The Turkish Journal of Pediatrics 2022; 64: 585-591
https://doi.org/10.24953/turkjped.2021.829

Case Report

Autosomal recessive hypophosphatemic rickets type 2; a novel mutation in the ENPP1 gene

Eda Çelebi Bitkin[1], Huri Sema Aymelek[2]

[1]Division of Pediatric Endocrinology, Department of Pediatrics and [2]Department of Medical Genetic, Van Yüzüncü Yıl University Faculty of Medicine Van, Turkey.

ABSTRACT

Background. Hypophosphatemic rickets (HR) is a rare disease caused by several genetic mutations in factors that cause an increase in fibroblast growth factor 23 (FGF23), and renal phosphate transporters. ENPP1 (ectonucleotide pyrophosphatase / phosphodiesterase 1) mutations cause autosomal recessive inheritance hypophosphatemic rickets type 2.

Case. In our study, we present a novel mutation in the ENPP1 gene detected in 4 siblings in a single family.

Conclusion. Our findings can be applied to further understand molecular pathogenesis and to establish a correlation between genotype and phenotype for HR.

Key words: hypophosphatemic rickets, ENPP1 gene, novel mutation.

Hypophosphatemic rickets (HR) is a disorder marked by renal phosphate wasting. It is caused by several genetic mutations in renal phosphate transporters and in pathways leading to increased fibroblast growth factor 23 (FGF23) signaling or secretion. The most common inherited form of HR is X-linked HR (XLH; OMIM: # 307800).[1] HR is a rare disease with a prevalence of 3.9 per 100,000 live births.[2] Hypophosphatemia occurs with kidney phosphate loss, which causes bone mineralization defects such as rickets and osteomalacia.

FGF23, the most important phosphaturic agent, is produced by osteocytes and osteoblasts.[3] FGF23 inhibits renal phosphate reabsorption by decreasing the expression of phosphate transporters in proximal renal tubules.[4] It also inhibits 25-OH vitamin D-1-hydroxylase and activates 25- hydroxyvitamin D-24-hydroxylase. This results in reduced 1,25 (OH) 2D levels and increases 24,25-dihydroxyvitamin D (24,25 (OH) 2D) levels. The most common cause of HR is an X-linked PHEX gene mutation. HR is also caused by autosomal dominant FGF23 gene mutations, DMP1 mutations, which give rise to autosomal recessive HR type 1, and ENPP1 mutations, resulting in autosomal recessive HR type 2.[3]

The majority of ENPP1 mutations cause either generalized arterial calcification of infancy (GACI). Myointimal proliferation is a component of GACI.[5] ENPP1 mutations trigger HR through a pathway that is yet to be discovered.[6]

ENPP1 plays an important role bone mineralization, soft tissue calcification, and regulation of pyrophosphate levels by producing inorganic pyrophosphate (PPi). Mineral accumulation in bones is determined by the ratio of phosphate to PPi regulated by ENPP1. When ENPP1 is mutated, it causes hypophosphatemic rickets due to high FGF23 levels. However, this mechanism is not fully understood.[5,6]

✉ Eda Çelebi Bitkin
edacelebitkin@gmail.com

Received 12th March 2021, revised 30th July 2021, 14th August 2021, 11th November 2021, accepted 29th November 2021.

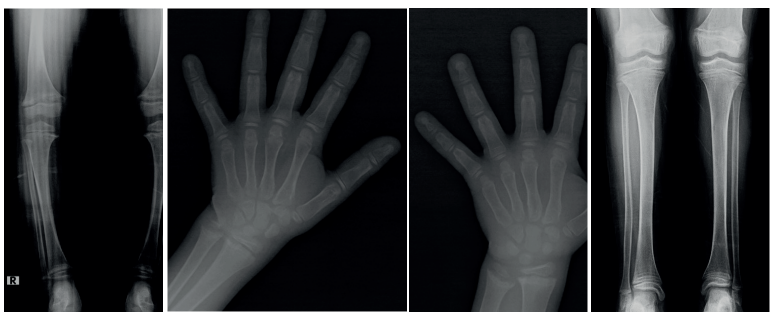
This paper describes a novel mutation in the ENPP1 gene that was observed in four siblings diagnosed with autosomal recessive HR type 2.

