endocrines

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Review

Pathogenic Variants of the *PHEX* Gene

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Abstract: Twenty-five years ago, a pathogenic variant of the phosphate-regulating endopeptidase homolog X-linked (*PHEX*) gene was identified as the cause of X-linked hypophosphatemic rickets (XLH). Subsequently, the overproduction of fibroblast growth factor 23 (FGF23) due to *PHEX* defects has been found to be associated with XLH pathophysiology. However, the mechanism by which PHEX deficiency contributes to the upregulation of FGF23 and the function of PHEX itself remain unclear. To date, over 700 pathogenic variants have been identified in patients with XLH, and functional assays and genotype–phenotype correlation analyses based on pathogenic variant data derived from XLH patients have been reported. Genetic testing for XLH is useful for the diagnosis. Not only have single-nucleotide variants causing missense, nonsense, and splicing variants and small deletion/insertion variants causing frameshift/non-frameshift alterations been observed, but also gross deletion/duplication variants causing copy number variants have been reported as pathogenic variants in *PHEX*. With the development of new technologies including next generation sequencing, it is expected that an increasing number of pathogenic variants will be identified. This chapter aimed to summarize the genotype of *PHEX* and related analyses and discusses the pathophysiology of PHEX defects to seek clues on unsolved questions.

Keywords: X-linked hypophosphatemic rickets; phosphate-regulating endopeptidase homolog X-linked; fibroblast growth factor 23; genotype–phenotype correlation; multiplex ligation-dependent probe amplification; nonsense-mediated decay; cryptic splice site; mosaicism; zinc-binding site; truncating variant

Citation: Ohata, Y.; Ishihara, Y. Pathogenic Variants of the *PHEX* Gene. *Endocrines* **2022**, *3*, 498–511. https://doi.org/10.3390/endocrines3030040

Academic Editors: Yukihiro Hasegawa and Seiji Fukumoto

Received: 21 May 2022
Accepted: 4 August 2022
Published: 8 August 2022

1. Introduction

Previously, genetic linkage analyses have revealed the pathogenic variants of the gene associated with the disorder X-linked hypophosphatemic rickets (XLH) located in Xp22 [1]. In 1995, the HYP consortium defined the XLH locus using a positional cloning approach and identified the phosphate-regulating endopeptidase homolog X-linked (*PHEX*) gene in this region [2]. *Hyp*, *Gy*, and *Ska1* mice have been identified as model mice for studying XLH, and it was later revealed that these mice harbor pathogenic variants in the mouse *Phex* homolog [3–5]. XLH is inherited in an X-linked dominant manner, with complete penetrance. The female-to-male ratio is approximately 2:1, and there is no male-to-male transmission. Genetic testing for XLH is available and can be used for differential diagnosis, especially when the inheritance pattern is unclear [6]. To date, 729 different *PHEX* variants available on the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php, accessed on 7 May 2022) have been reported as a cause of XLH. Recently, a new PHEX variant database (*PHEX* Locus Specific Database [LSDB] sponsored by UltraGenyx Pharmaceutical Inc.: https://www.rarediseasegenes.com/, accessed on 7 May 2022) has been established using four data sources including an old database [7],

results from a sponsored genetic testing program [8], unpublished variants identified in previous burosumab clinical studies, and published variant data collected in a literature review [9]. The number of reported *PHEX* pathogenic variants is increasing.

Patients with XLH have hypophosphatemia, phosphaturia, and low or inappropriately normal 1, 25-dihydroxy vitamin D (1,25$[OH]_2$D) levels caused by high levels of fibroblast growth factor 23 (FGF23) [10]. In renal tubules, FGF23 increases phosphate excretion in urine by downregulating type 2a and 2c sodium phosphate cotransporters, which reabsorb phosphate. In vitamin D metabolism, FGF23 downregulates 1-alpha-hydroxylase, which converts 25 hydroxy-vitamin D to 1,25$(OH)_2$D, the active form of vitamin D. FGF23 also upregulates 24-hydroxylase, which converts 1,25$(OH)_2$D to 24, 25-dihydroxy vitamin D, an inactive form of vitamin D. Therefore, excess FGF23 suppresses vitamin D activity and phosphate absorption from the intestine, which also contributes to hypophosphatemia in patients with XLH [11–13]. In addition to *PHEX*, the pathogenic variants of dentin matrix protein 1 (*DMP1*), *FGF23*, ectonucleotide pyrophosphatase phosphodiesterase-1 (*ENPP1*), and *FAM20C* can lead to the overproduction of FGF23 and cause hypophosphatemic rickets [14–19].

