X-linked hypophosphataemic rickets in children: clinical phenotype, therapeutic strategies, and molecular background

Monika Obara-Moszynska [1], Aleksandra Rojek [1], Zofia Kolesinska [1], Dorota Jurkiewicz [2], Krystyna H. Chrzanowska [2], Marek Niedziela [1]

[1]Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, Institute of Paediatrics, Poznan, Poland
[2]Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland

Abstract

Introduction: X-linked hypophosphataemic rickets (XLHR) is the most common form of hypophosphataemic rickets (HR), which is caused by mutations in the *PHEX* gene. The aim of this work was to investigate the clinical phenotype, therapeutic strategies, and molecular background of HR in children hospitalised in our clinic.
Material and methods: Eleven patients aged 5.7–18.25 years were included in this study. Molecular analysis was performed using polymerase chain reaction (PCR) and direct sequencing. The *PHEX* gene was examined in all of the patients, whereas the *FGF23* gene was analysed in 5 patients. All of them were treated with alphacalcidol and phosphorus, and 3 were additionally treated with recombinant human growth hormone (rhGH).
Results: The mean age at HR diagnosis was 4.05 ± 3.35 years. The mean htSDS was –2.99 ± 1.19. In 2 of the 3 patients treated with rhGH the height gain was +0.4SD and +0.3SD, respectively. In 10 of 11 patients, *PHEX* gene mutations were found. In 2 children, novel mutations in the *PHEX* gene were identified: c.325_326dupCA, N110Ifs*7 in one patient and c.899_900delTG, M300Kfs*4 in the remaining one, which coexisted with a known polymorphism c.1769-10C>T, rs3752433. In one patient, a novel deletion of exon 14 and 2 polymorphisms were detected: c.1646-46T>C, g.180417T>C, rs3213493 in intron 15 (known) and g.189156C>T in intron 17 (novel).
Conclusion: We report 3 novel mutations in the *PHEX* responsible for HR. Additionally, this study reports the effects of rhGH therapy for growth promotion in HR. **(Endokrynol Pol 2021; 72 (2): 108–119)**

Key words: hypophosphataemic rickets; hypophosphataemia; PHEX gene; recombinant human growth hormone therapy; rhGH

Introduction

Hypophosphataemic rickets (HR) belongs to a heterogeneous group of rare diseases that are caused by phosphate deregulation due to excessive renal phosphate wasting and decreased mineralisation of the growth plate in the growing child [1]. The mode of inheritance of HR varies depending on the affected gene, and the clinical manifestations of HR vary in severity. Some patients are minimally affected even in the absence of medical therapy, whereas others suffer from very severe rickets. The predominant skeletal deformities include progressive bowing of the legs, anteromedial rotational torsion of the tibiae, and short stature [2] along with dental, periodontal, and ear problems, which may also occur in patients with HR.

The most common form of HR (1:20,000 births) is X-linked HR (XLHR, X-linked dominant, OMIM #307800)

[3] caused by mutations in the *PHEX* gene (OMIM *300550, a phosphate-regulating gene with homology to neutral endopeptidases on the X chromosome) located on the X chromosome. The XLHR phenotype is characterised mainly by rickets with progressive bone deformities, flaring of the metaphyses, short stature, and dental anomalies [4], and the difference between the phenotypes of affected girls and boys may not be significant. XLHR belongs to FGF23-mediated rickets, where the level of intact FGF23 is increased in the serum. In turn, *FGF23* is expressed by osteocytes standing for a key regulator of phosphate reabsorption in the proximal renal tubules and which also inhibits the activity of vitamin D renal 1α-hydroxylase.

Early diagnosis of HR and its treatment with vitamin D and phosphorus supplementation, which is safe for patients, are crucial because they can help reduce the progression of bone deformations, prevent

✉ Aleksandra Rojek, Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, 2nd Chair of Paediatrics, 27/33 Szpitalna Street, 60–572 Poznan, Poland, tel/fax: (+48) 61 8491 424/(+48) 61 8480 291; e-mail: aleksandra.rojek@gmail.com

severe bone deformities, reduce the number of necessary surgeries, and improve the patient's final height. However, such treatment is not always effective, and careful monitoring to avoid toxicity is mandatory [2, 5]. The above-mentioned conventional treatment is limited by its renal (hypercalciuria, nephrocalcinosis) and gastrointestinal (abdominal pain, diarrhoea) side effects. Moreover, both phosphate and active vitamin D trigger further FGF23 expression in bones, resulting in a vicious circle, which may limit the efficacy and safety of the standard treatment [6]. Current outcomes of this therapy are still not fully satisfactory, and new therapies targeting the pathophysiology of the disease, i.e. FGF23 excess is the goal. In 2018, the US Food and Drug Administration and European Medicines Agency approved burosumab, a human anti-FGF23 monoclonal antibody that appears to be effective for the treatment of XLHR in children aged 1 year and older [7, 8]. In some cases, HR is diagnosed after infancy and may lead to severe growth deceleration. Recombinant human growth hormone (rhGH) therapy may be used along with supplementation with vitamin D and phosphorus because, if combined with insulin-like growth factor 1 (IGF-1), this therapy improves longitudinal growth and transiently stimulates phosphate reabsorption [9]. Several studies have shown that treatment with rhGH is able to improve growth in short children with XLHR for treatment periods of up to 3 years [10–13].

In addition to vitamin D analogues and phosphate supplements that improve tooth mineralisation, oral hygiene, active endodontic treatment of root abscesses, and preventive protection of teeth surfaces are recommended.