Cases Report

Three siblings (P2,P3,P4) had previously been admitted to another hospital due to bowing of legs and short stature. They had been treated irregularly for HR, and had not received treatment for the previous two years. The other sibling (P1) had bowing of legs and short stature but had not been previously diagnosed. This patient was evaluated and diagnosed with HR.

The age, gender and anthropometric measurements of the patients are given in Table I. The patients were born full term and had normal birth weights. After they started walking, bowing and short stature were noticed in the legs. All patients had disproportionate short stature. They received vitamin D prophylaxis until the age of one and HR treatment (calcitriol and phosphorus) irregularly. Their parents were cousins and there were no similar diseases observed in any other members of the family. Maternal height was 160.4 cm (-0.46 SDS), paternal height was 165 cm (-1.82 SDS). The family had a total of five children. Three of

Table I. Physical examination and test results of the patients.

Patient(P)	P1	P2	P3	P4
Gender	Female	Female	Male	Female
Age(years)	5.0	9.08	10.8	12.3
Height (SDS)	-3.08	-3.17	-3.73	-5.19
Weight (SDS)	-1.5	-1.12	-2.65	-2.67
Upper-to-lower body segment ratio (SDS)	1.4 (> +2)	1.3 (> +2)	1.1 (+2)	1.1 (+1)
Physical examination findings	Short stature Genu varum	Short stature Genu varum	Short stature Genu varum	Short stature Genu valgum Hypoplastic teeth and caries
Radiography (First application)				
Renal US	Normal	Urinary nephrocalcinosis	Urinary nephrocalcinosis	Normal
Renal Doppler US	Normal	Normal	Normal	Normal
Carotid Doppler US	Normal	Normal	Normal	Normal
Echocardiography	Normal	Minimal Tricuspid valve insufficiency, Minimal Mitral valve insufficiency, Minimal Aortic valve insufficiency	Minimal Aortic Valve Insufficiency	Pulmonary Valve Insufficiency, (Pulmonary Balloon Valvuloplasty performed)

the children were treated irregularly for HR, one child had bowing of legs and had not been diagnosed, and the other child was completely normal (Fig. 1). There were no findings in the parents either.

On the first admission (after two years without treatment), calcium (Ca), phosphorus (P), PTH, alkaline phosphatase (ALP), and 25-hydroxy vitamin D (25(OH)D) levels were measured. Tubular phosphate reabsorption (TRP), and urinary calcium and creatinine (U Ca / Cr) ratios were also calculated. The results of these measurements are summarized in Table II. Standard deviation of ALP are given.[7] Renal function was normal in all patients, and tubular dysfunction (except phosphaturia) was absent in all patients. Carotid and Renal Doppler ultrasonography (US) imaging was performed on all patients. On imaging, carotid and renal arteries appeared normal. Patients P2 and P3 had nephrocalcinosis and there were valve anomalies in the echocardiogram results of patients P2, P3, and P4. Patient P4 underwent pulmonary balloon valvuloplasty due to pulmonary valve insufficiency. There was improvement in valve insufficiency. Existing pathologies are summarized in Table I.

All patients had hypophosphatemia, hyperphosphaturia, elevated ALP levels, mild elevations in PTH levels, normocalcemia, normocalciuria, and rickets (enlargement and irregularity in epiphyseal plates, especially bowing of the bones in leg radiographs). Calcitriol (15 ng/kg/d) and oral phosphate (40 mg/kg/d) treatment was initiated following the diagnosis of HR. Biochemical values