Although it has been shown that PHEX deficiency leads to the overproduction of FGF23, which contributes to the pathogenesis of XLH, the mechanism by which an abnormality in PHEX causes an increase in FGF23 levels remains to be elucidated. Rowe et al. showed that PHEX bound to matrix extracellular phophoglycoprotein (MEPE), which belongs to a group of extracellular matrix proteins (small integrin-binding ligand, N-linked glycoproteins [SIBLINGs]) involved in bone mineralization. MEPE contains an acidic serine–aspartate-rich MEPE-associated motif (ASARM) and the ASARM peptide released from MEPE negatively affects mineralization and phosphate uptake [20]. The ASARM motif is also present in other SIBLINGs including DMP1 and osteopontin. Martin et al. reported that the degradation of SIBLINGs and release of ASARM peptides were responsible to the impaired mineralization in XLH [21]. *Hyp* mice harboring deletions in the 3′ region of *Phex* have high levels of FGF23 and hypophosphatemia with inappropriately normal 1,25$(OH)_2$D levels similar to that in patients with XLH [22]. *Fgf23* mRNA expression is increased in *Hyp* mouse bones and in osteoblasts and osteocytes isolated from these mice [22,23]. Sitara et al. generated hyperphosphatemic *Fgf23* null mice and crossed them with hypophosphatemic *Hyp* mice and showed the same phenotype [24], which suggested both defects are involved in the same pathway. These findings indicate that FGF23 may function downstream of PHEX; however, the precise mechanism is not fully understood. *PHEX* is predominantly expressed in osteoblasts, osteocytes, and odontoblasts, but not in kidney tubules [25], and it encodes a protein that structurally resembles the M13 family of membrane-binding metalloproteases. Neutral endopeptidase 24.11 or neprilysin (NEP) and endothelin converting enzyme-1 [2] belong to this family of metalloproteases, which are type II integral membrane glycoproteins containing a large extracellular domain that retains catalytic activity [25]. It has been postulated that the large extracellular domain of PHEX contains a zinc-binding motif which is essential for the catalytic activity in NEP [2]. Since the members of this family are known to cleave small peptides, FGF23 was initially considered to serve as a substrate for PHEX and degraded by PHEX [26]. However, several studies have revealed that FGF23 is not a substrate for PHEX [27–29], and the endogenous PHEX protein substrate remains to be verified. To identify any clues to clarify the function of PHEX and the pathogenesis of how PHEX deficiency causes XLH, we reviewed data on the *PHEX* genotype based on published papers.

2. Pathogenic Variants of the *PHEX* Gene

We reviewed 97 papers that were obtained from the HGMD database, including 55 case reports in which pathogenic variants of *PHEX* were described. Among these reports, 252 missense or nonsense, 117 splicing, 155 small deletions, 86 small insertions or duplications, 13 deletion/insertions (delins), 80 gross deletions, 16 gross insertions, 4 regulatory,

and 6 complex rearrangement variants have been reported to cause XLH. The analyses of pathogenic variants other than those in case reports are summarized in Table 1.

Table 1. List of studies describing *PHEX* genotypes other than case reports.