The aim of the study

The aim of this study was to investigate the clinical phenotype, therapeutic outcome, and molecular background of HR in children hospitalised in the Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences.

Material and methods

This study was carried out in accordance with the Declaration of Helsinki and was approved by the local Ethics Committee (Karol Marcinkowski Poznan University of Medical Sciences) (No. 504/13).

Patients

We analysed 11 patients (6 girls and 5 boys) aged 5.7–18.25 years (mean age 11.63 ± 4.31), who were diagnosed with HR due to their clinical and biochemical profiles. All of the patients were under care of the Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, and were treated with alphacalcidol and phosphorus. The average period of observation was 4.5 years (range, 0–7.3 years). Three patients with a diagnosis of growth hormone deficiency were also treated with recombinant human growth hormone (Omnitrope, Sandoz) (Tab. 1).

Methods

The height standard deviation score (htSDS) for chronological age was calculated using Polish references [14]. Bone age was estimated according to the Greulich and Pyle [15] method, while predicted adult height was calculated in line with the Bayley and Pinneau [16] method.

Blood samples were collected and frozen at –20°C until analysis. Genetic analysis of *PHEX* and *FGF23* was performed in the Molecular Endocrinology Laboratory of the Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, Poland. Routine biochemical measurements were performed in the Central Laboratory of Karol Jonscher's Clinical Hospital of the University using commercial kits. The studies were carried out using the standard polymerase chain reaction (PCR) method and DNA isolated from peripheral blood leucocytes using the QIAamp® DNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions. Oligonucleotides (Supplementary Table 1) were purchased from GenoMed, and their sequences were published previously [17]. PCRs were performed in 10-μl volumes using HotStarTaq® DNA Polymerase (QIAGEN) under the following parameters: denaturation at 95°C for 15 min, followed by 35 cycles of 95°C for 60 s, annealing at 52–62°C (depending on the primer pair) for 30 s, and elongation at 72°C for 30 s. A final amplification at 72°C for 10 min completed the PCR program.

The *FGF23* gene was analysed in the selected patients (Patient Nos. 1-5) and amplified using routine PCR and primers that encompass exon/intron sites (Supplementary Table 2) [18].

The PCR products were separated by electrophoresis on a 1% agarose gel in the presence of ethidium bromide (Merck), purified from the gel using a QIAquick® Gel Extraction Kit (QIAGEN) and directly sequenced on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems) using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Finally, sequences were analysed using Vector NTI 9.0 Software (Invitrogen) and compared to the NCBI Reference Sequences. Bioinformatic analysis of the DNA variants identified in the patients was conducted using Mutation Taster software (http://www.mutationtaster.org/). The DNA variant was annotated as a novel variant when it was not found in ExAC (http://exac.broadinstitute.org/), 1000G (http://www.internationalgenome.org/1000-genomes-browsers/) as well as gnomAD databases (https://gnomad.broadinstitute.org/).

Results

Clinical findings

The average time of HR diagnosis was from 1 and 1/12 to 9 years. In four patients (Nos. 2, 8, 9, and 11), rickets was recognised later (from 7 to 9 years), despite their mothers being affected. In all patients examined, deformities of the lower limbs were observed, and the dominant clinical feature was bowing of the legs with genu varum (10/11 patients, 91%).

In one patient, genu valgum was observed (1/11, 9%). The deformities of the lower leg were sometimes associated with other rickets symptoms, such as widening of the distal parts of the forearms, frontal bossing, or hyperlordosis. Characteristic symptoms of rickets in the examined patients are shown in Figure 1. In 4/11 patients (36%), periodontal problems were observed: gingivitis in two of the patients and advanced caries in two others. Short stature was observed in 9/11 (81.8%) patients. The mean htSDS was –2.99 ± 1.19 (min –4.7, max –1.3).

Table 1. *Clinical characteristics of the patients*

| Patient No.	Sex	Age [years/ months]	Age at diagnosis [years/ months]	HtSDS at diagnosis/ 1st stay at department	HtSDS at current/ most recent visit	Clinical symptoms/ /family interview	Ear problems	Dental /periodontal problems	Other clinical features	Max GH levels after stimulation (normal range > 10 ng/mL)	Treatment at current/ /most recent visit	Follow-up time [years]
1.	Female	9 7/12	2 3/12	–3.7	–3.0	Bowing of the lower limbs, short stature **Affected father**	No	No	No	5.2	rhGH: 0.033 mg/kg/d, phosphorus: 66 mg/kg/d, alfacalcidol: 40 ng/kg/d	7 4/12
2.	Female	13 4/12	7	–2.6	–2.2	Bowing of the lower limbs, short stature, lumbar hyperlordosis **Affected mother**	No	No	No	7.4	rhGH: 0.029 mg/kg/d, phosphorus: 33 mg/kg/d, alphacalcidol: 40 ng/kg/d, orthopedic surgery of lower limbs	6 4/12
3.	Male	8	2 3/12	–2.3	–1.8	Bowing of the lower limbs, short stature, frontal bossing, widening of the distal parts of the forearms	No	Gingivitis	No	7.1	rhGH: 0.029 mg/kg/d, phosphorus: 14 mg/kg/d, alfacalcidol: 69 ng/kg/d	5 9/12
4.	Male	9 6/12	2 8/12	–4.3	–4.2	Bowing of the lower limbs, short stature	No	Gingivitis	Transient hypogammaglobulinemia increased muscle tension	11.4	Phosphorus: 37 mg/kg/d, alphacalcidol: 60 ng/kg/d	6 10/12
5.	Male	7	1 1/12	–3.6	–3.7	Bowing of the lower limbs, short stature, deformation of chest, widening of the distal parts of the forearms	No	No	Immunodeficiency suspected; left cryptorchidism	Not tested	Phosphorus: 45 mg/kg/d, alphacalcidol: 88 ng/kg/d, orthopaedic surgery on the lower limbs	3 8/12
6.	Male	18 3/12	11/12	–2.1	–3.55	Bowing of the lower limbs, genu varum, lumbar hyperlordosis **Affected mother**	No	No	Chronic rhinitis, obesity, low level of cholesterol and triglycerides (as in the father)	10.3	Phosphorus: 11 mg/kg/d, alphacalcidol: 50 ng/kg/d, orthopaedic surgery on the lower limbs	4 5/12

