

## ORIGINAL ARTICLE

# X-linked hypophosphatemic rickets: Description of seven new variants in patients followed up in reference hospitals in Rio de Janeiro

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## Abstract

**Background:** X-linked hypophosphatemic rickets (XLHR) is a rare genetic disease, often delayed in diagnosis due to the low degree of suspicion and limited access to sophisticated diagnostic tools that confirm the diagnosis, such as genetic testing.

**Methods:** Through a cross-sectional and observational study, 26 patients with a previously presumptive diagnosis of X-linked hypophosphatemic rickets (based on clinical history, laboratory findings, and physical examination), were followed for approximately 12 months. During 12 months of follow-up, only 16 patients underwent genetic testing and enrolled in the study. Previous data were analyzed, such as clinical history (e.g., gender, current age, age of clinical diagnosis, age of admission to hospital, family history, and previous orthopedic surgery), physical exam, imaging tests (e.g., radiological changes) and laboratory tests (e.g., tubular maximum reabsorption rate of phosphate to glomerular filtration rate, alkaline phosphatase, and phosphate levels) at the time of the patient's admission to IEDE and UFRJ, to corroborate and substantiate our research. These data were extracted from the medical records of the patients.

**Results:** Among the 16 patients analyzed by molecular biology techniques, the new generation sequencing (NGS), using DNA samples from oral swabs, we obtained seven variants never previously described, which were verified by Sanger sequencing. Among the seven variants never previously described, the most common coding impact was the nonsense mutation. We found two frameshift, one intronic splicing variant, three nonsense, and one deletion splice junction loss. Among patients with new mutations who presented data in the medical record, 100% showed a reduction in TmP/GFR (average of 1.98 mg/dl), the most sensitive laboratory parameter at the time of diagnosis, as well as serum phosphorus (100% had hypophosphatemia on arrival at the referral hospitals—average of 2.4 mg/dl and median 2.3 mg/dl). We also performed NGS on three mothers of the patients

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with identified mutations. Among these mothers, only one tested negative for the mutation and no family history was reported as well. This mother had serum phosphate of 3.5 mg/dl (normal range: 2.5–4.5 mg/dl) at the time of genetic test collection. The others had a positive test, low serum phosphorus at the time of the molecular test, in addition to a positive family history.

**Conclusion:** This study describes seven new variants in the *PHEX* gene and aims to increase the knowledge of the scientific community about the types of mutations involving this gene, increasing information on the genetic basis of this condition, enabling future considerations about genotype–phenotype correlation, in addition to diagnosis accurate and early.

#### KEYWORDS

hypophosphatemic rickets, new generation sequencing, new variants, *PHEX*, X-linked hypophosphatemic rickets

## 1 | INTRODUCTION

X-linked hypophosphatemic rickets was described by Albright in 1937 as a form of rickets resistant to vitamin D, as high doses of vitamin D are needed for treatment. However, nowadays, the term “resistant” characterizes vitamin D-dependent rickets type II (Mechica, 1999). Autosomal recessive or dominant forms have also been described, but hypophosphatemic rickets with dominant transmission linked to the X chromosome is the most common, with an estimated incidence of 1 in 20,000 individuals (Mughal, 2011).

The responsible gene for the disease is located in Xp22.2-p22.1 and is called the *PHEX* phosphate regulator gene (OMIM # 300550). Its structure consists of 22 exons that encode a protein with 749 amino acids. Dixon et al. (1998) identified 31 mutations (seven nonsense, six deletions, two deletional insertions, one duplication, two insertions, four splice sites, eight missense, and one within the 5′ untranslated region), of which 30 were scattered throughout the putative extracellular domain, together with six polymorphisms that had heterozygosity frequencies ranging from less than 1% to 43%.

XLHR can be inherited in a dominant transmission, but it can also be sporadic (nonfamilial XLHR patients). Over 20% of the mutations were observed in nonfamilial XLHR patients, who represented de novo occurrences of *PHEX* mutations. More than 70% of the mutations are expected to be associated with functional loss of *PHEX* protein, rather than haploinsufficiency or a dominant negative effect. According to The Human Gene Mutation Database, 588 variants were described until now (Database THGM, n.d.).

Pathogenic variants in hemizygosity or heterozygosity in the *PHEX* gene are associated with hypophosphatemic rickets, which have a wide clinical presentation, from

isolated hypophosphatemia to severe lower limb deformities and fragility fractures. Symptoms most commonly begin in the first 2 years of life, when the child adopts the orthostatic posture, with a lower limb curvature. Therefore, we are addressing a genetically determined, X-linked inherited condition, which can lead to symptoms in both male and female individuals (Ruppe, n.d.).