Author	Year	Probands	Variant Positive	Variant Positivity Rate (%)	Variants	Reference
Rowe	1997	106	NR	83	NR	[30]
Francis	1997	43	33	77	26	[31]
Holm	1997	22	9	41	9	[32]
Dixon	1998	68	31	46	31	[33]
Filisetti	1999	22	22	100	22	[34]
Tyynismaa	2000	20	19	95	18	[35]
Popowska	2000	35	35	100	29	[36]
Holm	2001	41	22	54	20	[37]
Christie	2001	11 [a]	1	NA	NR	[38]
Cho	2005	17	8	47	7	[39]
Song	2007	15	9	60	8	[40]
Ichikawa	2008	26	26	100	18	[41]
Gaucher	2009	118	93	79	NR	[42]
Clausmeyer	2009	71 [b]	37	52	28	[43]
Morey	2011	36	36	100	34	[44]
Ruppe	2011	46	27	59	27	[45]
Jap	2011	9	5	56	5	[46]
Quinlan	2012	46	38	83	24	[47]
Beck-Nielsen	2012	24	21	88	20	[48]
Kinoshita	2012	27	26	96	17	[49]
Lee	2012	6	6 [c]	NA	4	[50]
Durmaz	2013	6	6	100	6	[51]
Yue	2014	9	9	100	10	[52]
Capelli	2015	26	22	84	19	[53]
Zhang	2015	13	9	69	9	[54]
Rafaelsen	2016	19	15	79	13	[55]
Li	2016	18	18	100	17	[56]
Guven	2017	9	7	78	7	[57]
Acar	2018	15	12	80	12	[58]
Chesher	2018	35	35	100	37	[59]
Gu	2018	86	7	NA	NR	[60]
Marik	2018	32	8	25	NR	[61]
Hernández-Frías	2019	22	22 [c]	NA	NR	[62]
Zhang	2019	216	216 [c]	NA	166	[63]
Lin	2020	76	61	80	51	[64]
Zheng	2020	53	53 [c]	NA	47	[65]
Baroncelli	2021	24	24	100	NR	[66]
Ishihara	2021	28	28 [c]	NA	23	[67]
Jiménez	2021	17	17	100	16	[68]
Lin	2021	105	105 [c]	NA	88	[69]
Park	2021	50	47	94	48	[70]
Rodríguez-Rubio	2021	39	39 [c]	83	NR	[71]

[a] The authors analyzed only probands for which no pathogenic variant was identified in the *PHEX* coding region. [b] All patients were counted. [c] The authors evaluated only probands for which pathogenic variants were confirmed. NA: not applicable. NR: not reported.

From these data, pathogenic variants of *PHEX* has been found to be located across the entire gene [44], which is consistent with data from the *PHEX* LSDB [9]. Sarafrazi et al. described the mapping data of *PHEX* pathogenic variants [9]. After excluding reports in which only genetic variants confirmed probands were analyzed, the median positive rate of genetic analysis was 83% (interquartile range: 59.3, 100). Rush et al. reported that approximately 10% of clinically diagnosed XLH patients had no variant of *PHEX* in a hypophosphatemia genetic testing program [8]. Owing to the high positivity

rate, genetic testing for XLH is useful for diagnosis. Not only single nucleotide variants causing missense, nonsense, and splicing variants and small deletion/insertion variants causing frameshift/non-frameshift alteration, but also gross deletion/duplication variants causing copy number variants (CNV) have been reported as pathogenic variants of *PHEX* [43,44,48,49,53,57–59,63,64,67]. The CNV ranges from 3.8–23% in these reports. Sanger sequencing-based entire gene analysis and gene panel tests are performed as a genetic testing tool for *PHEX*. Multiplex ligation-dependent probe amplification (MLPA) often complements these methods to detect CNV [72]. Since a certain number of CNV has been reported in XLH, MLPA should be considered if any variants are not identified by Sanger sequencing or gene panel testing. Advances in next generation sequencing (NGS)-based whole exome or whole genome sequencing are reducing the cost and time taken for sequencing [72]. In *PHEX* analysis, whole exome sequencing has been used in certain studies [68,73,74]. It is expected that NGS-based methods will eventually replace conventional sequencing methods.

