J. Appl. Genet. 42(1), 2001, pp. 73-88

X-linked hypophosphatemia in Polish patients. 2. Analysis of clinical features and genotype-phenotype correlation

Ewa POPOWSKA[1], Ewa PRONICKA[2], Anna SUŁEK[1], Dorota JURKIEWICZ[1], Elżbieta ROWIŃSKA[2], Jolanta SYKUT-CEGIELSKA[2], Zofia RUMP[3], Elżbieta ARASIMOWICZ[4], Małgorzata KRAJEWSKA-WALASEK[1]

[1]Department of Medical Genetics, [2]Department of Metabolic Diseases, [3]Department of Oral Medicine, [4]Department of Anthropology, Children's Memorial Health Institute, Warszawa, Poland

Abstract. Clinical and molecular data of 59 affected persons from 36 unrelated families with XLH (36 probands and 23 members of their families) were analysed. Characteristic phenotypic features (degree of leg deformities, growth failure, tooth abnormalities, tubular reabsorption of phosphate, serum phosphate and 1,25-dihydroxyvitamin D_3 concentrations, head length and hearing defect in some cases) were assessed in relation to the type and localisation of 29 different *PHEX* gene mutations. The severity of clinical symptoms did not strictly depend upon the type and localisation of the *PHEX* gene mutation. A hearing defect was correlated with mutations in the beginning fragment, while tooth abnormalities and increased head length with the mutations in the beginning and the terminal fragment of the gene. Phosphate and vitamin D_3 supplementation usually slowed progressive growth retardation and leg bowing. Our results point to the probability that alternative splicing occurs in the *PHEX* gene, producing several active forms of the PHEX protein. Some of them might be involved in bone turnover and dentin formation, others in renal phosphate uptake and vitamin D_3 metabolism.

Key words: clinical and molecular data, genotype-phenotype correlation, pharmacological treatment, *PHEX* gene, rickets, X-linked hypophosphatemia.

Introduction

X-linked hypophosphatemia (XLH) occurs with an incidence of 1:20000 and is manifested by growth retardation and bone deformities that are more apparent in

Received: July 17, 2000. Accepted: October 25, 2000.
Correspondence: E. POPOWSKA, Department of Medical Genetics, Children's Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warszawa, Poland, e-mail: epopowska@czd.waw.pl

the lower extremities. Pseudofractures, bone and/or joint abnormalities appear with age (McKUSICK 1994, RASMUSSEN, TENENHOUSE 1995, WHYTE et al. 1996). Biochemical studies revealed hypophosphatemia, normal or low plasma 1,25-dihydroxyvitamin D_3 concentrations and elevated plasma alkaline phosphatase activity resulting from decreased renal tubular phosphate reabsorption, abnormal regulation of renal 25-hydroxyvitamin D metabolism, and defective bone and tooth mineralization (SHIELDS et al. 1990, ECAROT et al. 1992, YAMAMOTO et al. 1992, RASMUSSEN, TENENHOUSE 1995). The usual therapy is a combination of phosphate supplementation and high doses of 1,25-dihydroxyvitamin D_3 (GLORIEUX et al. 1972, VERGE et al. 1991, PETERSEN et al. 1992, SEIKALY et al. 1994). According to current knowledge, the disease is a consequence of various mutations in the *PHEX* gene (HYP CONSORTIUM 1995, ROWE et al. 1997, FRANCIS et al. 1997, HOLM et al. 1997, SUŁEK et al. 1998, DIXON et al. 1998, FILISETTI et al.1999, TYYNISMAA et al. 2000) located at Xp22.1. Probably the *PHEX* gene product takes part in processing a circulating phosphaturic factor, MEPE, that influences osteoblast and renal cell function via a protein kinase C mediated pathway (BONEH, TENENHOUSE 1990, ROWE et al. 2000). The precise pathogenesis of XLH is not yet known.

In the second part of our work on X-linked hypophosphatemia in Polish patients we present biochemical, stomatological, audiological and anthropometrical characteristics of patients with established genotypes and analyse correlations between clinical symptoms and the type and localisation of the *PHEX* gene mutations.

Material and methods

Clinical features of 59 individuals from 36 unrelated families with X-linked hypophosphatemia were analysed. Twenty-two patients with XLH presented as a sporadic case in the family and 37 patients originated from 14 families in which more than one member was affected. This group included 45 children (15 boys and 30 girls) aged from 6 months to 16 yrs who were diagnosed and treated (some of them since 1977) at the Department of Metabolic Diseases CMHI, and 14 untreated affected adult members of the families (5 hypophosphatemic fathers and 9 hypophosphatemic mothers of the patients). Treatment consisted of oral administration of phosphate salts (2-4 g per day) and 1,25-dihydroxyvitamin D_3, plus orthopedic surgery if necessary. Changes in body height and leg deformities as well as calcium-phosphate homeostasis were systematically monitored during the treatment.

In the present study some retrospective biochemical, anthropometrical, stomatological and audiological data were chosen for the analysis of XLH phenotype. Three biochemical parameters measured before the treatment were assessed: serum phosphate concentration at fasting, 24-hour urinary excretion of phosphate

expressed as maximum threshold of phosphate reabsorption ($TmPO_4/GFR$), and 1,25-dihydroxyvitamin D_3 level (GRĄDZKA et al. 1988). Control values of serum phosphate concentrations in three distinguished age groups are: for <2 years 1.80-2.00 mmol/L, for 2-16 years 1.40-1.60 mmol/L, and for adults 0.90-1.30 mmol/L. Normal control values of $TmPO_4/GFR$ are 1.28-1.80 mmol/L of glomerular filtrate. $TmPO_4/GFR$ was not determined in affected adults. Laboratory control values for 1,25-dihydroxyvitamin D_3 level at normophosphataemia are 38-60 pg/mL.

Degree of leg deformation (bowing legs) was determined on a scale of 0 to 3, where 0 = no abnormalities, 1 = slight (3-6°), 2 = moderate (6-10°), 3 = severe deformation (>10°).

Body height was measured at the beginning (before the treatment) and several times during the treatment. Height was normalised and expressed as standard deviation (SD, Z-score) from normal values for chronological age (according to the charts for Polish healthy population).