Sophisticated diagnostic tools, such as genetic testing, help to confirm the diagnosis by detecting the specific mutation. The description of new mutations is important to improve our knowledge about such rare disease, its genetic bases and possible associated phenotypes, laboratory changes, and family history, in addition to genetic counseling. Perhaps, in this way, in the future, we may understand better the genotype–phenotype correlation.

## 2 | METHODS

### 2.1 | Editorial Policies and Ethical Considerations

This study was conducted according to the principles of the Declaration of Helsinki (World Medical Association Declaration of Helsinki 2013). All patients provided informed consent. The research was approved by IRB of the Instituto de Puericultura e Pediatria Martagão Gesteira, #CAAE 14944719.0.1001.5264 and of the Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione, #CAAE 26997919.1.0000.5266.

Through a cross-sectional and observational study, patients with a previously presumptive diagnosis (based on clinical history, laboratory findings, and physical examination) of X-linked hypophosphatemic rickets, were followed up in the Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione (IEDE), Rio de Janeiro/RJ—Brazil,

Medical Genetic Service of Instituto de Puericultura e Pediatria Martagão Gesteira (UFRJ), Rio de Janeiro/RJ—Brazil and Endocrinology Division, Universidade Federal do Rio de Janeiro, Rio de Janeiro/RJ—Brazil. They underwent genetic tests to find out the probable responsible mutations for the rickets.

In these reference centers, approximately 26 patients with a presumptive diagnosis of hypophosphatemic rickets are followed up, but during 12 months of follow-up, only 16 patients underwent genetic testing and were included in the study.

The convenience sample consisted of 16 patients with a previously presumptive diagnosis of X-linked hypophosphatemic rickets who were followed up for approximately 12 months. Patients who did not undergo genomic analysis techniques were excluded.

Previous data were analyzed, such as clinical history (e.g., gender, current age, age of diagnosis, age of admission to hospital, family history, previous orthopedic surgery), physical exam, imaging tests (e.g., radiological changes), and laboratory tests (e.g., tubular maximum reabsorption rate of phosphate to glomerular filtration rate, alkaline phosphatase, and phosphate levels) at the time of the patient's admission to IEDE and UFRJ, to corroborate and substantiate our research. These data were extracted from the medical records of the patients.

These patients were submitted to terms of free and informed consent and to the assent term, as required (Certificate of Presentation of Ethical Appreciation: 26997919.1.0000.5266 and 14944719.0.1001.5264).

### 2.1.1 | Genetic test

All participants were submitted to NGS through DNA extraction from mouth swab samples made available by the company Ultragenyx Brasil Farmacêutica Ltda. The samples were performed after arriving at the Laboratory Mendelics Análise Genômica SA and checking eligibility: confirmation of identification of the sample, quality control, sample quantification, and validation. The technique comprises a fully automated flow of laboratory preparation, sequencing, and bioinformatics. The mutations described for the first time in the literature were verified by Sanger sequencing.

New generation sequencing (NGS) was performed using a NovaSeq 6000 device (Illumina Platform). Alignment with the reference human genome using BWA-MEM (Burrows-Wheeler Aligner), genotyping of variants with the Genome Analysis Toolkit (GATK) in accordance with the best practices of the Broad Institute, as well as algorithmic copy number genotyping (large deletions and duplications—CNVs). As a sequencing quality parameter,

we can find a horizontal coverage of 95% of target bases with 10 or more readings, according to the College of American Pathologists guidelines, and average vertical coverage of 100× (Aziz et al., 2015).

The annotation and analysis of pathogenicity variants were carried out with proprietary development software Abracadabra®, which allows a fast and efficient interpretation of sequencing on a large scale, as well as Human Genome Mutation Database and the pathogenicity check with ClinVar, associated with population frequency (including own bank of Brazilian variants). The classification of detected variants follows the criteria established by the American College of Medical Genetics (ACMG) (Richards et al., 2015). The GenBank reference sequence was ENST00000379374.4. Finally, the reports were prepared by a team of certified clinical geneticists.

Lastly, the laboratory and bioinformatics script validation of the assay is performed using DNA as a reference germinative sample of whole blood or oral mucosa. DNA of somatic or germinative origin of other types of samples may not achieve satisfactory quality.

Laboratory accreditations: CAP # 8671464—College of American Pathologists; ISO 15189: 2015—CLC 0007; PALC # 32290508—Clinical Laboratory Accreditation Program.