2.1. Mosaicism

In some studies, several mosaicism cases have been found only in male patients with XLH [43,63,64,75–77]. In a Chinese cohort study, de novo mosaic variants have been identified in 6.15% of probands [64]. Lin et al. reported the first case of isolated germline mosaicism in which a heterozygous pathogenic variant was initially detected in the *PHEX* gene in a girl with XLH and was not found in her healthy parents based on gDNA from peripheral blood. Since her father had an occasional abnormality in his serum phosphate level, they conducted an additional genetic analysis using gDNA from eight different tissues of the father. They found the same pathogenic variant with the proband only in the sperm, while there was no variant in the hair, oral epithelium, saliva, nail, cuticle, whole blood, or urine [75]. Since the penetrance of XLH is considered to be 100%, the inheritance pattern can be determined from family history. However, the possibility of an isolated germline mosaic should be considered during genetic counseling [64]. Notably, the *PHEX* mosaic variants have been found in male patients alone. Since female patients usually harbor heterozygous pathogenic variants, mosaicism in women may be missed. In contrast, male patients usually have hemizygous variants and the mosaicism is apparent seen in heterozygous variants and can be detected.

2.2. Splice Site Variants

Using the HGMD database, 117 variants affecting mRNA splicing were identified. Almost all of these variants are located at the splicing junctions of the first two or last two nucleotides at the beginning or end of the exon, respectively. These variants result in exon skipping; if the number of nucleotides in the deleted exon is not a multiple of three, this alteration leads to a frameshift and produces a truncated protein owing to a new stop codon [72]. However, several *PHEX* variants have been reported to be located outside the canonical splicing junction. To clarify the effect of these variants on splicing, BinEssa et al. investigated 13 previously reported variants located outside the splicing junction consisting of canonical GT-AG dinucleotide splice donor or acceptor sites. The constructs were transfected into HEK293 cells and pre-mRNA splicing was analyzed using a reverse transcription polymerase chain reaction (RT-PCR) and sequencing. They found that 8 out of 13 variants, including c.1701-16T>A, result in complete exon skipping, and two variants (c.436+6T>C and c.1586+6T>C) cause a partial splicing error (60% exon skipping occurred in both variants). The c.1645+5G>A and c.1645+6 variants lead to 72 bp intron retention by activating the cryptic splice donor site located 70 bp downstream from the canonical splice donor site. Notably, c.437-3C>G resulted in an in-frame deletion due to activation of the adjacent cryptic splice acceptor site. The authors concluded that non-canonical spice site variants should not be missed when they are located within 50 bp from the exon–intron boundary [78]. Zou et al. described the c.633+12del variant of *PHEX* as a pathogenic variant for XLH. They analyzed *PHEX* mRNA extracted from the

peripheral blood leukocytes of a patient and revealed that c.633+12del leads to a frameshift resulting from alternative splicing using a cryptic donor splice site. They concluded that the c.633+12del variant activates nearby cryptic 5′ splice sites [79]. Such variants located in deep intron should be evaluated because they can alter mRNA transcription.

2.3. Nonsense Mediated mRNA Decay (NMD)

Many nonsense variants lead to disease by degrading mRNA via NMD [80–82]. Most pathogenic variants of PHEX detected in XLH are nonsense, frame-shift, splice site, and delins variants, which may result in either truncated proteins or degradation of mRNA via NMD [83]. NMD can be activated via several mechanisms. If a premature stop codon is located >50–55 nucleotides upstream from a final exon–exon junction, with an exon junction complex located approximately 24 nucleotides upstream of the junction, it is sufficiently far from the stop codon and cannot be removed by the terminating ribosome and NMD can occur [82]. However, Li et al. identified the p.Trp403* variant of an XLH family member located 939 nucleotides upstream from the last exon–exon junction, and they revealed that the variant does not undergo mRNA decay by showing that mRNA expression level was not reduced [84]. Functional analysis, as discuss later, is needed to determine whether NMD actually occurs in each nonsense variant. It is important to determine whether mRNA-containing pathogenic variants are degraded by NMD when we discuss the phenotype–genotype correlation. Therefore, the accumulation of such data is valuable.