Table 1. *Clinical characteristics of the patients*

Patient No.	Sex	Age [years/months]	Age at diagnosis [years/months]	HtSDS at diagnosis/1st stay at department	HtSDS at current/most recent visit	Clinical symptoms/family interview	Ear problems	Dental/periodontal problems	Other clinical features	Max GH levels after stimulation (normal range > 10 ng/mL)	Treatment at current/most recent visit	Follow-up time [years]
7. (sister of Patient No. 6)	Female	16 11/12	3/12 (therapy was started before clinical symptoms occurred)	–1.3	–3.0	Genu valgus, lumbar hyperlordosis **Affected mother**	No	No	Low level of cholesterol and triglycerides (as in the father)	14.3	Phosphorus: 15 mg/kg/d, alphacalcidol: 68 ng/kg/d, orthopaedic surgery on the lower limbs	4 3/12
8.	Male	11 9/12	8 2/12	–4.2	–3.2	Bowing of the lower limbs, lumbar hyperlordosis, asymmetry of the skull bones **Affected mother**	No	No	–	14.8	Phosphorus: 48 mg/kg/d, alphacalcidol: 57 ng/kg/d, orthopaedic surgery on the lower limbs	1 5/12
9. (sister of Patient No. 8)	Female	10 7/12	8 4/12	–4.7	–	Bowing of the lower limbs **Affected mother**	No	Advanced caries	–	Not tested	Phosphorus: 43 mg/kg/d, alphacalcidol: 68 ng/kg/d	Only one visit at age 8 4/12
10.	Female	5 8/12	2 7/12	–1.3	0.2	Bowing of the lower limbs, widening of the distal parts of the forearms	No	Advanced caries	–	Not tested	Phosphorus: 60 mg/kg/d, alphacalcidol: 41 ng/kg/d	3 1/12
11.	Female	17 4/12	9	–2.8	–4.5	Bowing of the lower limbs **Affected mother**	No	No	–	14.8	Phosphorus: 13 mg/kg/d, alphacalcidol: 40 ng/kg/d, orthopaedic surgery on the lower limbs	6 3/12

rhGH — human recombinant growth hormone

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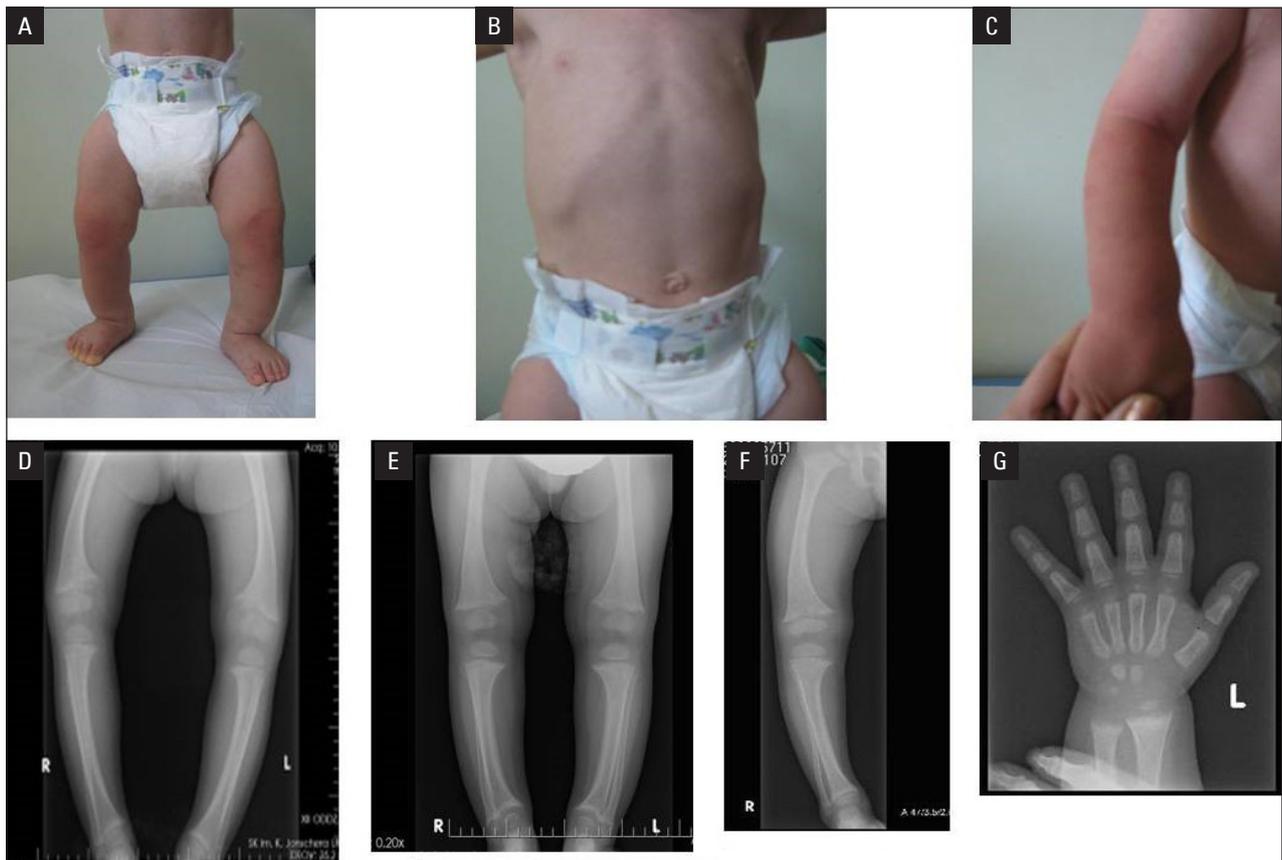
Figure 1. *Characteristic symptoms of rickets in the presented patients. Bowing of the lower limbs (A), deformation of the chest (B) and widening of the distal parts of the forearms (C) in Patient No. 5; X-ray of the lower limbs of Patient No. 1 (D), Patient No. 3 (E) and Patient No. 4 (F) at diagnosis, with characteristic bowing of the lower limbs and distal flaring of the long bones; X-ray of the left hand of Patient No. 3 with characteristic widening of the distal parts of the forearms (G)*