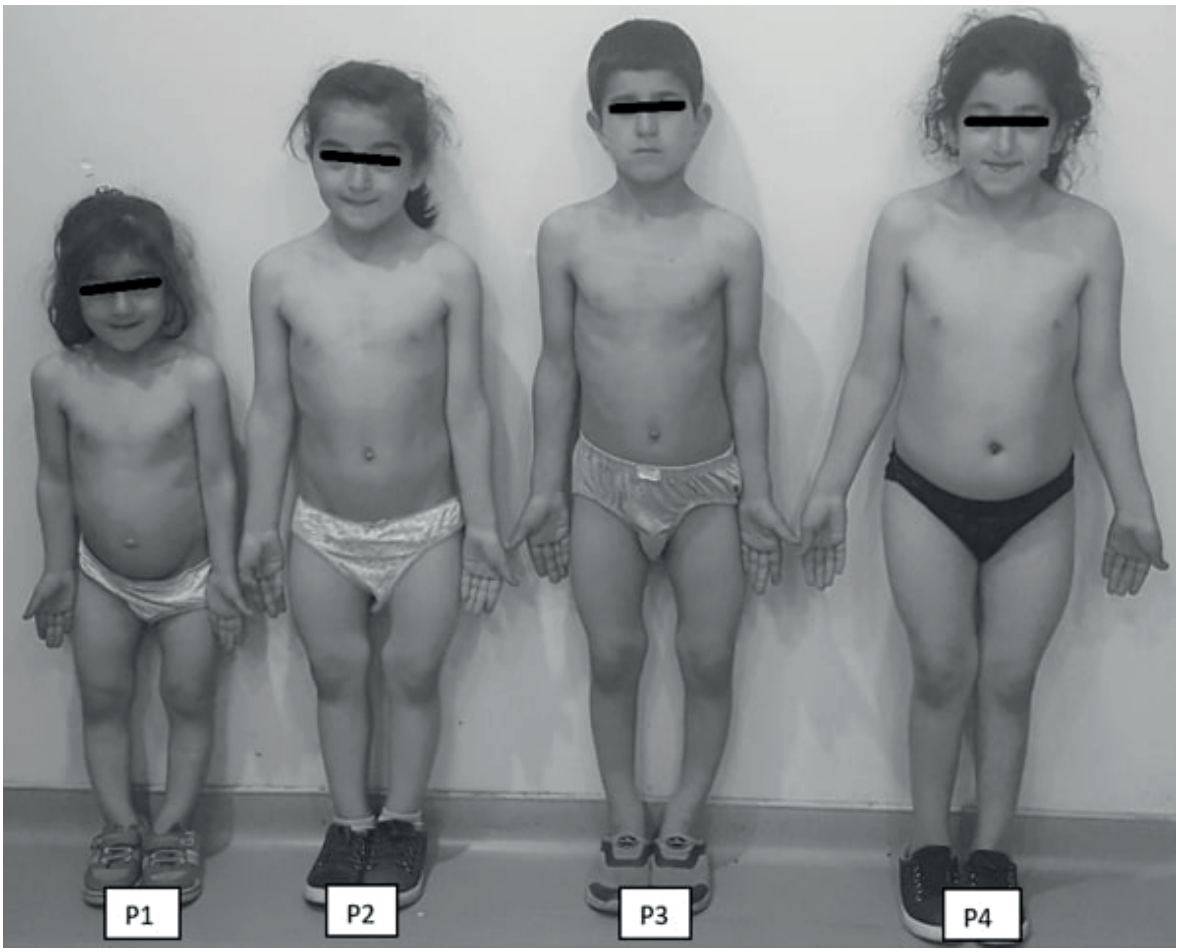


Fig. 1. Phenotype of patients.

Table II. Laboratory results of patients.

Patients	Age (years)	Ca (mg/dL), range (8.4-10)	P (mg/dL), range (3.4-6.2)	ALP (U/L), (-2SD , + 2SD)	PTH (pg/mL) range (15-68.3)	25 OHD (ng/mL), range (20-60)	TPR	U Ca / Cr
P1	5.0	9.6	2.5	562 (294-483)	136.4	31	%76	<0.01
P2	9.08	9.3	2.5	881 (326-769)	89.7	24	%75	<0.1
P3	10.8	9.7	2.4	1015 (400-938)	110.1	30.4	%74	<0.03
P4	12.3	9.6	2.6	960 (204-924)	99	27.8	%76	0.07