## 3 | RESULTS

Among the 16 patients analyzed by molecular biology techniques, the new generation sequencing, using DNA samples from oral swabs, we obtained seven variants never previously described. Of the undescribed variants, 71% of the patients were female, 42.85% had a positive family history, 57% had undergone orthopedic surgery at some point, 85.71% had high alkaline phosphatase (average of 935 U/L), 100% had a low tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) and 100% had hypophosphatemia on arrival at the referral hospitals (average of 2.4 mg/dl and median 2.3 mg/dl) as shown in Table 1.

Among the seven variants never previously described, the most common coding impact was the nonsense mutation. We found two frameshift, one intronic splicing variant, three nonsense, and one deletion splice junction loss. Table 2 shows the description of chromosome position, HGVS coding, HGVS protein, affected allele, coding impact, and ACGM classification of all mutations.

The seven new variants, never described in the medical literature, were confirmed by Sanger sequencing, and presented as a figure (see Figure 1).

Of the total number of patients analyzed, 81.25% were diagnosed in the first 5 years of life, and likewise, the majority

**TABLE 1** Clinical, laboratory, and radiological characteristics: Description of TmP/GFR, previous orthopedic surgery, alkaline phosphatase, phosphate levels at hospital admission and presence or absence of a new mutation in all patients analyzed

| Patient | Age of clinical diagnosis | Current age | Age of admission to hospital | Gender | Family history | Radiological changes on hospital admission  |
|---------|---------------------------|-------------|------------------------------|--------|----------------|---|
| 1       | 1 y 8 m                   | 22 y        | 1 y 8 m                      | Male   | + <sup>c</sup> | Cup-shaped ulna and femur epiphyses   |
| 2       | 2 y                       | 15 y        | 3 y                          | Female | + <sup>c</sup> | Increased thickness of the growth plates of the femurs and tibias associated with a slight enlargement of the metaphyses                          |
| 3       | Adulthood                 | 35 y        | 20 y                         | Female | - <sup>c</sup> | Osteomalacia  |
| 4       | 4 y                       | 14 y        | 4 y                          | Male   | + <sup>c</sup> | Wrist enlargement and curved lower limbs  |
| 5       | 1 y 6 m                   | 10 y        | 5 y                          | Female | - <sup>c</sup> | Enlargement and irregularity of the metaphyses, cup-shaped epiphysis, and valgus femurs   |
| 6       | 4 y                       | 7 y         | 4 y                          | Female | - <sup>c</sup> | Valgus femurs and discreet valgus in the lower portion of the tibias  |
| 7       | 6 y                       | 17 y        | 6 y                          | Female | - <sup>c</sup> | Low bone density, enlargement, and metaphyseal irregularity of long bones   |
| 8       | 2 y                       | 20 y        | 14 y                         | Female | - <sup>c</sup> | Enlarged wrist and curved lower limbs   |
| 9       | 1 y 6 m                   | 25 y        | 13 y                         | Female | - <sup>c</sup> | Morphostructural alteration of tibial diaphyses   |
| 10      | 9 y                       | 15 y        | 10 y                         | Male   | + <sup>c</sup> | Varus femurs and tibias, irregularity of contours of the calcification lines of the femurs and tibias   |
| 11      | 5 y                       | 10 y        | 5 y                          | Female | - <sup>c</sup> | Varus lower limbs, enlargement, and irregularities of distal metaphyses, irregularity of proximal metaphyses                                      |
| 12      | 1 y                       | 10 y        | 1 y                          | Male   | + <sup>c</sup> | Enlarged wrist and valgus lower limbs   |
| 13      | 1 y 8 m                   | 38 y        | 1 y 8 m                      | Female | + <sup>c</sup> | Varus lower limbs   |
| 14      | 3 y 9 m                   | 15 y        | 3 y 9 m                      | Female | - <sup>c</sup> | Valgus femurs, widening of wrists   |
| 15      | 3 y                       | 5 y         | 3 y                          | Male   | - <sup>c</sup> | Metaphyseal irregularity in superior and lower limbs, varus lower limbs, enlargement, and deformity of long bones, digitiform images in the skull |
| 16      | 4 y                       | 8 y         | 4 y                          | Male   | + <sup>c</sup> | Metaphyseal enlargement and irregularity of all long bones, varus femurs, pseudofracture of left humerus  |

Abbreviations: m, months; TmP/GFR, tubular maximum reabsorption rate of phosphate to glomerular filtration rate; y, years.

<sup>a</sup>TmP/TFG reference (mg/dl): < 3 months: 3.6–8.6/3–6 months: 3.7–8.25/6 months–2 years old: 2.9–6.5/2–15 years old: 2.9–6.5.

<sup>b</sup>... no results were found in the medical report.

<sup>c</sup>(↓) reduced/(↑) elevated/(N) normal/+ positive/- negative.

<sup>d</sup>■ New generation sequencing did not find a known pathogenic variant.