Head length (G-OP, distance between *glabella* and *opistokranion*) was estimated as a standard deviation (SD) score and normalised for age and sex (using charts for Polish healthy population). Head length has been recently identified by one of us (ARASIMOWICZ 2000) as a characteristic parameter for XLH among a number of anthropometric parameters of the head.

Stomatological examination included tooth abnormalities such as periodontal abscess, enamel hypoplasia or crowded teeth. Degree of tooth abnormalities was determined on a scale of 1 to 4, where 1 = slight, 2 = moderate, 3 = severe and 4 = very severe changes.

Some of the children with XLH underwent audiological examination and sensorineural hearing loss was detected in a few cases.

Mutation analysis of all Polish patients with familial hypophosphatemic rickets was performed and described in detail in our first paper (POPOWSKA et al. 2000). Thirty-six patients and 23 affected members of their families revealed 29 different *PHEX* gene mutations resulting from deletions, insertions and nucleotide substitutions. Fourteen out of all various mutations were present in more than one affected person (2-5 individuals), related or unrelated.

Results

Clinical and biochemical data on examined patients with X-linked hypophosphatemia

Selected clinical features and biochemical parameters were analysed to describe the phenotype of the examined hypophosphatemic patients (Table 1, Figures 1, 2, 3).

Pre-treatment serum inorganic phosphate concentration was low, ranging from 0.97 to 1.65 mmol/L (mean 1.20 ± 0.25 mmol/L) in 7 children aged from 1 to

Table 1. Clinical, biochemical and molecular data of patients with X-linked hypophosphatemia

Family No.	Case no. (relationship)	Sex	Age at diagnosis (years)	Period of treatment (years)	TmP/GFR (mmol/L)	Serum PO_4 (mmol/L)	$1,25D_3$ (pg/mL)	Deafness	Mutation	(number)
1	2	3	4	5	6	7	8	9	10	
i	1 (proband)	M	3	$9\ ^{7}/_{12}$*	0.93	1.09	–	yes	ex 1, R20X	(1)
	2 (mother)	F	adult	–	–	0.67	–	–		
ii	3 (proband)	M	16	2	–	0.50	42.6	–	IVS 1 +1 g>a	(2)
	4 (proband)	M	$4\ ^{7}/_{12}$	$10\ ^{5}/_{12}$*	0.93	0.93	42.1	yes		
iii	5 (sister)	F	$2\ ^{5}/_{12}$	$10\ ^{7}/_{12}$*	0.77	0.91	43.8	–	ex 2-4 del	(3)
	6 (mother)	F	adult	–	–	0.86	–	–		
iv	7 (proband)	M	$5\ ^{7}/_{12}$	$12\ ^{5}/_{12}$	0.81	0.89	16.3	no	ex 3, 201A-205A del	(4)
v	8 (proband)	M	$5\ ^{7}/_{12}$	$8\ ^{1}/_{12}$	0.32	0.63	46.5	–	ex 4, 412C del	(5)
	9 (proband)	M	2	12	0.93	0.93	–	yes		
vi	10 (brother)	M	$^{4}/_{12}$	9	0.41	1.46	–	–	ex 5, 610A-663G / IVS 5 +(1-50) del	(6)
	11 (mother)	F	adult	–	–	0.60	–	–		
vii	12 (proband)	F	$1^{2}/_{12}$	$2\ ^{6}/_{12}$	0.73	1.02	158.0	–	ex 6, 667C del	(7)
	13 (mother)	F	$5\ ^{10}/_{12}$	$10\ ^{11}/_{12}$*	0.93	0.93	–	–		
viii	14 (proband)	F	$4\ ^{4}/_{12}$	$13\ ^{8}/_{12}$*	0.32	0.57	–	–	ex 9, 1013A-1018G del	(8)
ix	15 (proband)	M	$5\ ^{3}/_{12}$	$12\ ^{7}/_{12}$	0.77	0.70	–	–	IVS 9 +1 g>a	(9)
x	16 (proband)	M	adult	–	–	0.61	–	–	IVS 9 +2 taag del	(10)
	17 (daughter)	F	$10\ ^{10}/_{12}$	$7\ ^{2}/_{12}$	0.77	0.89	29.0	–		
xi	18 (proband)	F	$7\ ^{2}/_{12}$	$9\ ^{10}/_{12}$	0.41	0.71	19.7	–	ex 10, W368X	(11)

1	2	3	4	5	6	7	8	9	10	
xii	19 (proband)	F	8	2 $^{4}/_{12}$	0.56	0.72	30.1	–	ex 11, 1185G del	(12)
	20 (proband)	M	adult	–	–	0.57	–	no		
xiii	21 (daught.1)	F	3	11 $^{3}/_{12}$	1.00	1.16	–	–	ex 11, W403X	(13)
	22 (daught.2)	F	2 $^{2}/_{12}$	13 $^{9}/_{12}$	0.95	1.11	–	–		
xiv	23 (proband)	F	6 $^{6}/_{12}$	9 $^{3}/_{12}$*	0.79	1.09	23.6	–	ex 11, Q428X	(14)
xv	24 (proband)	M	9 $^{8}/_{12}$	8 $^{4}/_{12}$	0.41	0.85	41.1	–	ex 12, 1400A-1404G del	(15)
xvi	25 (proband)	F	2 $^{11}/_{12}$	4 $^{5}/_{12}$*	0.44	0.81	28.9	–	ex 14, 1523A-1537T del	(16)
xvii	26 (proband)	F	4 $^{3}/_{12}$	13 $^{7}/_{12}$	0.74	0.89	41.2	–	ex 14, R510P.	(17)
xviii	27 (proband)	F	3 $^{9}/_{12}$	10 $^{3}/_{12}$	0.99	0.90	–	no		
	28 (mother)	F	adult	–	–	0.69	–	–		
xix	29 (proband)	F	13	4	1.07	1.07	–	–	ex 15, P534L	(18)
xx	30 (proband)	F	2 $^{9}/_{12}$	8 $^{1}/_{12}$	0.23	0.89	66.3	no		
xxi	31 (proband)	F	4	3 $^{2}/_{12}$	0.69	0.75	80.0	–	ex 15, R549X	(19)
xxii	32 (proband)	F	2 $^{6}/_{12}$	15 $^{6}/_{12}$*	1.07	1.03	38.3	no		
xxiii	33 (proband)	F	3 $^{4}/_{12}$	8	0.36	0.61	(2.0)	?**	IVS 15 +1 g>a	(20)
xxiv	34 (proband)	F	10	8	0.88	1.05	68.7	–		
	35 (proband)	F	11 $^{10}/_{12}$	2 $^{4}/_{12}$*	0.34	0.40	50.6	–		
xxv	36 (sister)	F	8 $^{8}/_{12}$	3 $^{2}/_{12}$*	0.56	0.67	59.3	–	ex 16, G562X	(21)
	37 (mother)	F	adult	–	–	0.43	–	–		
xxvi	38 (proband)	M	6	12	0.90	0.58	37.2	–		
	39 (mother)	F	adult	–	–	–	–	–	ex 17, G579R	(22)
xxvii	40 (proband)	M	adult	–	–	0.76	–	yes?**		