Ca: calcium, P: phosphate, ALP: alkaline phosphatase, PTH: parathyroid hormone, 25OHD: 25-hydroxyvitamin D, U Ca / Cr: urine calcium: creatinine ratio, TRP: tubular phosphate reabsorption

of the unaffected sibling and parents were normal. Since the parents were relatives and phenotypically normal, genetic mutations known to cause autosomal recessive HR were studied first. No mutations were detected in the DMP1 genes of the four affected siblings. The ENPP1 gene was examined in only one (P2) of the siblings and a homozygous c.1092-2A> C mutation was found (Fig. 2). Although the mutation detected in the ENPP1 gene did not lead to any change in the protein sequence, it is a splice site mutation that acts at the exon-intron boundary that can cause the formation of a non-functional protein. Four of the in silico algorithms (DANN, EIGEN, FATHMM-MKL and MutationTaster) that are used to predict the effects of mutations classified as pathogenic by the American College of Medical Genetics and Genomics (ACMG) were evaluated as pathogenic. The possibility of a pathogenic effect of the mutation, which has never been shown before in the literature, has been emphasized. Later, in the genetic analysis performed by the parents, heterozygous c.1092-2A> C genetic changes in the ENPP1 gene were detected in both (Fig. 2). Written consent was obtained from the family of the patients.

Genetic Analysis

Genomic DNA was extracted from the patients' peripheral blood lymphocytes using a QIA amp DNA mini kit (Qiagen, 51304, Duseldorf, Germany) according to the manufacturer's protocol and sequenced that comprised all exons and exon-intron junctions of ENPP1 and DMP1 genes on the Illumina-MiniSeq sequencing platform. The ACMG standards and reference to the Varsome, NCBI, ClinVar, and HGMD databases were used to classify the mutations into five categories: pathogenic, likely pathogenic, variants of uncertain significance, likely benign, or benign.

Discussion

Here, we present four cases of autosomal recessive HR type 2 caused by mutations in the ENPP1 gene in individuals from the same family. In the genetic examination of the patients, we found a novel mutation in ENPP1, which was not previously shown in the literature. The frequency of autosomal recessive HR type 2 is extremely rare. In a multi-center study in Turkey involving 166 patients with HR, 75 patients were genetically analyzed and none of these patients had a mutation in the ENPP1 gene.[1] There have not been any previously reported cases of ENPP1 gene mutations in Turkey prior to this case study.

Genetic disorders associated with the ENPP1 gene show phenotypic heterogeneity depending on the type and location of the mutation. GACI and HR result from biallelic mutations and exhibit recessive inheritance patterns. The nuclease domain or phosphodiesterase domain that mediates ENPP1 catalytic activity is affected by most mutations that cause ectopic calcification.[8]

With dominant or recessive inheritance, biallelic or heterozygous ENPP1 gene mutations have been shown to cause Cole's disease (irregular hypopigmentation, cutaneous calcifications, and punctate keratoderma), a