<sup>e</sup>Alkaline phosphatase reference: Male Female 1–3 years old: 124–410 U/L 129–376 U/L. 4–6 years old: 111–367 U/L 114–353 U/L. 7–9 years old: 102–374 U/L 82–396 U/L. 10–12 years old: 50–430 U/L 61–394 U/L. 13–15 years old: 88–463 U/L 60–193 U/L. 16–18 years old: 62–203 U/L 56–142 U/L. Adulthood: 36–110 U/L.

<sup>f</sup>Phosphate reference: 0–10 days old: 4.5–9.0 mg/dl. 11 days old–2 years old: 4.5–6.7 mg/dl. 3–12 years old: 4.5–5.5 mg/dl. 13 years old–adulthood: 2.5–4.5 mg/dl.

had deformities in the lower limbs seen on the radiological examination at admission. All 16 patients (100%) had low serum phosphorus on arrival at the referral centers.

The radiological alterations described, as well as the phosphorus levels, refer to the hospital admission of these patients (these data were detected on the first visit to the reference centers). It is noteworthy that 43.75% of them (patients 2, 4, 5, 8, 9, 10, and 16) had already started using active vitamin D when they arrived at the hospital, although 100% of them had a low serum phosphate on hospital admission.

Of the patients for whom we had access to TmP/GFR, only two had normal values (patients 10 and 16). Both patients were using calcitriol and phosphate before arriving at the reference centers, which could explain this result. The average TmP/GFR of all patients at diagnosis was 2.24 mg/dl ( $\pm 0.6$ ); the median was 2.06 (minimum value 1.73 mg/dl and maximum value 3.60 mg/dl). Among patients with reports of alkaline phosphatase (ALP) in the medical record at admission, 92.8% had elevated ALP and approximately half of patients had already undergone corrective orthopedic surgery at some

| TmP/GFR on hospital admission | Previous orthopedic surgery | Alkaline phosphatase on hospital admission | Phosphate levels at hospital admission | New mutation   |
|-------------------------------|-----------------------------|--|--|----------------|
| 2.19 mg/dl (↓) <sup>ac</sup>  | + <sup>c</sup>              | 556 U/L (↑) <sup>ce</sup>                  | 2 mg/dl (↓) <sup>cf</sup>              | Yes            |
| 2.11 mg/dl (↓) <sup>ac</sup>  | + <sup>c</sup>              | 812 U/L (↑) <sup>ce</sup>                  | 2.3 mg/dl (↓) <sup>cf</sup>            | Yes            |
| 2.1 mg/dl (↓) <sup>ac</sup>   | - <sup>c</sup>              | 250 U/L (↑) <sup>ce</sup>                  | 2.4 mg/dl (↓) <sup>cf</sup>            | Yes            |
| ... <sup>b</sup>              | + <sup>c</sup>              | 471 U/L (↑) <sup>ce</sup>                  | 2.8 mg/dl (↓) <sup>cf</sup>            | Yes            |
| 1.9 mg/dl (↓) <sup>ac</sup>   | - <sup>c</sup>              | 506 U/L (↑) <sup>ce</sup>                  | 2.3 mg/dl (↓) <sup>cf</sup>            | Yes            |
| 1.9 mg/dl (↓) <sup>ac</sup>   | - <sup>c</sup>              | 3771 U/L (↑) <sup>ce</sup>                 | 3.2 mg/dl (↓) <sup>cf</sup>            | Yes            |
| 1.73 mg/dl (↓) <sup>ac</sup>  | + <sup>c</sup>              | 181 U/L (N) <sup>ce</sup>                  | 2 mg/dl (↓) <sup>cf</sup>              | Yes            |
| 1.89 mg/dl (↓) <sup>ac</sup>  | + <sup>c</sup>              | 380 U/L (↑) <sup>ce</sup>                  | 2.3 mg/dl (↓) <sup>cf</sup>            | No             |
| 2.06 mg/dl (↓) <sup>ac</sup>  | + <sup>c</sup>              | 391 U/L (↑) <sup>ce</sup>                  | 2.4 mg/dl (↓) <sup>cf</sup>            | No             |
| 3.24 mg/dl (N) <sup>ac</sup>  | + <sup>c</sup>              | 1129 U/L (↑) <sup>ce</sup>                 | 2.9 mg/dl (↓) <sup>cf</sup>            | No             |
| 1.94 mg/dl (↓) <sup>ac</sup>  | - <sup>c</sup>              | 726 U/L (↑) <sup>ce</sup>                  | 2.1 mg/dl (↓) <sup>cf</sup>            | No             |
| ... <sup>b</sup>              | - <sup>c</sup>              | 562 U/L (↑) <sup>ce</sup>                  | 3.4 mg/dl (↓) <sup>cf</sup>            | ■ <sup>d</sup> |
| ... <sup>b</sup>              | + <sup>c</sup>              | 385 U/L (↑) <sup>ce</sup>                  | 2.7 mg/dl (↓) <sup>cf</sup>            | ■ <sup>d</sup> |
| ... <sup>b</sup>              | - <sup>c</sup>              | ... <sup>b</sup>                           | 1.9 mg/dl (↓) <sup>cf</sup>            | ■ <sup>d</sup> |
| ... <sup>b</sup>              | - <sup>c</sup>              | ... <sup>b</sup>                           | 2.8 mg/dl (↓) <sup>cf</sup>            | No             |
| 3.6 mg/dl (N) <sup>ac</sup>   | + <sup>c</sup>              | 502 U/L (↑) <sup>ce</sup>                  | 2.6 mg/dl (↓) <sup>cf</sup>            | No             |