The clinical characteristics of the studied group are listed in Table 1.

The dominant biochemical abnormality at the time of diagnosis/during the first stay at the department was hypophosphataemia (11/11, 100% of patients) and high levels of alkaline phosphatase (10/11, 90.9%). In most patients urine phosphorus excretion was decreased (6/11; 54.5%), in 2 patients it was increased (2/11; 18.2%), and in 3 patients it was within the laboratory normal range (3/11, 27.3%). In all patients, tubular reabsorption of phosphate (TRP) at the time of the diagnosis/during the first stay at the department was normal but dropped below the range during follow-up. The concentrations of total calcium, parathyroid hormone, and 25(OH)D3 (a metabolite of vitamin D3) at diagnosis/during the first stay at the department were within normal ranges in most patients (10/11, 90.9%; 8/11, 72.7%; 10/11, 90.9%, respectively). Normalisation of alkaline phosphatase during therapy was observed in 6 children (6/11, 54.5%). The biochemical characteristics of the studied patients are presented in the supplementary materials (Supplementary Table 3).

In one patient (No. 3), due to the presence of frontal bossing, MRI of the head was performed, which revealed a Chiari type 1 malformation.

Six children underwent conventional orthopaedic surgery because of advanced deformities of the lower limbs, and in 3 patients (Nos. 1, 3, and 4) improvement of lower limb deformation was observed by applying only pharmacological treatment. The intercondylar distance changed from 12 cm to 2 cm in Patient No. 1 (Fig. 2), from 8 to 3 cm in Patient No. 3, and from 7 to 0 cm in Patient No. 4. In Patient No. 10 the intercondylar distance, despite an early diagnosis (2 years and 7 months) and 3 years of pharmacological treatment, decreased only from 8 cm to 5.5 cm, and because of severe bowing of the tibial bones, orthopaedic surgery is planned. In other patients, the effects of pharmacologic treatment were difficult to estimate because they underwent orthopaedic surgery or the clinical observation period at our department was too short.

Three patients (Nos. 1, 2, and 3) are being treated with rhGH because they presented GH deficiency. In two of them, we achieved an improvement in height

Figure 2. *Lower limbs of Patient No. 1. Bowing of the lower limbs at age 3 years and 3 months (**A**) and the current status at age 9 years and 7 months (**B**)*

Table 2. *Auxological parameters of patients treated with recombinant human growth hormone (rhGH)*

Patient	Sex	Age at the time of HR diagnosis [years, months]	Age at the onset of rhGH therapy [years, months]	Current age [years, months]	HtSDS at the onset of rhGH therapy	PAH at the onset of rhGH therapy [cm]	Current HtSDS	Current PAH [cm]
1	Female	2 3/12	6 9/12	9 7/12	–3.4	149.7	–3.0	152.6
2	Female	7	10 6/12	13 4/12	–2.0	153.6	–2.2	152.6
3	Male	2 3/12	4 9/12	8	–3.1	164.8	–1.8	168

HtSDS — height standard deviation score; PAH — predicted adult height

and adult height prediction. In one patient (No. 2), after nearly 3 years of rhGH treatment, the height deficit and the predicted adult height were slightly worse than they were at the starting point of the therapy. The auxological details of these patients are given in Table 2 and on growth charts (Fig. 3A–C).

Molecular findings

In 10 out of 11 patients studied, *PHEX* gene mutations were found, except for Patient No. 4, in whom only one known polymorphism in intron 15 (c.1646-46T>C, g.180417T>C, rs3213493) was detected and described as of unknown clinical significance. Unfortunately, we were not able to sequence the entirety of exons 12 and 19.

In 2 of the patients, we detected 2 novel mutations (Fig. 4): c.325_326dupCA, N110Ifs*7 in exon 3 in Patient No. 1 and her affected father, and c.899_900delTG, M300Kfs*4 in exon 8 in Patient No. 11. In 1 of these patients a novel mutation coexisted with a known polymorphism: c.1769-10C>T, rs3752433 in intron 17 (No. 11).

