 2016;174(2):125-136.

5. Hawley S, Shaw NJ, Delmestri A, et al. Prevalence and mortality of individuals with X-linked hypophosphatemia: a United Kingdom real-world data analysis. J Clin Endocrinol Metab. 2020;105(3):e871-e878.

6. Carpenter TO, Imel HA, Holm IA, Jan de Beur SM, Insogna KL. A clinician’s guide to X-linked hypophosphatemia. J Bone Miner Res. 2011;26(7):1381-1388.

7. Francis F, Henning S, Korn B, et al. A gene (PEX) with homologies to the cationic alkaline phosphatase gene in dental matrix protein 1. Implications in biomineralization and gene transcription by activation of intracellular Ca2+-store. J Biol Chem. 2003;278(19):17500-17508.

8. Koshida R, Yamaguchi H, Yamasaki K, Tsuchimochi W, Yonekawa T. Dysregulated gene expression of 25-hydroxyvitamin D and cause an atypical form of vitamin D deficiency. J Clin Endocrinol Metab. 2015;100(7):E1005-E1013.

9. Molin A, Wiedemann A, Demers N, et al. Vitamin D-dependent rickets type 1B (25-hydroxylase deficiency): a rare condition or a misdiagnosed condition? J Bone Miner Res. 2017;32(9):1893-1899.

10. Wang JT, Lin CJ, Burridge SM, et al. Genetics of vitamin D 1alpha-hydroxylase deficiency in 17 families. Am J Hum Genet. 1998;63(6):1694-1702.

11. Simon S, Resch H, Klaushofer K, Roschger P, Zwerina J, Kocijan R. Mutation spectrum in patients with X-linked hypophosphatemia-related morbidity. Orphanet J Rare Dis. 2019;14(1):58.

12. Carpenter TO. The expanding family of hypophosphatemic syndromes. J Bone Miner Metab. 2012;30(1):1-9.

13. ADHR Consortium. Autosomal dominant hypophosphatemic rickets is associated with mutations in FGF23. Nat Genet. 2000;26(3):345-348.

14. Simon S, Resch H, Klaushofer K, Roschger P, Zwerina J, Kocijan R. Hypophosphatemia: from diagnosis to treatment. Curr Rheumatol Rep. 2018;20(11):69.

15. Mornet E. Hypophosphatasia. Orphanet J Rare Dis. 2007;2:40.

16. Mornet E, Taillandier A, Peyramaure S, et al. Identification of fifteen novel mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in European patients with severe hypophosphatasia. Eur J Hum Genet. 1998;6(4):308-314.

17. Hu JC, Paletke R, Mornet E, et al. Characterization of a family with dominant hypophosphatasa. Eur J Oral Sci. 2000;108(3):189-194.

18. Taillandier A, Cozien E, Muller F, et al. Identification of fifteen new mutations in the TNSALP gene in tissue-nonspecific alkaline phosphatase (TNSALP) gene in patients with hypophosphatasia. Hum Mutat. 2000;15(3):293.

19. Mornet E. Hypophosphatasia. Orphanet J Rare Dis. 2007;2:40.

20. Fauvert D, Brun-Heath I, Lia-Baldini A-S, et al. Mild forms of hypophosphatasia mostly result from dominant negative effect of severe alleles or from compound heterozygosity for severe and moderate alleles. BMC Med Genet. 2009;10:51.

21. Foster BL, Sheen CR, Hatch NE, et al. Periodontal defects in the A116T knock-in murine model of odontohypophosphatasia. J Dent Res. 2015;94(5):706-714.

22. Whyte MP, Zhang F, Wenkert D, et al. Hypophosphatasia: validation and expansion of the clinical nosology for children with 25 years experience with 173 pediatric patients. Bone. 2015;75:229-239.

23. Hoopes RR Jr, Hueber PA, Reid RJ Jr, et al. CLCN5 chloride-channel mutations in six new North American families with X-linked nephropathy. Kidney Int. 1998;54(3):698-705.