point (56.25%). These data are described in more detail in [Table 1](#).

Of the 16 patients described all of them, upon arriving at the hospital, had conventional treatment performed or optimized (43.75% already used active vitamin D), with active vitamin D and phosphate. Currently, the majority (62.5%) continues with conventional treatment, only six patients (patients 2, 6, 7, 10, 15, and 16) are using burosumab, a recombinant monoclonal antibody to FGF23.

## 4 | DISCUSSION

As far as we know, this study brought to light seven new variants of the PHEX gene. Among the 16 patients who underwent to the genomic analysis technique through new generation sequencing (NGS), 81% (13 patients) had identified pathogenic and/or probably pathogenic variants, 53.84% (seven patients) had never been described before, with the following code impact: frameshift (two), intronic splicing variant (one), nonsense (three), and

TABLE 2 Description of chromosome position, HGVS coding, HGVS protein, affected allele, coding impact, and ACMG classification of all patients' mutations

| Patient | Chromosome position (chrX) | HGVS coding          | HGVS protein       | Affected allele situation | Coding impact                              | ACMG Classification |
|---------|----------------------------|----------------------|--------------------|---------------------------|--|---------------------|
| 1       | 22.051.142<br>22.051.144   | c.20_21delGC         | p.Ser7LysTer43     | Hemizygosis               | Frameshift                                 | Pathogenic          |
| 2       | 22.065.330                 | c.349 + 1G > A       | -                  | Heterozygosis             | Intronic, splicing variant                 | Pathogenic          |
| 3       | 22.129.673<br>22.129.674   | c.1169delC           | p.Ser390Ter        | Heterozygosis             | Nonsense                                   | Pathogenic          |
| 4       | 22.237.193                 | c.1741G > T          | p.Glu581Ter        | Hemizygosis               | Nonsense                                   | Pathogenic          |
| 5       | 22.095.749<br>22.095.750   | c.593delA            | p.Tyr198SerfsTer23 | Heterozygosis             | Frameshift                                 | Pathogenic          |
| 6       | 22.112.216<br>-            | c.849_849 + 24delGGT | -                  | Heterozygosis             | Deletion, splice junction loss             | Pathogenic          |
| 7       | 22.237.166                 | c.1714G > T          | p.Gly572Cys        | Heterozygosis             | Nonsense                                   | Probably Pathogenic |
| 8       | 22.196.490<br>22.196.492   | c.1586_1586 + 1delAG | p.Glu529ValfsTer52 | Heterozygosis             | Splicing variant                           | Pathogenic          |
| 9       | 22.132.619                 | c.1217G > A          | p.Cys406Tyr        | Heterozygosis             | Missense                                   | Pathogenic          |
| 10      | 22.151.509<br>22.196.513   | -                    | -                  | Hemizygosis               | Duplication of exons 12 to 14              | Probably Pathogenic |
| 11      | 22.237.221<br>22.237.222   | c.1768 + 2delIT      | -                  | Heterozygosis             | Intronic, splicing variant                 | Pathogenic          |
| 15      | 22.245.716                 | c.2060_2063dupGTTA   | p.Tyr688Ter        | Hemizygosis               | Insertion, nonsense                        | Pathogenic          |
| 16      | 22.095.388<br>-22.095.832  | -                    | -                  | Hemizygosis               | CNV, intragenic deletion comprising exon 5 | Pathogenic          |

Notes: The Ensemble Transcript was ENST00000379374.4 for all mutations. Mutation nomenclatures are in accordance with the HGVS nomenclature. Den Dunnen, J.T., et al. (2016). HGVS recommendations for the description of sequence variants: 2016 update. Human Mutation, 37(6), 564–569. The GenBank reference sequence was ENST00000379374.4.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; CNV, copy number variation; F, female; HGVS, Human Genome Variation Society; M, male.