1	2	3	4	5	6	7	8	9	10	
	41 (daughter)	F	5 $^{10}/_{12}$	10 $^{2}/_{12}$	0.61	0.77	– /	no		
xxviii	42 (proband)	F	3	15*	1.02	0.78	38.5	–	ex 17, G579R	(22)
xxix	43 (proband)	M	2 $^{1}/_{12}$	11 $^{11}/_{12}$	0.72	0.93	63.2	–		
xxx	44 (proband)	F	$6^{3}/_{12}$	8 $^{9}/_{12}$	0.93	1.30	47.2	–	IVS 17 +3 t ins	(23)
xxxi	45 (proband)	M	3	3	0.34	0.67	25.7	–	IVS 17 +5 g>a	(24)
xxxii	46 (proband)	F	2 $^{4}/_{12}$	6 $^{3}/_{12}$	0.72	0.78	25.3	–	ex 18, W627X	(25)
	47 (mother)	F	adult	–	–	0.58	–	–		
xxxiii	48 (proband)	F	$2^{1}/_{12}$	$2^{10}/_{12}$	0.56	0.82	80.4	no	ex 20, 1990TGAC ins	(26)
	49 (father)	M	adult	–	–	0.83	□	–		
xxxiv	50 (proband)	M	1 $^{7}/_{12}$	4 $^{4}/_{12}$	0.44	0.97	36.2	–	IVS 20 -1 g>a	(27)
	51 (proband)	M	$^{9}/_{12}$	7 $^{6}/_{12}$*	0.58	1.13	29.1	–		
	52 (brother)	M	3	8 $^{2}/_{12}$	0.43	0.90	56.2	no		
xxxv	53 (sister)	F	$^{1}/_{12}$	5 $^{6}/_{12}$*	0.96	1.65	28.2	–	ex 22, R747X	(28)
	54 (cousin)	F	10	$15^{10}/_{12}$*	0.68	0.83	18.6	–		
	55 (mother)	F	adult	–	–	0.71	–	–		
	56 (cousin's mother)	F	adult	–	–	0.84	–	–		
	57 (proband)	M	adult	–	–	–	–	no		
xxxvi	58 (daught.1)	F	1	14 $^{3/12}$	0.94	1.04	–	–	ex 22, 2245ACTC ins	(29)
	59 (daught.2)	F	1	2 $^{9}/_{12}$	1.09	1.17	–	–		

* no systematic treatment

** probably no primary defect

19 months, 0.40 to 1.30 mmol/L (mean 0.84 ± 0.19 mmol/L) in 38 children aged from 2 to 16 yrs, and from 0.43 to 0.86 mmol/L (mean 0.69 ± 0.12 mmol/L) in 14 adults. The mean phosphate level did not differ in males and females of the same age group. Pre-treatment urinary phosphate excretion expressed as $TmPO_4/GFR$ was lowered to values ranging from 0.23 to 1.09 mmol/L (mean 0.69 ± 0.22 mmol/L). Concentration of 1,25-dihydroxyvitamin D_3 was 31.2 ± 4.4 pg/mL in younger children and 42.5 ± 17.8 pg/mL in older children. The level of 1,25-dihydroxyvitamin D_3 was abnormally low in comparison with the decreased level of phosphate in affected persons. Serum phosphate concentration, urinary phosphate excretion (TmP/GFR) and 1,25-dihydroxyvitamin D_3 level varied markedly among affected families and also among affected members of the family, without strict correlation with disease severity.