24. Kriz W, Gretz N, Lemley KV. Progression of glomerular diseases: is the podocyte the culprit? Kidney Int. 1998;54(3):687-697.

25. Piwow N, Günther W, Schwake M, Bös ML, Jentsch TJ. CIC5 CI-channel disruption impairs endocytosis in a mouse model for Dent’s disease. Nature. 2000;408(6810):369-373.

26. Hoopes RR Jr, Raja KM, Koich A, et al. Evidence for genetic heterogeneity in Dent’s disease. Kidney Int. 2004;65(5):1615-1620.
43. St-Louis M, Tanguay RM. Mutations in the fumarylacetoacetate hydrolase gene causing hereditary tyrosinemia type I: overview. Hum Mutat. 1997;9(4):291-299.

44. Bergman AJ, van den Berg IE, Brink W, Poll-The BT, Ploos van Amstel JK, Berger R. Spectrum of mutations in the fumarylacetoacetate hydrolase gene of tyrosinemia type I patients in northwestern Europe and Mediterranean countries. Hum Mutat. 1998;12(1):19-26.

45. Ijaz S, Zahoor MY, Imran M, et al. Direct sequencing of FAH gene in Pakistani tyrosinemia type 1 families reveals a novel mutation. J Pediatr Endocrinol Metabol. 2016;29(3):327-332.

46. Simpson MA, Hsu R, Keir LS, et al. Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. Am J Hum Genet. 2007;81(5):906-912.

47. Kochar GS, Choudhary A, Gadodia A, et al. Raine syndrome: a clinical, radiographic and genetic investigation of a case from the Indian subcontinent. Clin Dysmorphol. 2010;19(3):153-156.

48. Wang X, Wang S, Li C, et al. Inactivation of a novel FGFR3 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet. 2012;8(5):e1002708.

49. Vogel P, Hansen GM, Read RW, et al. Amelogenesis imperfecta and other bimodularization defects in Fam20a and Fam20c null mice. Vet Pathol. 2012;49(6):998-1017.

50. Rafaelsen SH, Raeder H, Fagerheim AK, et al. Exome sequencing reveals FAM20c mutations associated with fibroblast growth factor 23-related hypophosphatemia, dental anomalies, and ectopic calcification. J Bone Miner Res. 2013;28(6):1378-1385.

51. Kimushita Y, Horii M, Taguchi M, Fukumoto S. Functional analysis of mutant FAM20C in Raine syndrome with FGFR3-related hypophosphatemia. Bone. 2014;67:145-151.

52. Acevedo AC, Poulter JA, Alves PG, et al. Variability of systemic and oro-dental phenotype in two families with non-lethal Raine syndrome with FAM20C mutations. BMC Med Genet. 2015;16:8.

53. Econs MJ, McEnery PT, Lennon F, Speer MC. Autosomal dominant hypophosphatemic rickets is resistant to proteolytic cleavage and causes hypophosphatemia in vivo. Endocrinology. 2002;143(3):3179-3182.

54. Shimada T, Muto T, Urakawa I, et al. Mutant FGFR-23 responsible for autosomal dominant hypophosphatemic rickets is resistant to proteolytic cleavage and causes hypophosphatemia in vivo. Endocrinology. 2002;143(3):3179-3182.

55. Yamaizaki Y, Okazaki R, Urakawa I, et al. Processing and stability of type IIc sodium-dependent phosphate cotransporter mutations in patients with hereditary hypophosphatemic rickets with hypercalciuria predict a key role for the sodium-phosphate cotransporter NaPi-IIc in maintaining phosphate homeostasis. Am J Hum Genet. 2006;78(2):179-192.

56. Holm IA, Huang X, Kunkel LM. Mutational analysis of the PHEX gene in patients with X-linked hypophosphatemic rickets. Am J Hum Genet. 1997;60(4):790-797.