*2.4. c.*231A>G Variant*

Four variants causing regulatory abnormalities have been reported: c.*231A>G [41,85,86], c.349+11149A>T, c.1482+3997G>A, and c.1646-9276T>G [62]. Ichikawa et al. initially reported six XLH probands harboring c.*231A>G, a novel non-coding single nucleotide substitution variant located in the 3′-untranslated region (UTR) and 3 bp upstream of the putative polyadenylation signal. They also conducted allele-specific PCR in 440 healthy individuals and showed that no controls harbor c.*231A>G. Although this variant has been postulated to affect posttranscriptional transport and translation of mRNA, they did not perform functional analysis to determine whether it can alter the polyadenylation of *PHEX* mRNA [41]. Mumm et al. reported that all individuals with c.*231A>G have exon 13–15 duplication [87]. Rush et al. suggested that exon 13–15 duplication may contribute to the pathogenesis of XLH in these patients because one patient carried the duplication without *231A>G [8]. However, it is possible that *231A>G may facilitate the duplication of exon 13–15 and indirectly contribute to the pathophysiology of XLH. Further investigations are required to clarify the pathogenicity of *231A>G.

3. Functional Analysis Based on Pathogenic Variants Associated with XLH

Functional analyses of pathogenic variants have been conducted in certain studies. Based on the amino acid sequence of PHEX, it has been hypothesized that PHEX is a transmembrane glycoprotein containing a short N-terminal cytoplasmic region, single N-terminal transmembrane region, and large extracellular C-terminal domain [88]. The PHEX protein is thought to contain multiple glycosylation, enzymatic active, and zinc-binding sites [9]. Although the precise function of PHEX has not been determined, the glycosylation status, endopeptidase activity, and intracellular trafficking have been investigated in mutant PHEX via functional analysis because it is homologous to the M13 zinc metallopeptidases, which function as extramembrane endopeptidases [89]. Sabbagh et al. generated three disease-causing missense variant *PHEX* cDNAs via PCR mutagenesis, including p.Cys85Arg, p.Gly579Arg, and p.Ser711Arg, identified in patients with XLH. They transfected wild-type and mutant PHEX cDNAs into HEK293 cells and showed that these mutants were not appropriately glycosylated because they were fully sensitive to endoglycosidase H digestion. They also showed that these mutants accumulate in the endoplasmic reticulum (ER) and targeting to the plasma membrane is disrupted [88].

Zheng et al. also analyzed 10 *PHEX* variants in the expression of mutant proteins, cellular trafficking, and endopeptidase activity. They showed that certain nonsense variants, including p.Arg567*, p.Gln714*, and p.Arg747*, are not degraded by NMD and produce mutant proteins with relatively lower molecular weights that have trafficking defects. They also evaluated seven non-truncating variants and revealed that p.Cys77Tyr, p.Cys85Ser, p.Ile281Lys, p.Ile333del, p.Ala514Pro, and p.Gly572Ser mutants accumulate in cells and are not secreted into the medium, whereas the p.Gly553Glu mutant is normally secreted; however, the endopeptidase activity is reduced [65]. Since this variant has been predicted as a pathogenic variant using the American College of Medical Genetics interpretation software [90,91], their results indicated that such defects in endopeptidase activity can result in XLH pathogenesis. Li et al. identified a novel missense variant (p.Phe727Leu) in *PHEX* in patients with XLH and revealed that the mutant is glycosylated inappropriately. They showed that the intracellular transport is blocked and the mutant protein is retained in the ER. Finally, they measured the concentration of FGF23 in the conditioned medium and reported that the level of FGF23 is elevated in the medium with mutant transfected cells when compared to that in the control sample [74]. These findings suggested that those PHEX deficiencies, including abnormalities in glycosylation, can cause an increase of FGF23 expression.