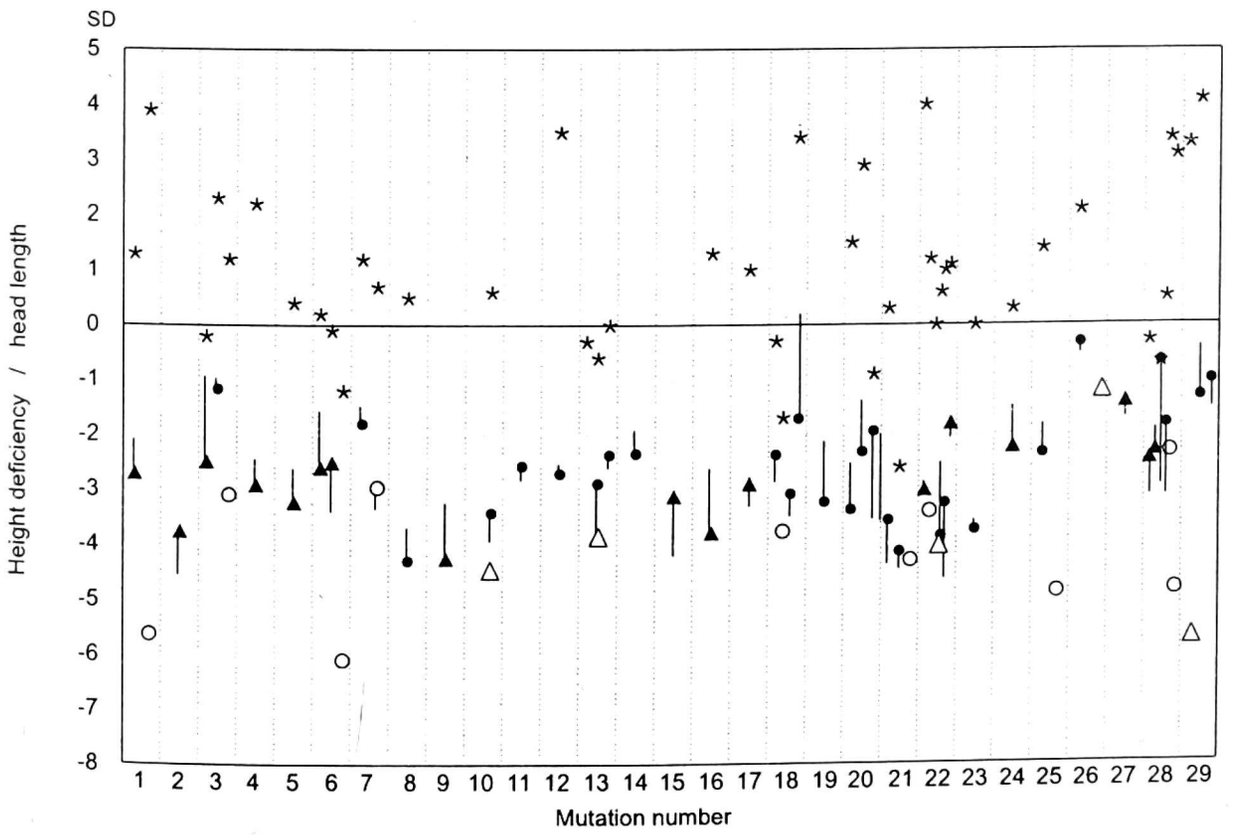

Figure 1. Correlation of height deficiency and changes in head length with location of the *PHEX* gene mutations in XLH patients

Height values are expressed in SD and shown separately for affected girls (●), boys (▲) and their affected mothers (○) or fathers (△). Black lines directed down or up indicate the direction and size of growth changes after pharmacological treatment. Stars (✶) represent head length values (in SD) in affected patients.

Degree of leg deformities was used as a simple indicator of severity of the disease, resulting directly from the impairment of bone mineralization (rickets, osteomalacia). Most of the affected patients, including children and adults, showed severe or moderate deformations. In general, they responded well to early and systematic anti-rachitic treatment. In a few cases leg deformities were mild (one boy no. 50 and three girls nos. 19, 58, 59 before treatment, and two related untreated affected mothers nos. 55, 56)

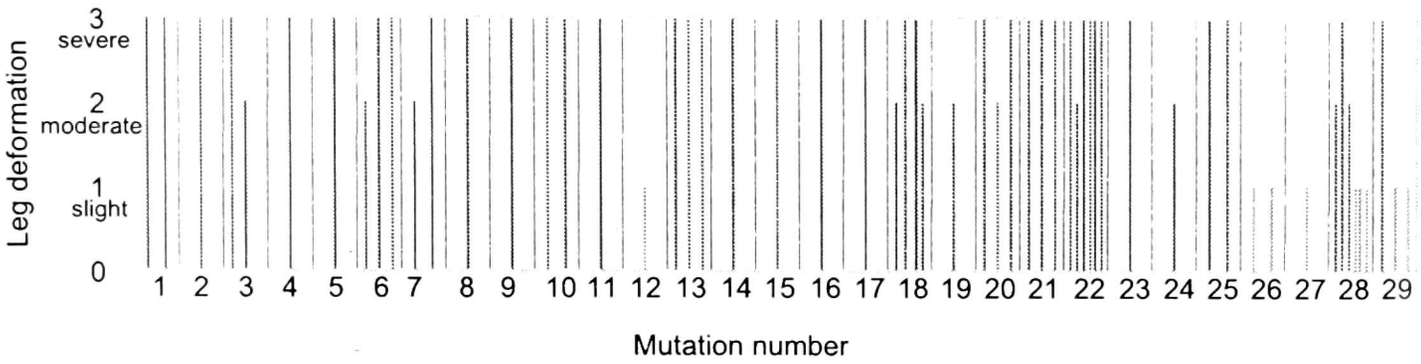

Figure 2. Analysis of degree of leg deformation in relation to localisation of the *PHEX* gene mutations identified in XLH patients

Almost all patients showed growth retardation. In younger children height ranged from –0.69 to –2.46 SD (mean value –1.45 ± 0.62 SD) before treatment, and from –0.44 to –3.16 SD (mean value –1.90 ± 1.03 SD) after treatment. In older children height ranged from –0.34 to –4.29 SD (mean value –2.80 ± 0.83 SD), and from +0.16 to –4.55 SD (mean –2.71 ± 1.15 SD) before and after treatment, respectively. The affected (untreated) adults had a mean height SD score –3.94 ± 1.35, ranging from –1.22 to –6.09. Growth retardation at the beginning seemed to be less expressed in some patients (nos. 5, 30, 34, 43, 50, 54, 58 and 59) and/or responded unusually well to the treatment (in siblings nos. 4 and 5 [brother and sister], and nos. 25, 30, 33 and 59 [all girls]). In two families (iii and xxxv) with affected children of both sexes, the boys revealed markedly more severe growth retardation before the treatment (no. 4: –2.49 SD, and nos. 51, 52: –2.46 SD, –2.31 SD, respectively) than their affected sisters (no. 5: –1.17 SD, and no. 53: –0.69 SD, respectively).