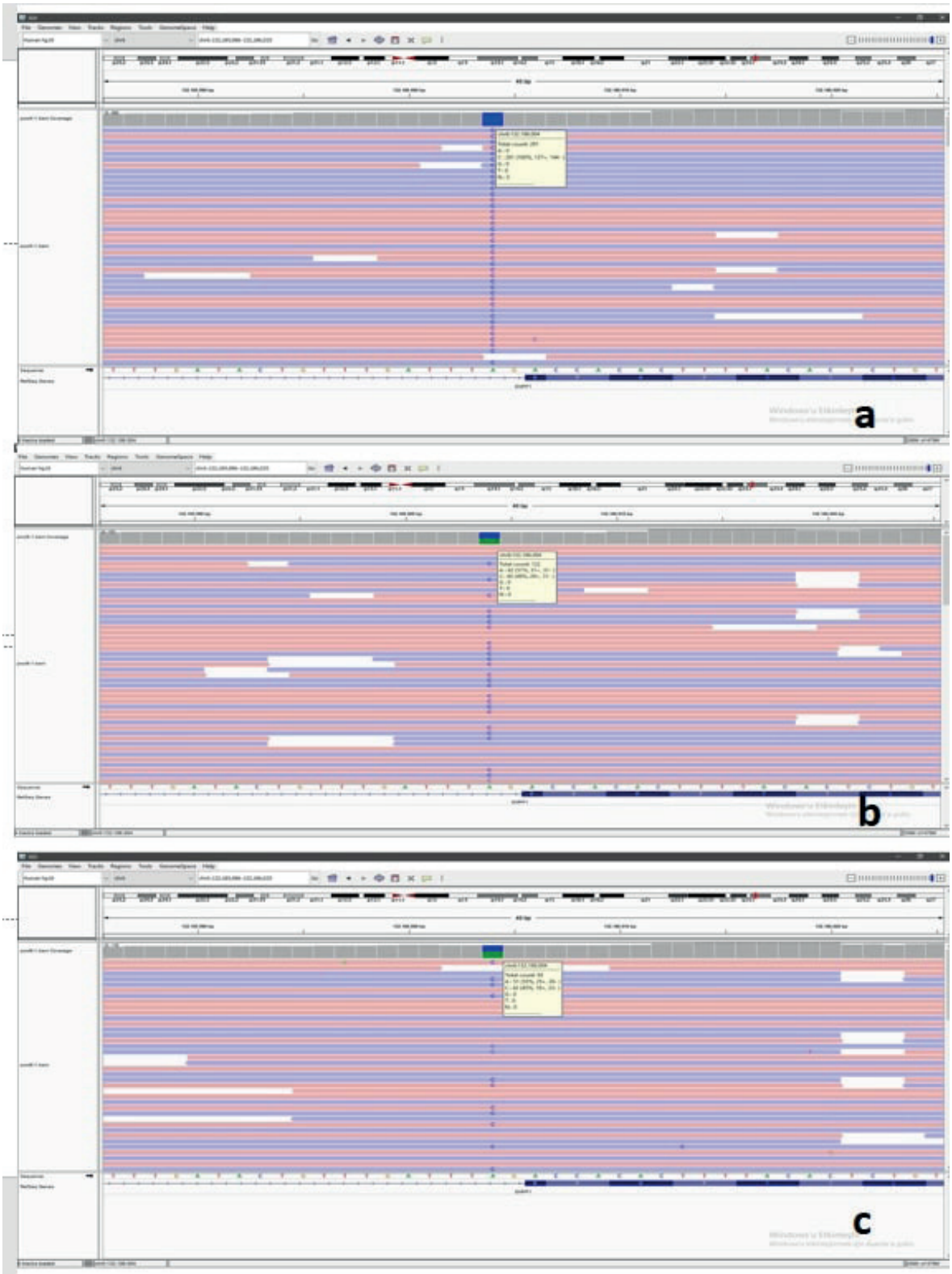


Fig. 2. ENPP1 Gene c.1092-2A> C Homozygous mutation (Patient P2 (a)), ENPP1 Gene c.1092-2A> C Heterozygous mutation (Mother (b) and Father (c)).

rare genodermatosis.[9] Brunod et al.[10] reported that some cases of GACI were associated with hypophosphatemia and even HR. It has been found that hypophosphatemia is associated with a milder phenotype and a better prognosis.[10] Physiopathology is not well understood. It is assumed that hypophosphatemia in GACI may occur through a physiological compensatory mechanism rather than the primary defect.[5] However, patients with mutations in the ENPP1 gene have an autosomal recessive phenotype for HR without any arterial calcification, suggesting a different pathway in the development of the disorder.[10] Mild pulmonary stenosis and thickening of the aortic valves have been observed in HR patients with ENPP1 gene mutations. The resulting changes in the heart are thought to occur due to arterial calcifications. Current studies show that these patients are susceptible to heart valve anomalies.[6] The patients in this study had no evidence of carotid and renal arterial calcification, but the changes observed in heart valves may be a result of arterial calcification. However, we think that this situation should be supported by studies with more patients.

There are two mechanisms that may explain why loss-of-function mutations in ENPP1 result in HR in some patients and GACI in others. The first explanation is that affected individuals exhibit various disease phenotypes based on genetic and environmental factors. This explanation has been proposed because patients showing a mild GACI phenotype accompanied by arterial calcifications, hypophosphatemia and hyperphosphaturia have been identified.[10] In addition, two members of the same family carrying the same homozygous ENPP1 mutation had HR without arterial calcification in one and GACI with hypophosphatemia in the other case. The other explanation is that different mutant alleles are associated with functional differences. There is no evidence for different isoforms of the ENPP1 protein and mutations are spread throughout the gene in both diseases. However, the effect of mutations at the protein level may be different for the two phenotypes.[6]

Although the change we detected in the ENPP1 gene does not lead to any change in the protein sequence, it is a splice site mutation that can affect the exon-intron segment and cause the formation of a non-functional protein.