Li et al. reported a p.Trp403* variant in a large Chinese family with XLH. To evaluate the function of this variant, they examined the p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathways, because Greenblatt et al. showed that these pathways are involved in osteoblastic differentiation and maintenance of bone structure and function [92]. They overexpressed wild-type or mutant *PHEX* in HEK293 cells and confirmed that phosphorylation of p38 MAPK is significantly decreased in cells transfected with mutant *PHEX*, while the phosphorylation of ERK1/2 is comparable. Based on these data, they concluded that this variant of PHEX causes XLH by downregulating the p38 MAPK signaling pathway [84]. Further studies are needed to confirm whether defects in the p38 MAPK signaling are derived from mutant *PHEX* and cause XLH.

4. Genotype–Phenotype Relationship

4.1. Gene Dosage Effect

Previously, comprehensive clinical studies on untreated adults with XLH suggested that radiographic abnormalities are generally more severe in men than in women, which is explained by X-chromosome inactivation [93]. Theoretically, heterozygous females should have a less severe phenotype because approximately half of the normal alleles remain, whereas males have none [56]. However, it is not clear whether such a gene dosage effect is involved in the XLH phenotype. To analyze the genetic influences on the XLH phenotype, several studies have evaluated the effect of sex on disease severity (Table 2).

Holm et al. tested the skeletal and dental phenotypes and found no significant correlation between these parameters. They then sub-grouped the population into prepuberty and postpuberty and found a trend toward more severe dental disease in males in the postpubertal group (male: 10, female: 15; $p = 0.064$) [37]. Morey et al. compared the clinical features between men and women with XLH independent of the *PHEX* pathogenic variant type and reported that women develop nephrocalcinosis to a lower extent than in men ($p = 0.03$) [44]. However, the gene dosage effect has not been verified, even in a relatively large population [63].

4.2. Location of Pathogenic Variant

To test the hypothesis that patients harboring pathogenic variants located at the N-terminal side have a relatively more severe phenotype, Holm et al. assigned patients into groups with variants at the N-terminal and C-terminal regions, and compared the severity of the phenotype. In this study, the authors found no significant differences in skeletal and dental severities [37]. Several studies performed similar analyses (Table 3).

Table 2. Summary of gene dosage effect analyses.

Author	Year	Subject (Male, Female)	Analyzed Phenotype	*p* Value	Reference
Whyte	1996	30 (7, 23)	serum Pi	0.34	[94]
			serum ionized Ca	0.89	
			serum Ca	0.99	
			serum $Ca^{2+} \times Pi$	0.30	
			serum ALP	0.075	
			serum iPTH	0.91	
			urinary Ca/Cr	0.65	
			urinary Pi/Cr	0.51	
			% TRP	0.79	
			Tmp/GFR	0.59	
Holm	2001	27 (9, 18)	height z-score	0.11	[37]
		76 (26, 50)	skeletal severity	0.145	
		60 (19, 41)	dental severity	0.272	
Cho	2005	8 (3, 5)	biochemical parameters skeletal severity dental severity	n.s.	[39]
Song	2007	9 (1, 8)	no description	n.s.	[40]
Morey	2011	46 (11, 35)	nephrocalcinosis	**0.03**	[44]
Quinlan	2012	23 (11, 12)	height z-score	n.s.	[47]
Zhang	2019	139 (46, 93)	serum Pi	0.251	[63]
		174 (60, 114)	onset age for any signs	0.284	
		150 (55, 95)	age for first walking	0.844	
		124 (46, 78)	onset age for lower limb deformity	0.817	
		164 (59, 108)	height z-score	0.094	
		47 (19, 28)	RSS	0.850	
		230 (72, 158)	serum i-FGF23	0.696	
Ishihara	2021	26 (5, 21)	RSS	0.11	[67]
		24 (4, 20)	serum iFGF23	0.54	
		29 (6, 23)	height z-score	0.23	
		29 (6, 23)	serum phosphate	0.47	
		28 (5, 23)	serum ALP	**0.048**	
		27 (7, 23)	Tmp/GFR	0.47	
Rodríguez-Rubio	2021	48 (15, 33)	clinical manifestation growth impairment biochemical parameters	n.s.	[71]

Ca, calcium; Pi, phosphate; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; TRP, tubular reabsorption of phosphorus; Tmp/GFR, tubular maximum phosphate reabsorption per glomerular filtration rate; RSS, rickets severity score; n.s., not significant, FGF23, fibroblast growth factor 23. The value smaller than 0.05 should be highlighted with bold font.