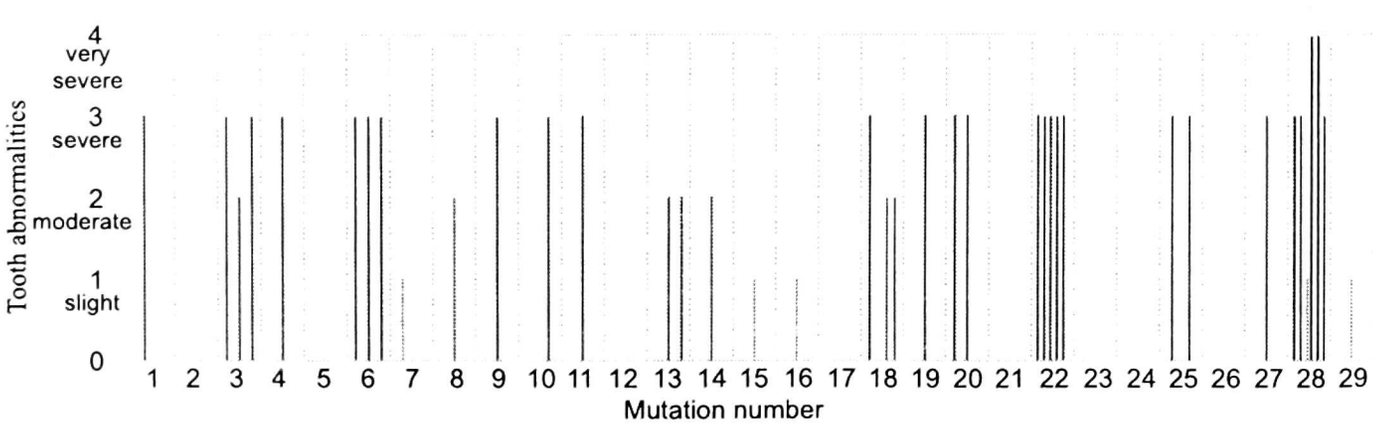

Figure 3. Comparison of tooth abnormalities in patients with different *PHEX* gene mutations

Head length, in general, was increased. A broad range of values in the examined children (from –2.6 to +4.1 SD; mean +0.93 ± 1.08 SD) and in adults (from –1.7 to +3.9 SD; mean +1.14 ± 1.70 SD) was observed. Many patients (nos. 2, 5, 7,

19, 30, 33, 38, 55, 56, 57 and 58) showed a marked increase in head length, but some others (nos. 4, 8, 14, 17, 27, 34, 45, all members of families vi, xiii, xxvii, and nos. 51-53 from family xxxv) revealed values similar to controls. It was noticed that in some cases members of the same family (e.g. iii, xxxv) presented distinctly different values of head length.

Most of the patients had moderate or severe tooth abnormalities with no differences in expression between children and their affected parents. For example in family vi two affected sons and their mother had severe tooth abnormalities. Similar observations were made in two other families, xxvi and xxxii (both carrying the same *PHEX* gene mutation). Besides, affected girls (families iii and xxxv) showed lower degrees of abnormalities (moderate and slight, respectively) than their affected brothers (severe, in each family).

Some of the children with XLH underwent audiological examination and in three boys (nos. 1, 4 and 9) sensorineural hearing loss was observed.

Correlation between type of mutations and clinical symptoms of the disease

Among the analysed patients, deletions were the most frequent causes of the disease. In two patients (nos. 14 and 25) with in-frame deletions of 6 and 15 nucleotides, respectively, severe height retardation, leg deformities and moderate/mild tooth abnormalities were observed. Only slow improvement was observed after long-term pharmacological treatment. The severe/moderate clinical symptoms support the molecular prediction that shorter proteins will be produced, one without three conserved amino acids in positions 338-340 (patient 14) and the other without five amino acids in positions 508-512 (patient 25). In one of the families (iii) with the large in-frame deletion the course of the disease was rather mild. The affected girl (no. 5) showed slightly decreased growth (–1.17 SD).The growth of her affected brother was lower but responded very well to pharmacological treatment (improvement from –2.49 to –1.01 SD).

In patients with frameshift deletions, shorter or longer fragments of the PHEX protein are expected to be synthesised. The catalytic domain and substrate binding domain are located in exons 17, 19 and 22, so all deletions placed upstream from the above region would result in a fully inactive *PHEX* gene product. In agreement with that statement, almost all of our patients having frameshift deletions in exons 3-14 showed severe or moderate leg deformities and tooth abnormalities. Height deficiency was also marked and ranged from –1.8 to –3.8 SD in affected children and from –3.1 to –6.1 SD in affected (untreated) adults.

Insertions were the least frequent type of mutations and were observed in the *PHEX* gene of three patients. In family xxxiii with duplication of a 4 bp fragment in exon 20, translation of a shorter protein without several conserved amino acids located in exons 21 and 22 is expected. Two affected members of the family (father and daughter) showed very mild leg deformities and almost normal height.

In family xxxv with another 4 bp duplication inserted in exon 22 the mutated protein contains almost the entire sequence with catalytic and substrate binding domains characteristic of the endonuclease. The unspecific C-terminal tail may to some extent influence the protein structure and reduce the PHEX enzymatic activity. Two affected sisters (nos. 58 and 59) revealed mild symptoms of the disease with slight leg deformations, low deficit in height (–1.33 SD and –1.03 SD, respectively) and slight tooth abnormalities. Both girls were diagnosed at the age of one year and the pharmacological treatment was introduced very early, when each child started to walk. The affected father of these girls (no. 57), who had never been treated, revealed much more severe clinical symptoms of the disease. It is worth noticing that this man was less handicapped than other affected fathers (nos. 16 and 40).

Splicing mutations were observed in a group of ten unrelated patients. In most cases they resulted from nucleotide substitutions and led to adjacent exon skipping, frameshift and appearance of stop codons. A G→A substitution at the beginning of intron 15 was identified in three unrelated sporadic cases and it was one of the three most frequent mutations in our group of patients. The mutated PHEX protein would be devoid of a large part of the polypeptide with catalytic and substrate binding domains. Three affected girls (nos. 32-34) with that mutation exhibited severe leg deformations and tooth abnormalities and moderate/severe growth deficiency. In agreement with predicted translation of the inactive PHEX protein, two of the girls who underwent early long-term systematic treatment revealed only slow improvement in body height and shape of legs.

Eight out of 36 unrelated patients carried nonsense mutations distributed along the gene. Depending on the stop codon localisation, a shorter or longer fragment of the PHEX protein without or with partial enzymatic activity would be formed. One nonsense mutation resulted from a substitution in nucleotide sequence coding arginine-747 situated two amino acid residues away from the end of the coding gene sequence. The mutation was identified in patient no. 51 and in five members of family xxxv. Clinical symptoms of the disease showed some intra-familial variation. Some of the differences might depend on the patient's age at diagnosis and use of pharmacological treatment. In the siblings (except a very young girl), growth deficiency at the moment of diagnosis was moderate (from –1.83 to –2.46 SD). Except one case (no. 52), growth retardation has worsened in spite of the treatment during the last 5-8 years, reaching values from –3.01 to –3.18 SD. This unsatisfactory outcome may have resulted partially from poor compliance in the regular use of phosphate and vitamin D. The leg deformities and tooth abnormalities varied in the examined children from mild to severe and both parameters were not always parallel. Two affected adult females (nos. 55, 56) showed severe tooth abnormalities and almost no visible leg deformities. The degree of growth failure showed a pronounced difference in both women (–2.34 SD and –4.84 SD). On the basis of this family it might be concluded that different phenotypic parameters in affected members of the same family are also the result of other factors

(other genes, environmental factors including life style and treatment compliance).