In one patient (No. 5), a novel deletion of exon 14 and two polymorphisms were detected: a known polymorphism in intron 15 (c.1646-46T>C, g.180417T>C, rs3213493) and a novel polymorphism (g.189156C>T) in intron 17, both of which may lead to aberrant splicing of the *PHEX* transcript and aberrant function of the PHEX protein (sequencing of the entire exon 19 was unsuccessful). This novel deletion is not shown in Fig. 4.

Moreover, in the remaining patients, known mutations were observed: c.663+1G>T in intron 5 in 2 siblings (No. 8 and No. 9) and their affected mother [19], and c.1483-1G>A in intron 13 in 2 other siblings (No. 6 and No. 7) and their affected mother [20].

In Patient No. 2, the c.1645+1G>A mutation in intron 15 coexisted with the polymorphism c.1769-10C>T, rs3752433 in the splicing site of intron 17. Both DNA changes, which may cause aberrant splicing of the *PHEX* transcript, were also found in the girl's affected mother. In Patient No. 3, c.1801_2250del encompassing exon 17-22, which leads to partial loss of the gene, was present.
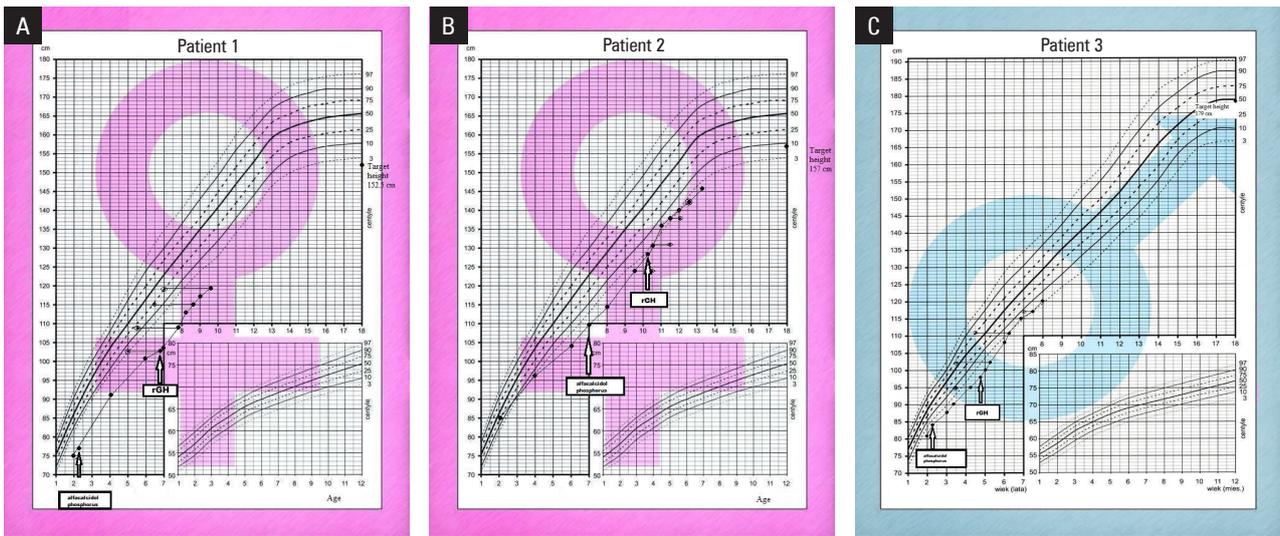
Figure 3. *Growth charts of patients treated with recombinant growth factor (rGH).* **A***. Patient No. 1;* **B.** *Patient No. 2;* **C.** *Patient No. 3*
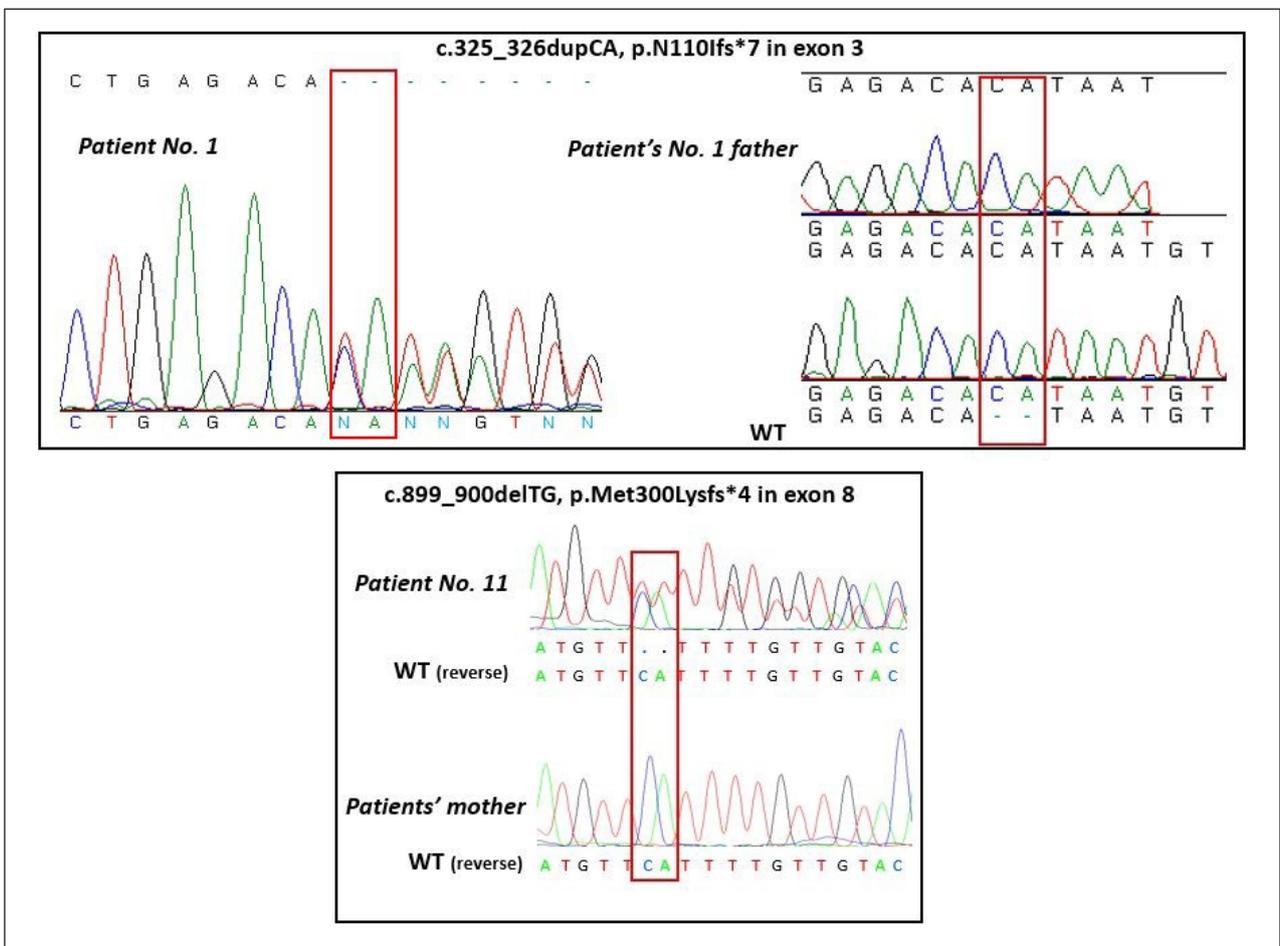
Figure 4. *Novel mutations found in the analysed patients. The figure shows only 2 point mutations found in Patients 1 and 11, except for Patient No. 5, in which a deletion of exon 14 was found. Polymorphisms are not shown*

