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Research Article

Whole Exome Sequencing Reveals Novel *PHEX* Mutations in Patients of Sporadic Hypophosphatemic Rickets

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Abstract

Hypophosphatemic rickets is a form of refractory rickets that cannot be treated by usual calcium and vitamin D supplementation. It can be inherited or acquired and caused due to mutations in phosphate regulating genes such as *PHEX, FGF23, DMP1* and *ENPP1* etc. Whole exome sequencing in patients with sporadic hypophosphatemic rickets revealed 3 novel mutations in *PHEX* gene. This article emphasizes the importance of genetic testing for precise diagnosis, timely initiation of treatment, and better management of the condition, especially in developing countries.

Keywords: Genetic Testing; Genes; Mutations; Phosphate; Refractory Rickets

Introduction

Hypophosphatemic rickets (HR) is a genetic disorder characterized by hypophosphatemia and osteomalacia. It is caused due to excessive excretion of phosphate in urine due to mutations in genes involved in renal phosphate reabsorption. It can be inherited or acquired. X-linked HR (XLHR) due to inactivating *PHEX* mutations is the predominant form and an account for approximately 80% of the familial cases [1]. XLHR is inherited in a dominant manner. Autosomal dominant and autosomal recessive forms of HR due to *FGF23, DMP1, ENPP1* and *SLC24A3* mutations are also documented but their occurrences are rare. Acquired forms include sporadic HR and tumor induced osteomalacia. Clinical features are almost similar in all forms of HR but their treatment modalities are different. Genetic testing can only provide a correct diagnosis. Screening of individual genes is time consuming. Whole exome sequencing (WES) approach allows rapid identification of causative mutations and aids in an accurate diagnosis. We report here the mutations identified in patients with sporadic HR.

Materials and Methods

Five clinically diagnosed sporadic HR female patients without any family history, born out of non-consanguineous marriage were recruited. Detailed clinical and family history was noted, and 5 ml blood samples collected after taking informed consent. Genomic DNA was isolated and WES was done for all the samples on Illumina HiSeq 4000 sequencer. Data was analyzed using standard bioinformatics approach. Mutations were validated by Sanger sequencing. Crystal structures of the wild-type and the mutant PHEX protein were obtained using the SWISS-MODEL online software (https://swissmodel.expasy.org/). Comparison of the structure of the normal and the mutant PHEX protein was done using the TM-align protein alignment *in silico* tool (http://zhanglab.ccmb.med.umich.edu/TM-align/).

Results and Discussion

The patients had short stature, skeletal deformities and hypophosphatemia with mean age of onset at 2.8+1.3 years.

WES identified five PHEX mutations in five patients (Table 1) - a known missense (c.1970A>G→Y657C) [2,3], a known nonsense (c.1979G>A→W660*) [3,4], a novel deletion-insertion (c.1337delinsAATAA→F446*) and two novel deletions (c.651_654delACAT→H218Sfs*2, c.665_674delTGGACCAAGC→L222Qfs*7). The novel deletions and deletion-insertion mutations lead to frameshift which produced premature termination codons respectively resulting in the formation of truncated and non-functional PHEX product (Figure 1). All the mutations identified in the patients were de novo as their parents carried wild type alleles. Variability in disease severity was also observed. The patient having the missense mutation was mildly affected with delayed onset of disease and healing rickets.

Conclusion

This study reports three novel *PHEX* mutations and suggests that *PHEX* may be mainly responsible for sporadic hypophosphatemic rickets in India. Genetic testing provides a precise diagnosis of hypophosphatemic rickets, which allows initiation of specific therapy which can minimize skeletal deformities and help in better treatment and management of the condition, especially in developing countries like India.

Figure 1: Novel PHEX mutations identified in our study. (a–c) Chromatograms containing novel mutations are shown in comparison to the normal sequences. (a) c.651_654delACAT in exon 5, (b)c.665_674delTGGACCAAGC in exon 6, and (c) c.1337delinsAATAA in exon 12. Superimposed images of normal (blue) and mutant PHEX protein (red). The novel PHEX mutations (d) c.651_654delACAT in exon 5, (e) c.665_674delTGGACCAAGC in exon 6, (f) c.1337delinsAATAA in exon 12 resulted in truncated PHEX protein (red).

S. No	c.DNA change/ Amino acid change	*PHEX* Exon	Age/ Sex	Type of Mutation	Zygosity	Serum Phosphate/ Calcium	Parathyroid hormone	25-OH Vitamin D
I.	c.651_654delACAT/ H218Sfs*2	5	14Y/F	Novel deletion	Het	2.4/8.9	160.8	13.2
II.	c.665_674delTGGACCAAGC/ L222Qfs*7	6	18Y/F	Novel deletion	Het	2.3/9.6	34.7	40
III.	c.1337delinsAATAA/ F446*	12	22Y/F	Novel deletion-insertion	Het	2/9.4	329	-
1V.	c.1970A>G/ Y657C	20	8Y/F	Reported Missense	Het	2/9.4	171.3	21.4
V.	c.1979G>A/ W660*	20	10Y/F	Reported Nonsense	Het	2.4/9.6	34.3	30.3

Table 1: Mutations identified in the patients and their clinical characteristics.

Het-Heterozygous; Serum phosphate levels -mg/dl, normal range: 3-5mg/dl; Serum calcium levels-mg/dl, normal range-9-11.5 mg/dl; Serum intact parathyroid hormone levels-pg/ml, normal range-15-68pg/ml; Serum 25-OH vitamin D levels-ng/ml, normal range-25-80 ng/ml

Conflict of Interest

None.

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Bibliography

1. Bajpai A., *et al*. "Non-azotemic refractory rickets in Indian children". *Indian Pediatrics* 2005;42(01): 23-30.

2. Gaucher C., *et al*. "PHEX analysis in 118 pedigrees reveals new genetic clues in hypophosphatemic rickets". *Human Genetics* 125.4 (2009): 401-411.

3. Marik B., *et al*. "Genetics of Refractory Rickets: Identification of Novel PHEX Mutations in Indian Patients and a Literature Update". *The Journal of Pediatric Genetics* 7 (2018): 47-59.

4. Francis F., *et al*. "Genomic organization of the human PEX gene mutated in X-linked dominant hypophosphatemic rickets". *Genome Research* 7.6 (1997): 573-585.

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