Patient no. 1 with a stop codon in exon 1 exhibited an inner ear defect and consequent deafness. In two other patients (nos. 4 and 9), with deletions involving the 5' terminal region of the *PHEX* gene, hearing defects were also observed. Unfortunately, not all of our patients were available for hearing examination and no final conclusion could be proposed.

In eight probands with XLH, three different missense mutations located in exons 14, 15 and 17 were identified. One of them (R510P) was carried by a female patient (no. 26) with severe leg bowing and moderate growth deficiency. Two others (G579R and P534L) belong to the most frequent mutations in the *PHEX* gene. The mutation G579R was identified in four Polish unrelated XLH patients (nos. 38, 41-43), two of them with a positive family history (families xxvi, xxvii). The course of the disease was rather severe. Leg deformities ranged from moderate to severe. Tooth abnormalities were severe in all diagnosed persons. Severe growth deficiency was resistant to the treatment. The second recurrent mutation, C1601→T, resulted in substitution of a leucine for a proline-534. Our patients carrying the mutation (nos. 27-30) showed rather severe growth deficiency (except one case) and moderate leg deformities and tooth abnormalities.

It was observed that the clinical symptoms of XLH in patients with missense mutations might depend on the conservation of mutated regions and expected residual activity of the PHEX protein. In patients with mutations resulting in frameshift and stop codons, moderate or severe clinical symptoms were observed, which in some cases did not strictly correlate with predicted PHEX protein translation and its enzymatic activity.

Discussion

The analysed patients and affected members of their families exhibited typical X-linked hypophosphatemic phenotypes, which were characterised by several biochemical and clinical features. In general, patients showed growth retardation, lowered renal tubular reabsorption of phosphate, low serum phosphate level, normal or low serum 1,25-dihydroxyvitamin D_3 concentration, increase in head length, leg deformities and tooth abnormalities (periodontal abscesses, enamel hypoplasia and crowded teeth). The degree of the symptoms was slightly influenced by early systematic treatment and to a lesser extent seemed to depend upon the type and localisation of the *PHEX* gene mutations.

In our own experience based on long-term research on more than one hundred XLH patients, growth failure is a primary defect of XLH and is only slowly influenced by treatment, irrespective of its kind, compliance, age at the beginning and duration (SYKUT-CEGIELSKA 1995). Growth retardation was rather severe in most patients and it was only slightly influenced by the type of mutation. Standard

deviation of body height for the same mutation in patients of the same sex reached 1.5 SD in the case of different families and only 0.1-0.5 SD in the case of members of the same family. In two families with affected children of both sexes, the boys displayed more severe growth retardation than their sisters, which seems to point to a gene dosage effect. Deficiency in body height was less pronounced in treated children than in untreated adults, showing that pharmacological treatment partially influenced the final body height of the patients.

Similarly, tooth abnormalities were severe in almost all examined patients with XLH. There were no marked differences between children and their affected mothers and fathers. Moderate abnormalities in teeth were observed in patients carrying one of the mutations localised in exons 6, 9, 11, 12, 14 or 15. Besides, affected girls (from two families) showed a lower degree of abnormalities than their affected brothers, which correlated with lesser growth retardation in those girls.

Anthropometric measurement showed increased head length in several patients with XLH. Most of the patients with mutations in the first three exons (region with transmembrane domain) or the last nine exons (region with specific substrate-binding and catalytic domains) revealed a pronounced increase in head length (from +1.0 to +4.0 SD). The mutations in the middle region of the *PHEX* gene seemed to have only a slight influence on this parameter (from –1.0 to +1.0 SD). The correlation is not quite as strong in all cases. Clearly distinct values of head length observed in some affected members of the same family carrying the same mutation seem to indicate that the parameter is influenced not only by PHEX protein activity, but by other genetic and environmental conditions, as well.

The hearing defect observed in three boys was linked with mutations located in the beginning fragment of the gene (exons 1-5). Unfortunately, only one affected patient from each family was available for the audiological examination, so we were not able to show a strict correlation between deafness, sex of the affected patient and type of mutation. The occurrence of deafness in a boy with nucleotide substitution in exon 3 (C77S) has been reported by ROWE et al. 1997. Inner ear defect was also observed in the Gy mouse with classic hypophosphatemic rickets in which a deletion of exons 1-3 in *Phex* gene and a large deletion in the spermine synthase gene were identified (DU et al. 1996, STROM et al. 1997, MEYER et al. 1998).

Most of the mutations identified in Polish patients and localised to the beginning of the *PHEX* gene would be expected to result in frameshift or stop codons and, as a consequence, in a severe form of the disease. That is true for several parameters reflecting proper bone remodelling, turnover and mineralization, such as body height, head length, leg deformities and tooth abnormalities. Changes observed in all of these characteristics were assessed as severe or, at least, moderate.

The exact function of the *PHEX* gene is still unknown. It was found that *PHEX/Phex* mRNA is expressed predominantly in human foetal and murine adult calvaria, long bone and tooth, and, to a lesser extent, also in foetal lung, skeletal muscle, brain, ovary, testis and liver (DU et al. 1996, BECK et al. 1997, GRIEFF et

al. 1997, LIPMAN et al. 1998, RUCHON et al. 1998). Level of *PHEX* mRNA expression in human foetal tissue is at least two orders of magnitude lower than of other genes like β-actin. This may be the reason for the observed difficulties in determining the correct *PHEX/Phex* gene expression pattern. The product of the *PHEX* gene is a membrane-bound endopeptidase (LIPMAN et al. 1998). It presumably participates in the processing of phosphaturic factor, MEPE (ROWE et al. 2000), which is expected to control mineralization and regulate renal phosphate reabsorption and 1,25-dihydroxyvitamin D_3 metabolism.