In Patient No. 10, c.871C>T, p.R291* in exon 8 was found. The mutation leads to the formation of a premature stop codon and premature termination of PHEX protein translation.

Table 3. *Genetic characteristics of the patients. In patient No. 1, a known c.C716>T, p.T239M heterozygous polymorphism (rs7955866) in exon 3 of FGF23 was found, which was absent in the patient's father. The FGF23 gene was intact in patients Nos. 2-5; patients Nos. 6-11 were not tested for FGF23*

Patient No.	PHEX
1.	**A novel heterozygous mutation** (c.325_326dupCA, N110Ifs*7) in exon 3. This variant was found in neither the ExAC nor the 1000G database and was also present in the patient's affected father. The mutation causes a frameshift and premature termination of PHEX protein translation
2.	A known heterozygous splicing mutation (c.1645+1G>A) in intron 15 and a heterozygous polymorphism (c.1769-10C>T, rs3752433) in the splice site of intron 17. Both DNA changes, which may cause aberrant splicing of the PHEX transcript, were also found in the girl's affected mother
3.	A known hemizygous deletion (c.1801_2250del) leading to partial loss of the *PHEX* gene (exon 17 to exon 22)
4.	A known polymorphism in intron 15 (c.1646-46T>C, g.180417T>C, rs3213493), which might lead to aberrant splicing of the PHEX transcript and aberrant function of the PHEX protein (sequencing of the entire exon 12 and 19 was unsuccessful)
5.	A **novel** deletion of exon 14 and two polymorphisms were detected: a known polymorphism in intron 15 (c.1646-46T>C, g.180417T>C, rs3213493) and a novel polymorphism (g.189156C>T) in intron 17, both of which may lead to aberrant splicing of the PHEX transcript and aberrant function of the PHEX protein (sequencing of the entire exon 19 was unsuccessful)
6.	A known hemizygous mutation (c.1483-1G>A) in intron 13 leading to changes in the transcription of the *PHEX* gene (also present in the patient's affected mother and younger sister (Patient No. 7) [33]
8.	A known hemizygous mutation (c.663+1G>T) in intron 5 [32] and a known polymorphism (c.849+3A>G, rs200585038) in intron 7. Both mutations lead to aberrant splicing of the PHEX transcript and aberrant function of the PHEX protein. Both mutations were present in the patient's affected mother and younger sister (Patient No 9)
10.	A known heterozygous nonsense mutation (c.871C>T, p.R291X) in exon 8 leading to the formation of a premature stop codon and premature termination of translation of the PHEX protein
11.	**A novel heterozygous mutation** (c.899_900delTG) in exon 8, M300Kfs*4, a splicing mutation leading to a frameshift and premature termination of the PHEX transcript and a known polymorphism c.1769-10C>T, rs3752433 in intron 17

In one patient (No. 4), we found only one known polymorphism in intron 15; namely, c.1646-46T>C, g.180417T>C, rs3213493. The *FGF23* gene was intact, which suggests that HR in this case is caused by mutations in genes other than *PHEX* and *FGF23*.