<sup>a</sup>In patients 12, 13, and 14, new generation sequencing did not find a pathogenic variant known. So, they were not reported in this table.

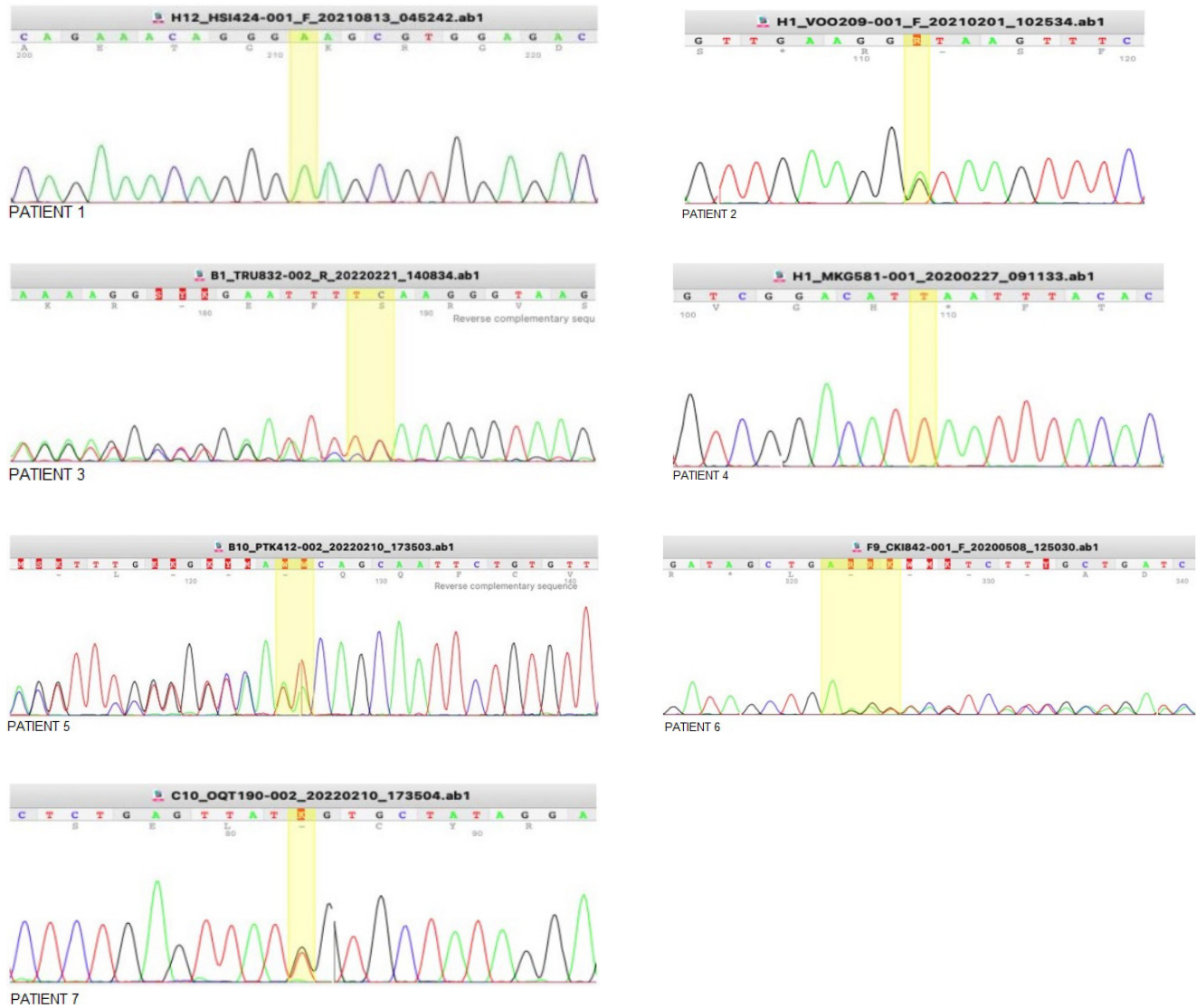


FIGURE 1 Confirmation of the seven new variants by Sanger sequencing

deletion splice junction loss (one). These new seven variants were verified by Sanger sequencing. In the remaining patients, a pathogenic variant was not detected, which does not allow us to exclude the diagnosis.

The discovery of new variants brings necessary knowledge to the study of rare diseases, making it possible to correlate the genetic basis, the clinical history, and laboratory findings, thus establishing an effective and early treatment in addition to genetic counseling. For that, it is necessary to have access to genetic tests, which is a limiting factor in many centers, mainly due to the high cost and low availability in some countries. In our study we use NGS and Sanger sequencing in some cases.