57. Solanou M, Soumounou Y, Martel J, et al. Pex/Pex/Pex tissue distribution and evidence for a deletion in the 3' region of the Pex gene in X-linked hypophosphatemic mice. J Clin Invest. 1997;99(6):1200-1209.

58. Seton M, Jüppner H. Autosomal dominant hypophosphatemic rickets/osteomalacia. J Clin Endocrinol Metab. 2002;87(11):4048-4057.

59. Malloy PJ, Tasic V, Taha D, et al. Vitamin D receptor mutations in patients with hereditary 1,25-dihydroxyvitamin D-resistant rickets. Mol Genet Metab. 2014;111(1):33-40.

60. Lincoln SE, Kobayashi Y, Anderson MJ, et al. A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients. J Mol Diagn. 2015;17(5):533-544.

61. Hutti K, Soumounou Y, Martel J, et al. Pex/Pex/Pex tissue distribution and evidence for a deletion in the 3' region of the Pex gene in X-linked hypophosphatemic mice. J Clin Invest. 1997;99(6):1200-1209.

62. Holm IA, Huang X, Kunkel LM. Mutational analysis of the PEX gene in patients with X-linked hypophosphatemic rickets. Am J Hum Genet. 1997;60(4):790-797.

63. Solanou M, Soumounou Y, Martel J, et al. Pex/Pex/Pex tissue distribution and evidence for a deletion in the 3' region of the Pex gene in X-linked hypophosphatemic mice. J Clin Invest. 1997;99(6):1200-1209.

64. Malloy PJ, Tasic V, Taha D, et al. Vitamin D receptor mutations in patients with hereditary 1,25-dihydroxyvitamin D-resistant rickets. Mol Genet Metab. 2014;111(1):33-40.

65. Kimushita Y, Saito T, Shimizu Y, et al. Mutational analysis of patients with X-linked hypophosphatemic rickets. Eur J Pediatr. 2012;171(2):165-172.
83. Pekkarinen T, Lorenz-Depiereux B, Lohman M, Mäkitie O. Unusually severe hypophosphatemic rickets caused by a novel and complex re-arrangement of the PHEX gene. Am J Med Genet A. 2014;164A(11):2931-2937.

84. Vidmar AP, Miyazaki B, Sanchez-Lara PA, Pitukcheewanont P. X-linked hypophosphatemic rickets, del(2)(q37.1;q37.3) deletion syndrome and mosaic turner syndrome, mos 45,X/46,X, del(2)(q37.1;q37.3) in a 3-year-old female. J Bone Metab. 2017;24(4):257-261.

85. Ichikawa S, Traxler EA, Estwick SA, et al. Mutational survey of the PHEX gene in patients with X-linked hypophosphatemic rickets. Bone. 2008;43(4):663-666.

86. Mumm S, Huskey M, Cajic A, et al. PHEX 3'-UTR c.*231A>G near the polyadenylation signal is a relatively common, mild, American mutation that masquerades as sporadic or X-linked recessive hypophosphatemic rickets. J Bone Miner Res. 2015;30(1):137-143.

87. Newman S, Hermetz KE, Weckselblatt B, Rudd MK. Next-generation sequencing of duplication CNVs reveals that most are tandem and some create fusion genes at breakpoints. Am J Hum Genet. 2015;96(2):208-220.

88. Smith PS, Gottesman GS, Zhang F, et al. X-linked hypophosphatemia: uniquely mild disease associated with PHEX 3'-UTR mutation c.*231A>G (a retrospective case-control study). J Bone Miner Res. 2020;35(5):920-931.

89. Williams A, Black M, Dahari K. SAT-360 out of sight, out of mind: PHEX 3'-UTR C.*231A>G X-linked hypophosphatemia in adults: a case study of one family pedigree with a widely variable phenotype. J Endocrinol Soc. 2020;12(6):e8594.