Because HR can be caused by mutations in other genes a mutational analysis of *FGF23* was performed in the selected patients (Nos. 1–5), but we did not find any pathogenic variants in any of them. In one patient (No. 1), we detected a known c.C716>T, p.T239M heterozygous polymorphism in exon 3 rs7955866 (which was absent in the patient's affected father), without clinical significance. Thus, we concluded that the identified novel mutation c.325_326dupCA, N110Ifs*7 in exon 3 of the *PHEX* gene is a molecular cause of HR in this family. In patients Nos. 6-11, the *FGF23* gene was not tested because we did not have funds for further analysis, and the molecular background of HR was confirmed by mutations in *PHEX* gene.

The molecular results for the cohort are presented in Table 3.

Discussion

Clinical picture
The time of HR diagnosis was relatively early; however, it was late despite a positive family history in four patients. Most children are diagnosed with X-linked hypophosphataemia in the first year of life, provided that there is a known family history of the disorder [2]. Children presenting *de novo* HR symptoms are usually detected when poor weight gain and growth coexisting with progressive bowing of legs are observed [21]. Our cases show that the genetic counselling of affected families, and education of parents and general practitioners play a very important role in improving patient health and well-being. In the presented patients with late diagnosis and positive family history, the family members were not aware that rickets might be inheritable and that early diagnosis and treatment initiation improve the disease's outcome. Unfortunately, in three patients, the late diagnosis was associated with the necessity to perform several orthopaedic surgeries of the lower limbs. In affected mothers of three patients with a late diagnosis, the molecular tests were performed along with the genetic analysis of the children, and they were informed about the inheritance of the disease afterwards.

The dominant dysfunction in our patients was deformation of the legs (especially genu varum), which is in line with the current literature [2, 22]. General softening of the bone due to defective mineralisation, together with the weight of the child and muscles pulling on weak bones, led to lower limb bending [23]. The cancellous compartment of long bones, particularly the tibia, is undermineralised [24]. Some studies

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show that females with XLHR have less related bone involvement than males [23]. Whyte et al. [25] failed to show any evidence for genetic heterogeneity or for effects based on gender, race, anticipation, or parent of origin on XLHR expression in children. Our case study also does not allow us to say that boys suffer in a more severe way. Early conventional medical treatment may prevent or reduce long-bone deformities and facilitate the healing of pseudofractures [26]. The objective in pharmacological treatment should be attained after 3–4 years of leg straightening, which is 1 cm in intercondylar distance every 6 months [22]. Unfortunately, early diagnosis and supplementation with phosphorus and alphacalcidol did not guarantee the reduction of skeletal abnormalities in our studied group. The patient's compliance should also be taken into account, especially because the phosphorus intake can be problematic. Oral phosphate supplements should be taken 4–5 times daily due to rapid absorption and excretion. The oral intake of phosphorus leads to a rapid increase in serum and reaches the baseline level within 1.5 hours [27]. The compliance of phosphorus intake in the studied group, especially at the beginning of therapy, could be not satisfactory, but evident irregularity in the treatment was not registered.

Almost all of our patients were short at diagnosis and during therapy. Only one girl (Patient No. 10) had a normal height (current htSDS 0.2) with the target height calculated on the basis of her parents' height estimated to be 167 cm. She is overweight, which probably has a promoting impact on her growth. Her height standard deviation score improved on conventional treatment. Somatic growth in obese patients appears to be mainly GH-independent [28]. Increased insulin action on the IGF-1 receptor [29] and insulin resistance suppressing IGF-binding proteins leading to greater IGF-1 bioavailability [30] are the mechanisms stimulating the growth process. Altered sex steroid concentrations and adipokines released by the adipose tissue could also play a role [28]. Leptin appears to be an additional factor for stimulating growth [31]. Furthermore, increased aromatisation of androgens into oestrogens in adipose tissue may be another mechanism regulating growth [32].Short stature in HR is secondary to growth restriction of the lower extremities rather than generalised growth failure [33]. The degree of growth impairment is not dependent on the magnitude of hypophosphataemia or the extent of leg bowing, and the chronic administration of phosphate supplements and alphacalcidol is usually not able to normalise the height [34]. Adults with XLHR have a final height that is significantly reduced by up to 20 cm, with a mean htSDS of –1.9 [352]. Adults who have begun earlier treatment with phosphate and calcitriol manage to grow taller despite having a similar degree of hypophosphataemia [36].

Our presented group is heterogeneous, and because of the different types of therapy and sometimes a short observation period, it is hard to clearly specify the conclusions regarding growth, time of diagnosis and laboratory findings.

In two out of three patients with an early diagnosis of HR and treated with rhGH, a reduction in the height deficit with improved adult height prediction and reduction in lower limb bowing were observed. One girl with a late diagnosis, who started rhGH therapy after pubertal onset, did not improve her height. It seems that the time of therapy introduction is the key point. Recombinant human growth hormone influences patient growth as well as bone mineral metabolism. The GH-related increase in phosphate reabsorption is thought to be mediated by IGF-1. In healthy individuals, the latter increases the renal production of 1,25(OH)2 vitamin D3 as well as intestinal and renal phosphate absorption [36, 37]. In view of the GH-induced rise in IGF-1 serum levels, this pathway seems to be still operational in XLHR, although this was not sufficient to normalise TmP/GFR (tubular maximum reabsorption of PO_4/ /glomerular filtration rate) and serum phosphate levels [13]. There are many studies concerning the usefulness of rhGH therapy in XLHR. The randomised study of Živičnjak et al. [13] resulted in a linear growth increase (+1.1 height SDS) in 16 short prepubertal patients with XLHR treated with rhGH for three years. Rothenbuhler et al. [38] also proved that two-year rhGH treatment is effective in treating short stature in XLHR children and that prepubertal children respond better to rhGH. However, the studies are still based on small groups, and there are doubts as to whether rhGH treatment has a positive impact on body proportions.