In contrast to Sanger sequencing, the speed of sequencing and amounts of DNA sequence data generated with NGS are exponentially greater. It uses parallel sequencing of multiple small fragments of DNA to determine

sequence, allowing a dramatic increase in the speed and a decrease in the cost. It is appropriate to consider exome sequencing or targeted NGS gene panels when a large number of pathogenic genes need to be screened (Adams & Eng, 2018). In the XLH, the genetic test can be helpful in nonfamilial cases, atypical presentations, disease presenting at older age or for those who desire genetic counseling. NGS might present slightly inaccurate results when detecting specific types of mutations, such as, detection of chromosomal copy number changes and/or large gains, translocations, or losses. This limitation is related to the short DNA sequence read lengths, resulting in failure to detect chromosomal deletions or insertions. In these cases, other diagnostic tools can be used depending on the specific clinical conditions being evaluated, such as comparative genomic hybridization (CGH) microarray, multiplex ligation-dependent probe amplification (MLPA),

fluorescence in situ hybridization (FISH), or cytogenetics (Stuppia et al., 2012).

Patients with clinical history and laboratory findings compatible with X-linked hypophosphatemic rickets and submitted to NGS with negative results could be candidates for MLPA, since the genetic variants located in intronic regions, in regulatory regions, in repetitive regions, from homopolymers, high homology, and pseudogene regions may not be detected due to limitations of the NGS technique. Whole-exome sequencing provides coverage of more than 95% of the exons, which contains 85% of disease-causing mutations in Mendelian disorders. So, it is estimated that approximately 15–20% of cases can be lost (Rabbani et al., 2014). Therefore, when we cannot find the mutation by NGS, we cannot exclude this diagnosis; given the strong suspicion, it is worth complementing it with MLPA. Of the three patients who had no mutations found, patient number 12 underwent MLPA, but no deletions and duplications in the *PHEX* gene were identified. This patient was also submitted to a search for mutations in the *ALPL* (OMIM # 171760), *CLCN5* (OMIM # 300008), *CYP27B1* (OMIM # 609506), *CYP2R1* (OMIM # 608713), *DMP1* (OMIM # 600980), *ENPP1* (OMIM # 173335), *FAH* (OMIM # 613871), *FGF23* (OMIM # 605380), *KL* (OMIM # 604824), *SLC34A1* (OMIM # 182309), *SLC34A3* (OMIM # 609826), and *VDR* (OMIM # 601769) genes with a negative result. Among the three patients who had no identified mutations, we chose patient 12 for further tests, as he had a rich family history, with involvement of family members in all generations, suggesting an autosomal dominant pattern of inheritance, in addition to hypophosphatemia and radiological changes on hospital admission. Although we did not perform MLPA in all negative cases, we emphasize the relevance of this diagnostic tool in specific situations.

Among the most common laboratory changes found, hypophosphatemia and loss of renal phosphate are best measured by the tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR). TmP/GFR corresponds to the theoretical lower limit of serum phosphate below which all filtered phosphate would be reabsorbed (Beck et al., 1998). Among patients with new mutations who presented data in the medical record, 100% showed a reduction in TmP/GFR (average of 1.98 mg/dl), the most sensitive laboratory parameter at the time of diagnosis, as well as serum phosphorus.

In our study, among the 16 patients analyzed, only 11 had information on TmP/GFR, and among these, only two had normal values (patients 10 and 16). The patients 10 had been previously treated with calcitriol for 12 months before arriving at the hospital as well as with exogenous phosphate administration. It is known that calcitriol increases the absorption of phosphorus at the intestinal level (Jacquillet & Unwin, 2019) and, in addition to exogenous

phosphate administration, it may have overestimated the result, since the formula uses the value of serum and urinary phosphorus. The patient 16 had already been treated with calcitriol and phosphate before the exams. In our study, 43.75% of patients (patients 2, 4, 5, 8, 9, 10, and 16) had already started conventional treatment when they arrived at the hospital, all were taking active vitamin D, but only three patients (patients 8, 10, and 16) used phosphate associated with vitamin D. The non-optimized treatment of some patients (patients 2, 4, 5, 8, and 9) and often given with vitamin D in its inactive form when arriving at the hospital, may explain the suboptimal values of laboratory results at hospital admission.

Alkaline phosphatase participates in the mineralization of bone and growth plate cartilage and is an excellent marker of rickets activity and consequently, a marker of decompensated bone disease (Seibel, 2005). It was elevated in most patients who had their dosage performed at admission (14 patients). We have highlighted the great variability in the alkaline phosphatase reference value, according to the kits used in different laboratories.