Zhang et al. analyzed the severity of XLH in patients harboring pathogenic variants in the first 649 amino acids (N-terminal) and those with variants located from 650 amino acids to 3′ ends (C-terminal), similar to the study of Holm et al. They found that patients with variants in the N-terminal region showed relatively more severity with any signs at an earlier age ($p = 0.015$) and had higher serum i-FGF23 levels ($p = 0.045$) [63]. In contrast, other studies did not show significant differences in any of these parameters. Lin et al. evaluated a large population and stratified them using the same method as that of Holm et al. and Zhang et al.; however, there was no significant difference in onset age and serum i-FGF23 levels [69]. Further studies with relatively larger sample sizes are needed to determine the effect of the location of pathogenic variants on the phenotype.

4.3. Truncating and Non-Truncating Variants

Nonsense, frameshift, and splicing variants result in truncating mutants which may cause more severe functional defects than those caused by non-truncating mutants due to missense variants. To assess the influence of the type of variant on the phenotype, several

studies have compared the severity of phenotypes between truncating and non-truncating variants (Table 4).

Table 3. Summary of analyses on *PHEX* variant location.

Author	Year	Subject (N Terminal, C Terminal)	Analyzed Phenotype	*p* Value	Reference
Holm	2001	23, 6	skeletal severity	1.000	[37]
		22, 5	dental severity	0.621	
			onset age	n.s.	
Song	2007	2, 7	skeletal severity	0.083	[40]
			dental severity	n.s.	
Zhang	2019	113, 26	serum Pi	0.573	[63]
		141, 33	onset age for any signs	**0.015**	
		119, 31	age for first walking	0.478	
		104, 20	onset age for lower limb deformity	0.055	
		132, 25	height z-score	0.692	
		37, 10	RSS	0.711	
		187, 46	serum i-FGF23	0.045	
Baroncelli	2021	24 [a]	dental severity height z-score skeletal severity biochemical parameters	n.s.	[66]
Lin	2021	105, 24	onset age	0.360	[69]
			height z-score	0.759	
			serum Pi	0.286	
			serum ALP	0.077	
			serum i-FGF23	0.485	
			RSS	0.538	

[a] No subject number of subgroup (N-terminal and C-terminal) described. Pi, phosphate; ALP, alkaline phosphatase; RSS, rickets severity score; n.s., not significant; PHEX, phosphate regulating endopeptidase homolog X-linked. The value smaller than 0.05 should be highlighted with bold font.

As predicted from the mutant structure caused by pathogenic variants, Morey et al. reported that truncating variants lead to a relatively more severe phenotype in the percentage of tubular reabsorption of phosphorus (%TRP) and $1,25(OH)_2D$ levels [44]. Jiménez et al. detected the severity of height z-score in patients harboring truncating variants [68]. Nevertheless, other studies that analyzed a relatively larger number of subjects showed no significant difference in phenotypes between truncating and non-truncating variants [63,65,69,70]. Thus, the influence of variant type seems to be limited.

4.4. Preservation of Zinc-Binding Sites in Mutant PHEX

PHEX has a high amino acid sequence homology with NEP. Since NEP is a zinc-dependent metalloprotease, it has been postulated that PHEX also possesses a zinc-binding site and functions as a zinc-dependent metalloprotease [2,30,95–97]. We hypothesized that the preservation of the zinc-binding site structure is effective in improving the severity of XLH; we predicted three-dimensional structures of mutant PHEX and sub-grouped them with and without zinc-binding sites. Notably, the level of serum i-FGF23 was significantly higher in patients with variants that cause defective zinc-binding sites than that in patients with variants which preserve the three-dimensional structure of the zinc-binding site of PHEX [67]. Although a relatively larger sample size should be evaluated, these data may indicate the importance of zinc-binding sites and help clarify the function of PHEX.

Table 4. Summary of analyses on *PHEX* variants.