Some disproportion in the involvement of different phenotypic parameters observed by us seems to point to the probability of alternative splicing of the *PHEX* gene. For example, severe growth deficiency but relatively slight influence on teeth and the shape of the head was observed in some families (family xiii, W403X; family xviii, P534L). This might indicate that the later processes are modulated by a shorter form of transcript devoid of its middle part. Alternative splicing and formation of several transcripts has been described for many genes, for example the *DMD* gene located on the short arm of chromosome X (WORTON, BROOKE 1995).

GUO and QUARLES (1997) showed that the *PHEX* gene is expressed in differentiated osteoblasts. An osteoblast defect in XLH patients may be a reason for the ineffectiveness of treatment with phosphate and vitamin D_3 in normalising bone mineralization. Our XLH patients, receiving an active metabolite of vitamin D_3 and phosphate supplementation from the time of diagnosis to puberty, showed only partial correction of skeletal lesions. Improvement was manifested by a slight increase in the children's growth (not in all patients) and more significant normalisation of bowing legs

In summary, we can conclude that almost all patients with XLH revealed growth retardation, leg deformities, tooth abnormalities, change in head length, low serum phosphate level, lowered renal tubular reabsorption of phosphate, and normal or low serum 1,25-dihydroxyvitamin D_3 concentration. Comparison of patient genotype and phenotype revealed the existence of only slight correlation between clinical symptoms and the type and localisation of *PHEX* gene mutations. Severe or moderate growth retardation in almost all patients indicate that the whole gene sequence is necessary for correct mineralization in longitudinal bones. Tooth structure and head length were more influenced by mutations localised at the beginning (region with transmembrane domain) and the end of the gene (region with specific substrate-binding and catalytic domains). Hearing defect was observed only in patients with mutations located in the beginning fragment of the gene. The above results seem to point to the probability of the alternative splicing of the *PHEX* gene in various tissues.

Identification of the *PHEX* gene mutations in the XLH patients generally indicates that the gene plays an important role in regulating phosphate uptake, vitamin D_3 metabolism and bone remodelling and mineralization processes. The exact pathogenesis of the disease with aspects of *PHEX* gene expression tissues, proba-

bility of different transcript occurrence and types of PHEX substrates still need to be elucidated.

Acknowledgements. This work was partially supported by the State Committee for Scientific Research in Poland, Grant No. 4PO5E13714.

REFERENCES

ARASIMOWICZ E. (2000). Shape and proportion of the head in patients with hypophosphatemic rickets. Ph. D. Thesis, Adam Mickiewicz University, Poland.

BECK L., SOUMOUNOU Y., MARTEL J., KRISHNAMURTHY G., GAUTHIER C., GOODYER C.G., TENENHOUSE H.S. (1997). 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. 99: 1200-1209.

BONEH A., TENENHOUSE H.S. (1990). Protein kinase C in mouse: effect of the hyp mutation and phosphate deprivation. Kidney Int. 37: 682-688.

DIXON P.H., CHRISTIE P.T., WOODING C., TRUMP D., GRIEFF M., HOLM I., GERTNER J.M., SCHMIDTKE J., SHAH B., SHAW N., SMITH C., TAU C., SCHLESSINGER D., WHYTE M.P., THAKKER R.V. (1998). Mutational analysis of *PHEX* gene in X-linked hypophosphatemia. J. Clin. Endocrinol. Metab. 83: 3615-3623.

DU L., DESBARATS M., VIEL J., GLORIEUX F.H., CAWTHORN C., ECAROT B. (1996). cDNA cloning of the murine *Pex* gene implicated in X-linked hypophosphatemia and evidence for expression in bone. Genomics 36: 22-28.

ECAROT B., GLORIEUX F.H., DESBARATS M., TRAVERS R., LABELLE L. (1992). Defective bone-formation by Hyp mouse bone-cells transplanted into normal mice: Evidence in favor of an intrinsic osteoblast defect. J. Bone. Miner. Res. 7: 215-220.

FILISETTI D., OSTERMANN G., VON BREDOW M., STROM T., FILLER G., EHRICH J., PANNETIER S., GARNIER J.M., ROWE P., FRANCIS F., JULIENNE A., HANAUER A., ECONS M.J., OUDET C. (1999). Non random distribution of mutations in the *PHEX* gene, and under-detected missense mutations at non-conserved residues. Eur. J. Hum. Genet. 7: 615-619.

FRANCIS F., STROM T.M., HENNIG S., BODDRICH A., LORENZ B., BRANDAU O., MOHNIKE K.L., CAGNOLI M., STEFFENS C., KLAGES S., BORZYM K., POHL T., OUDET C., ECONS M.J., ROWE P.S.N., REIHARDT R., MEITINGER T., LEHRACH H. (1997). Genomic organization of the human *PEX* gene mutated in X-linked dominant hypophosphatemic rickets. Genome Research 7: 573-585.

GLORIEUX F.H., SCRIVER C.R., READE T.M., GOLDMAN H., ROSENBOROUGH A. (1972). Use of phosphate and vitamin D to present dwarfism and rickets in X-linked hypophosphatemia. N. Eng. J. Med. 287: 481-487.

GRĄDZKA I., ŁUKASZKIEWICZ J., LORENC R. (1988). Application of the rat thymus receptor in a specific sensitive and simplified assay of 1,25 dihydroxyvitamin D in blood serum. Biochim. Biophys. Acta 965: 52-59.

GRIEFF M., MUMM S., WAELTZ P., MAZZARELLA R., WHYTE M.P., THAKKER R.V., SCHLESSINGER D. (1997). Expression and cloning of the human X-linked hypophosphatemia gene cDNA.Biochem Biohys. Res. Commun. 231: 635-639.

GUO R., QUARLES L.D. (1997). Cloning and sequencing of human *PEX* from a bone cDNA library: Evidence for its developmental stage-specific regulation in osteoblasts. J. Bone. Miner. Res. 12: 1009-1017.

HOLM I.A., HUANG X., KUNKEL L.M. (1997). Mutational analysis of the *PEX* gene in patients with X-linked hypophosphatemic rickets. Am. J. Hum. Genet. 60: 790-797.