We reported that the diagnosis was made between 1 and 5 years old in 81.25% of cases, and no diagnosis was made before 1 year of age, probably due to the difficulty in perceiving the symptoms in this age range, since skeletal deformities become more evident when the child starts walking. Emma et al. (2019) developed a questionnaire to collect data on XLH epidemiology, diagnosis, and treatment in Italy. Data from 10 Italian centers (nine of which were pediatric) and 175 patients, followed between 1998 and 2017, were included in the survey. The diagnosis was made before the age of 1 and between 1 and 5 years in 11% and 50% of cases, respectively. Clinically apparent bone deformities were present in 95% of patients. We emphasize the importance of basic laboratory investigations in the first months of life in patients with a positive family history in order to obtain an early diagnosis of hypophosphatemia.

Among 81% of patients who had an identified variant (13 patients), only 38.46% (5 patients) had a positive family history. We performed NGS on three mothers of the patients with identified mutations (mothers of patients 4, 10, and 11), and only one (mother of patient 11) did not have report of family history. We looked for the specific variant in the mother of patient 11, but we did not find it (absence of the c.1768 + 2delT variant in the *PHEX* gene). This mother had serum phosphate of 3.5 mg/dl (normal range: 2.5–4.5 mg/dl) at the time of genetic test collection. In the mother of patient 4, the presence, in heterozygosis, of the p. Glu581 \* variant was detected in the *PHEX* gene. The mother of patient 4 was diagnosed at 34 years of age, only after the diagnosis of her eldest daughter at 5 years of age, at the time she had a serum phosphorus of 2.0 mg/dl (normal range: 2.5–4.5 mg/dl). Patient 4 has an older



sister with a diagnosis of XLH established at age 5, which helped in his diagnosis in childhood, although treatment could have been started earlier. In the mother of patient 10, duplication of exons 12 to 14 (ENST00000379374) was detected. The mother of patient 10 was diagnosed with rickets at 38 years of age, only after the birth of her child. At the time of diagnosis, she had a serum phosphorus of 2.1 mg/dl (normal range: 2.5–4.5 mg/dl) and TmP/GFR = 2.19 (low/lower limit of normality).

Other studies suggest that screening for different family members should be mandatory when a *PHEX* pathogenic or probably pathogenic variant is confirmed in a sporadic case. Gaucher et al. (2009) analyzed gene *PHEX* in a large cohort of 118 pedigrees representing 56 familial cases and 62 sporadic cases. *PHEX* mutations were found in 87% of familial cases but also in 72% of sporadic cases.

Finally, among patients with recognized *PHEX* mutation (13 patients), 61.53% (eight patients) underwent orthopedic surgery at some point of life, highlighting the negative impact on mobility and quality of life of those affected. In these patients, different code impact was described (one frameshift, two splicing variant, two nonsense, one missense, one duplication of exons 12 to 14, and one intragenic deletion compromising exon 5). Further studies are needed to confirm the relationship between the type of mutation, phenotype, and physical impairment.

## 5 | CONCLUSION

X-linked hypophosphatemic rickets is a rare condition that requires a high degree of suspicion from the examiner for the correct diagnosis. Sophisticated tools that allow a more accurate diagnosis, such as genetic tests, are expensive and, therefore, constitute a limiting factor to the diagnosis, which consequently limits scientific knowledge about the genetic basis of this condition.

This study describes seven new variants in the *PHEX* gene and aims to increase the knowledge of the scientific community about the types of mutations involving this gene, increasing information on the genetic basis of this condition, enabling future considerations about genotype–phenotype correlation and precise treatment that acts on the pathophysiology of the disease with a consequent positive impact on the quality of life of affected people, better mobility, less motor sequelae, and deformities, in addition to greater social inclusion and genetic counseling.

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## ETHICS STATEMENT

This study was conducted according to the principles of the Declaration of Helsinki (World Medical Association Declaration of Helsinki 2013). All patients provided informed consent. The research was approved by IRB of the Instituto de Puericultura e Pediatria Martagão Gesteira, #CAAE 14944719.0.1001.5264 and of the Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione, #CAAE 26997919.1.0000.5266.

## AUTHOR CONTRIBUTIONS

*Conceived the presented idea:* Iara Sant' Ana. *Developed the theory and verified the analytical methods:* Iara Sant' Ana, Renato Torrini, Maria Caroline Alves Coelho, Joyce Cantoni, Miguel Madeira and Márcia Ribeiro. *Encouraged investigate about XLH and and oversaw the results of this work:* Márcia Ribeiro, Miguel Madeira and Iara Sant' Ana. All authors discussed the results and contributed to the final manuscript.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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