GACI is an autosomal recessive disease and has a hypermineralizing phenotype. This disease is known to be caused by inactivating mutations in the ENPP1 gene. That both GACI and hypophosphatemic rickets are caused by loss-of-function mutations is most strongly supported by the observation reported in a single family, in which father suffered from and his son suffered from GACI plus hypophosphatemia, although both had the same homozygous ENPP1 mutation in the same family.[6] However, individuals with the same homozygous loss-of-function ENPP1 mutation in our patients had the same clinical findings.

Although the mechanism by which mutations in the ENPP1 gene increase FGF23 levels and cause hypophosphatemic rickets cannot be fully explained, there have been studies showing that loss of function mutations in this gene reduce ENPP1 activity and lead to hypophosphatemic rickets with an increase in FGF23 levels.[6] However, FGF23 levels could not be determined in our cases.

In summary, we uncovered a novel mutation in the ENPP1 gene that occurred in siblings diagnosed with autosomal recessive HR type 2. Our findings can be applied to further understand molecular pathogenesis and to establish a correlation between genotype and phenotype for HR.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ECB; data collection:ECB, HSA; analysis and interpretation of results: ECB, HSA; draft

manuscript preparation: ECB, HSA. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Şıklar Z, Turan S, Bereket A, et al. Nationwide Turkish Cohort Study of Hypophosphatemic Rickets. J Clin Res Pediatr Endocrinol 2020; 12: 150-159. https://doi.org/10.4274/jcrpe.galenos.2019.2019.0098
2. Guven A, Al-Rijjal RA, BinEssa HA, et al. Mutational analysis of PHEX, FGF23 and CLCN5 in patients with hypophosphataemic rickets. Clin Endocrinol (Oxf) 2017; 87: 103-112. https://doi.org/10.1111/cen.13347
3. Acar S, Demir K, Shi Y. Genetic causes of rickets. J Clin Res Pediatr Endocrinol 2017; 30: 88-105. https://doi.org/10.4274/jcrpe.2017.S008
4. Prié D, Friedlander G. Genetic disorders of renal phosphate transport. N Engl J Med 2010; 362: 2399-2409. https://doi.org/10.1056/NEJMra0904186
5. Rutsch F, Ruf N, Vaingankar S, et al. Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification. Nat Genet 2003; 34: 379-381. https://doi.org/10.1038/ng1221
6. Lorenz-Depiereux B, Schnabel D, Tiosano D, Häusler G, Strom TM. Loss-of-function ENPP1 mutations cause both generalized arterial calcification of infancy and autosomal-recessive hypophosphatemic rickets. Am J Hum Genet 2010; 86: 267-272. https://doi.org/10.1016/j.ajhg.2010.01.006
7. Turan S, Topcu B, Gökçe İ, et al. Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z- scores in different types of rickets. J Clin Res Pediatr Endocrinol 2011; 3: 7-11. https://doi.org/10.4274/jcrpe.v3i1.02
8. Staretz-Chacham O, Shukrun R, Barel O, et al. Novel homozygous ENPP1 mutation causes generalized arterial calcifications of infancy, thrombocytopenia, and cardiovascular and central nervous system syndrome. Am J Med Genet A 2019; 179: 2112-2118. https://doi.org/10.1002/ajmg.a.61334
9. Chourabi M, Liew MS, Lim S, et al. ENPP1 Mutation Causes Recessive Cole Disease by Altering Melanogenesis. J Invest Dermatol 2018; 138: 291-300. https://doi.org/10.1016/j.jid.2017.08.045
10. Brunod I, Tosello B, Hassid S, Gire C, Thomachot L, Panuel M. Generalized arterial calcification of infancy with a novel ENPP1 mutation: a case report. BMC Pediatr 2018; 18: 217. https://doi.org/10.1186/s12887-018-1198-4