HYP CONSORTIUM, FRANCIS F., HENNIG S., KORN B., REINHARDT R., DE JONG D., POUSTKA A., LEHRAH H., ROWE P.S.N., GOULDING J.N., SUMMERFIELD T., MOUNTFORD R.C., READ A.P., POPOWSKA E., PRONICKA E., DAVIES K.E., O'RIORDAN J.L.H., ECONS M.J., NESBITT T., DREZNER M.K., OUDET C., PANNETIER S., HANAUER A., STROM T.M., MEINDL A., LORENZ B., CAGNOLI M., MOHNIKE K.L., MURKEN J., MEITINGER T. (1995). A gene (*PEX*) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. Nature Genet. 11: 130-136.

LIPMAN M.L., PANDA D., BENNETT H.P., HENDERSON J.E., SHANE E., SHEN Y., GOLTZMAN D., KARAPLIS A.C. (1998). Cloning of human *PEX* cDNA. Expression, subcellular localization, and endopeptidase activity. J. Biol. Chem. 273: 13729-13737.

MCKUSICK V.A. (1994). Mendelian inheritance in man: Catalogues of human genes and genetic disorders., 11th edn. Johns Hopkins University Press, Baltimore.

MEYER R.A., HENLEY C.M., MEYER M.H., MORGAN P.L., MCDONALD A.G., MILLS C., PRICE D.K. (1998). Partial deletion of both the spermine synthase gene and the *PEX* gene in the X-linked hypophosphatemic, gyro (Gy) mouse. Genomics 48: 289-295.

PETERSEN D.J., BONIFACE A.M., SCHRANCK F.W., RUPICH R.C., WHYTE M.P. (1992). X-linked hypophosphatemic rickets: a study (with literature review) of linear growth response to calcitriol and phosphate therapy. J. Bone Miner. Res. 583-597.

POPOWSKA E., PRONICKA E., SUŁEK A., JURKIEWICZ D., ROWE P., ROWIŃSKA E., KRAJEWSKA-WALASEK M. (2000). X-linked hypophosphatemia in Polish patients. 1. Mutations in the *PHEX* gene. J. Appl. Genet. 41: 293-302.

RASMUSSEN H., TENENHOUSE H.S. (1995). Mendelian hypophosphatemias. In: The metabolic and molecular bases of inherited disease. 7th edn. (Scriver C.R., Beaudet A.L., Sly W.S., Valle D., eds), McGraw-Hill, New York: 3717-3745.

ROWE P.S.N., DE ZOYSA P.A. DONG R., WANG H.R., WHITE K.E., ECONS M.J., OUDET C.L. (2000). *MEPE*, a new gene expressed in bone marrow and tumors causing osteomalacia. Genomics 67: 54-68.

ROWE P.S.N., OUDET C.L., FRANCIS F., SINDING C., PANNETIER S., ECONS M.J., STROM T.M., MEITINGER T., GARABEDIAN M., DAVID A., MACHER M.-A., QUESTIAUX E., POPOWSKA E., PRONICKA E., READ A.P., MOKRZYCKI A., GLORIEUX F.H., DREZNER M.K., HANAUER A., LEHRACH H., GOULDING J.N., O'RIORDAN J.L.H. (1997). Distribution of mutations in the *PEX* gene in families with X-linked hypophosphatemic rickets (HYP). Hum. Mol. Genet. 6: 539-549.

RUCHON A.F., MARCINKIEWICZ M., SIEGFRIED G., TENENHOUSE H.S., DESGROSEILLERS L., CRINE P., BOILEAU G. (1998). *Pex* mRNA is localized in developing mouse osteoblasts and odontoblasts. J. Histochem. Cytochem. 6: 459-468.

SEIKALY M.G., BROWNE R.H., BAUM M. (1994). The effect of phosphate supplementation on linear growth in children with X-linked hypophosphatemia. Pediatrics 94: 478-81.

SHIELDS E.D., SCRIVER C.R., READE T., FUJIWARA T.M., MORGAN K., CHIAMPI A., SCHWARTZ S. (1990). X-linked hypophosphatemia: The mutant gene is expressed in teeth as well as in kidney. Am. J. Hum. Genet. 46: 434-42.

STROM T.M., FRANCIS F., LORENZ B., BODDRICH A., ECONS M.J., LEHRACH H., MEITINGER T. (1997). *Pex* gene deletions in Gy and Hyp mice provide mouse models for X-linked hypophosphatemia. Hum. Mol. Genet. 6: 165-171.

SUŁEK A., POPOWSKA E., ROWE P.S.N., GOULDING J., ROWIŃSKA E., PRONICKA E. (1998). Molecular diagnosis of *PEX* gene mutations in Polish patients with X-linked hypophosphatemic rickets. Acta Med. Lituanica 5: 64-67.

SYKUT-CEGIELSKA J. (1995). Effect of different medical treatment on growth and leg deformities in children with hypophosphatemic rickets and other bone deformities. MD Thesis, Children's Memorial Health Institute, Warsaw, Poland.

TYYNISMAA H., KAITILA I., NANTO-SALONEN K., ALA-HOUHALA M., ALITALO T. (2000). Identification of 15 novel *PHEX* gene mutations in Finnish patients with hypophosphatemic rickets. Hum. Mutat. (Online) 15: 383-384.

VERGE C.F., LAM A., SIMPSON J.M., COWELL C.T., HOWARD N.J., SILINK M. (1991). Effects of therapy in X-linked hypophosphatemic rickets. N. Engl. J. Med. 325: 1843-1848.

WHYTE M.P., SCHRANCK F.W., ARMAMENTO-VILLAREAL T. (1996). X-linked hypophosphatemia: A search for gender, race, anticipation, or parent of origin effects on disease expression in children. J. Clin. Endocrinol. Metab. 81: 4075-4080.

WORTON R.G., BROOKE M.H. (1995). The X-linked muscular dystrophies. In: The metabolic and molecular bases of inherited disease. 7th ed. (Scriver C.R., Beaudet A.L., Sly W.S., Valle D., eds), McGraw - Hill, New York: 4195-4226.

YAMAMOTO T., ECAROT B., GLORIEUX F.H. (1992). Abnormal response of osteoblasts from Hyp mice to 1,25-dihydroxyvitamin D_3. Bone 13: 209-215.