Author	Year	Subject (Truncating, Non-Truncating)	Analyzed Phenotype	*p* Value	Reference
Holm	2001	21, 8	skeletal severity	0.112	[37]
		20, 7	dental severity	1.000	
Cho	2005	5, 3	biochemical parameters skeletal severity dental severity onset age	n.s.	[39]
Song	2007	3, 6	skeletal severity dental severity	n.s.	[40]
Morey	2011	28, 6	onset age	0.08	[44]
		24, 6	height z-score	0.11	
		24, 6	serum Pi	0.53	
		22, 5	% TRP	**0.028**	
		16, 6	$1,25(OH)_2D$	**0.013**	
		14, 6	25(OH)D	0.30	
		20, 6	serum PTH	0.06	
		22, 6	serum ALP	0.48	
Rafaelsen	2016	21 [a]	height z-score skeletal severity dental severity	n.s.	[55]
Zhang	2019	107, 32	serum Pi	0.674	[63]
		143, 31	onset age for any signs	0.641	
		121, 29	age for first walking	0.235	
		106, 18	onset age for lower limb deformity	0.312	
		133, 34	height z-score	0.379	
		42, 5	RSS	0.724	
		184, 49	serum i-FGF23	0.777	
Zheng	2020	39, 14	height z-score	0.42	[65]
		39, 14	serum Pi	0.94	
		38, 13	Tmp/GFR	0.42	
		39, 14	serum ALP	0.37	
Park	2021	39, 9 [a]	onset age	0.561	[70]
			height z-score	0.793	
			serum Pi	0.672	
			serum Ca	0.750	
			serum ALP	0.916	
			serum 25(OH)D	**0.023**	
			serum PTH	0.235	
			%TRP	0.362	
			Tmp/GFR	0.362	
			urine Ca/Cr	0.644	
Baroncelli	2021	24 [b]	dental severity height z-score skeletal severity biochemical parameters	n.s.	[66]
Jiménez	2021	17 [b]	height z-score	**<0.05**	[68]
			onset age	n.s.	
			serum i-FGF23	n.s.	
			skeletal severity	n.s.	
Ishihara	2021	21, 4	RSS	0.53	[67]
		19, 4	serum i-FGF23	0.60	
		22, 6	height z-score	0.29	
		22, 6	serum Pi	0.25	
		21, 6	serum ALP	0.49	
		21, 5	Tmp/GFR	0.35	
Lin	2021	124, 29	onset age	0.996	[69]
			height z-score	0.510	
			serum Pi	0.925	
			serum ALP	0.700	
			serum i-FGF23	0.695	
			RSS	0.895	

[a] The detailed number of subjects with defects has not been described. [b] No subject number of subgroups (truncating and non-truncating) described. Ca, calcium; Pi, phosphate; ALP, alkaline phosphatase; PTH, parathyroid hormone; Cr, creatinine; TRP, tubular reabsorption of phosphorus; Tmp/GFR, tubular maximum phosphate reabsorption per glomerular filtration rate; RSS, rickets severity score; n.s., not significant; PHEX, phosphate-regulating endopeptidase homolog X-linked. The value smaller than 0.05 should be highlighted with bold font.

5. Conclusions

Twenty-five years have passed since the pathogenic variant of *PHEX* was determined to cause XLH. Subsequently, PHEX impairment has been found to lead to the elevation of FGF23 level, which is involved in the pathogenesis of XLH. To date, a novel treatment to inhibit excessive FGF23 levels has been developed and clinically approved. Although our understanding of the pathophysiology of XLH and the development of therapeutic strategies based on molecular pathology are remarkable, the function of PHEX itself remains unclear. Further investigations associated with the *PHEX* genotype, including functional assays and genotype–phenotype analyses are expected to provide clues to this unsolved problem and lead to further elucidation of the pathophysiology of XLH.

Author Contributions: Conceptualization, writing—original draft preparation, writing—review and editing, Y.O.; Data curation and editing, Y.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Health, Labor, and Welfare (grant number 21FC1010 and 22FC1012).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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