The initial dominant biochemical abnormality in our patients was hypophosphataemia and elevated alkaline phosphatase, which is a typical abnormality found in XLHR patients occurring in the first months of their life, as a result of FGF23-driven phosphaturia [24]. Urine phosphorus excretion was decreased, and TRP was normal at the time of diagnosis/during the first stay at the department in most patients. Low phosphorus excretion is a result of low chronic hypophosphataemia. Tubular reabsorption of phosphate improves as serum phosphorus and the filtered load of PO_4 declines, and the calculation of the tubular maximum reabsorption of PO_4 (TmP/GFR) is needed to identify the mutant renal phenotype [24].

In one of our patients, a Chiari type 1 malformation was revealed. XLHR children are at risk of developing cranial vault and craniovertebral anomalies, such as early closure of the cranial sutures and Chiari type

1 malformation. The association of craniosynostosis to rickets has been documented as early as 1964, and several reports of scaphocephaly in patients with rickets have been described [39]. Rothenbuhler et al. [40] found that 59% of XLHR children had a complete or partial fusion of the sagittal suture, and 25% of XLHR children showed protrusion of the cerebellar tonsils. A history of dental abscesses was related to craniosynostosis, which in turn was associated with abnormal descent of cerebellar tonsils. Only 2 patients from that study showed neurological symptoms. Our patient presented deformation of the head with frontal bossing and had a chronic problem with dental abscesses. Although he had an episode of seizures, there were no pathological signs in neurological evaluation, and epilepsy was finally excluded.

There are several limitations to our study. The first is the size of the study group and the relatively short time of observation. As bones' mineralisation continues beyond the period where the final height is reached, it would be valuable to extend the study to a longer period. Finally, we cannot exclude periodic non-compliance with phosphorus and alphacalcidol intake in some of our HR patients, affecting the results.

Molecular analysis

The *PHEX* gene consists of 22 exons [41, 42, 43, 44] and is translated into a 749 amino acid protein (https://www.uniprot.org/uniprot/P78562). Extensive mutation analysis showed that the spectrum of the *PHEX* gene mutations is very wide, including nonsense, missense, and splicing site mutations, as well as insertions and deletions in different positions. Current data from the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php; access date: 09.10.2020) include 588 mutations in *PHEX*. The most common types of mutations are missense/nonsense mutations whereas other types are less common. There are no hot-spot mutations, which makes the analysis more time-consuming and expensive. Moreover, the gene undergoes alternative splicing, which may lead to the production of several active forms of the PHEX protein (https://www.genecards.org/cgi-bin/carddisp.pl?gene=PHEX). It is possible that some of them might be involved in bone turnover and dentin formation, whereas others may play an important role in renal phosphate uptake and vitamin D3 metabolism. Considering that splice prediction programs cannot reliably predict the outcome of splice mutations, the effects of mutations in such, probably active alternative forms of PHEX, are very difficult to foresee.

There is also no clear genotype-phenotype correlation of the *PHEX* gene mutation in XLHR, and the severity of clinical symptoms does not strictly depend on the type of the *PHEX* gene mutation and its location. However, some trends in families with XLHR may be observed in the literature. For example, Popowska et al. [45] described 59 patients with XLHR and observed that hearing defects and dental abnormalities were correlated with mutations located at the beginning of the gene, whereas mutations located in its terminal region were associated with increased head length. Other studies showed more severe skeletal malformations in patients with nonsense mutations leading to a truncation of the PHEX protein [41] or with a mutation in the region encoding for its C-terminal part [42]. Lower 1,25(OH)2D3 levels and TRP have also been found in patients with nonsense mutations, which suggest that the phenotypic severity of the disease may be dependent on the type of *PHEX* mutation [46]. However, further and thorough molecular studies are needed to confirm this hypothesis.

Several studies have shown that elevated levels of circulating FGF23 in serum were associated with *PHEX* mutations in XLHR patients [47] and suggest that overexpression of FGF23 might stand for an ultimate linkage in the pathogenesis of XLHR. Unfortunately, we were unable to determine the serum level of FGF23 due to a lack of initial patient serum samples.

Conclusions

The study presents the clinical picture and biochemical profile of patients with XLHR. Three novel mutations in the *PHEX* gene responsible for HR are reported. Early diagnosis and implementation of conventional treatment and rhGH can improve patient height and minimise bone deformities. Molecular analysis is necessary to confirm the clinical diagnosis of HR and conduct appropriate genetic counselling in families with HR patients.

Authors' contributions

M.O.M. and A.R contributed equally to this paper.
All the authors have read the manuscript, accept responsibility for its entire content, and approve its submission. M.O.M — study concept and design, clinical evaluation of the patients, manuscript drafting; A.R. — study concept and design, molecular genetic studies, analysis and interpretation of molecular data, manuscript drafting, funding procurement; ZK — clinical evaluation of the patients; D.J. — molecular genetic studies, analysis and interpretation of molecular data; K.H.C.H. — clinical evaluation of the patients, analysis and interpretation of molecular data, critical revision of the manuscript for important intellectual content; M.N. — study concept and design, clinical evaluation of the patients, critical revision of the manuscript for

important intellectual content, funding procurement, study supervision.

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