Lifelong deformities in an adult caused by vitamin D-dependent rickets type 1A: A case report

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Abstract. Vitamin D-dependent rickets (VDDR) type 1A is a rare autosomal recessive disorder caused by cytochrome P450 family 27 subfamily B member 1 (CYP27B1) mutations and can lead to deficiencies in 1α-hydroxylase activity. The present study describes the case of a 39-year-old male patient who presented with rickets and deformities of limbs. Blood biochemical analysis revealed hypocalcemia and high serum parathyroid hormone (PTH) levels. Whole-exome Sanger sequencing using peripheral venous blood of this patient and his parents revealed exon1 c.182T>C, a novel mutation. Through physical examination, laboratory tests, imaging including lower limbs and lumbar spine X-ray and pelvis CT scan, and genetic testing, the patient was diagnosed with VDDR-1A. Following 1 month of treatment with 0.5 μg 1,25-dihydroxy-vitamin D3 twice daily and 0.6 g calcium carbonate once daily, follow-up examinations revealed that the patient's PTH and serum calcium levels had returned to normal. As the patient was diagnosed in his adulthood and missed the optimal treatment period, he developed irreversible deformities. If VDDR-1A can be diagnosed during infancy and childhood, skeletal deformities may be prevented. Therefore, the present report supports the proposal of early genetic sequencing in children with calcium deficiencies for the early diagnosis of rare diseases such as VDDR-1A, -1B and -2A and hereditary hypophosphatemic rickets. Since VDDR-1A diagnosed in adults is rare, the present case may provide clinicians with further insights into the characteristics of this rare disease.

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Introduction

Vitamin D exists as two main forms in the body: Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (1). Vitamin D2 is derived from the diet, such as ryegrass, beef and lamb. Whilst vitamin D3 is formed from the exposure of 7-dehydrocholesterol in the skin to ultraviolet B (280-310 nm) light. Vitamin D is metabolized and converted into its active form 1,25-dihydroxy-vitamin D3 [1,25$(OH)_2$D] by several enzymes, mainly microsomal CYP2R1 in the liver and 25-OH vitamin D-1α-hydroxylase in kidneys, which promotes calcium absorption from the gut into the bloodstream and regulates serum calcium concentration maintained at 2.25 to 2.75 mmol/l. Deficiencies in vitamin D metabolism or action can cause hypocalcemia and high serum parathyroid hormone (PTH) levels (2). These can adversely affect the growth and development of the human musculoskeletal and nervous systems, eventually resulting in vitamin D-dependent rickets (VDDR) (3).

Cytochrome P450 family 27 subfamily B member 1 (CYP27B1) mutations may cause deficiencies in 1α-hydroxylase activity, resulting in a vitamin D deficiency and causes a rare autosomal recessive disorder known as VDDR type 1A (VDDR-1A) (4). The clinical features of VDDR-1A during childhood include muscle weakness, growth disorders, joint pain, genu valgum, seizures (5), symptoms of rickets and increased susceptibility to bone fractures (6). In adulthood, the main characteristic of VDDR-1A is osteomalacia (7). The laboratory findings of this condition typically reveal hypocalcemia, high serum PTH concentrations, elevated serum alkaline phosphatase levels, low serum 1,25$(OH)_2$D levels and normal or elevated serum 25-hydroxyvitamin D (25OH-D) concentrations (8). The present report describes the case of a patient with VDDR-1A caused by a novel mutation in the *CYP27B1* gene, which was diagnosed in adulthood.

Case report

A male 39-year-old patient presented to the Department of Endocrinology, Affiliated Hangzhou First People's Hospital,

Zhejiang University School of Medicine (Hangzhou, China) and asked to be hospitalized due to his short stature in September, 2021. He had a 37-year history of rickets induced by calcium deficiency, taking calcium carbonate orally at 600 mg each time for ~5 times per week, and vitamin D3 orally at 400 IU each time for 4 times per week. A physical examination revealed bilateral deformities of the lower extremities, with an X-shaped lower left limb and O-shaped lower right limb (height, 148.0 cm). His thorax exhibited pectus carinatum and a rib flare. His forehead was slightly raised. Laboratory tests indicated elevated serum PTH [electrochemiluminescence immunoassay (ECLIA) using COBAS e 601; cat. no. 11972103122; Roche Diagnostics GmbH] concentrations (115 pg/ml; normal range, 15-65 pg/ml) coupled with low serum 25OH-D (ECLIA using COBAS e 601; cat. no. 05894913190; Roche Diagnostics GmbH) (18.31 μg/l; normal range, 20-70 μg/l), serum calcium (ASAIII, 11111371701, DiaSys Diagnostic System Co. Ltd., Beckman coulter AU680) (1.67 mmol/l; normal range, 2.0-2.6 mmol/l) and 24-h urine calcium (ASAIII, 11111371701, DiaSys Diagnostic Systems GmbH; Beckman Coulter, Inc. AU680) (0.56 mmol/day; normal range, 1.0-7.5 mmol/day) concentrations. Additional radiographic examinations revealed that both of his lower limbs were bent to the right (Fig. 1). The total lengths of his right and left lower limbs were 690 and 663 cm, respectively. Lateral joint space narrowing was observed in the left knee joint. Osteopenia was evident. A plain CT scan (Optima CT-540; GE Healthcare) revealed osteopenia in all components of the pelvic bone and scoliosis. A lumbar spine X-ray revealed that the spinal alignment was curved to the right. The T-scores for bone mineral density (BMD) (Lunar Prodigy; GE Healthcare) were -2.0 at the left femoral neck and -2.2 at the left hip, which indicates osteopenia.

Whole-exome Sanger sequencing was performed in the peripheral venous blood of this patient and his parents owing to a suspected genetic contribution. Peripheral venous blood from this patient and his parents was taken. DNA was isolated from peripheral blood with CWE9600 Automated Nucleic Acid Extraction System using CWE2100 Blood DNA Kit V2 (cat. no. CW2553; CoWin Biosciences). The primer 1-F (5'-TGTGGCCAGTAGGGGACTT-3'), 1-R (5'-CCAGACGCT GGTCACTCTG-3'), and the primer 2-F (5'-ATCTGCAGG ATCTCCACACC-3'), 2-R (5'-CATGACCCAGACCCTCAA GT-3') were designed for *CYP27B1* gene using Primer Premier 5.0 (Premier Biosoft International) before PCR (Phanta Max Super-Fidelity DNA Polymerase; Vazyme Biotech Co., Ltd) was performed to amplify the fragments covering the mutated sites in a LifeECO Thermal Cycler TC-96/G/H(b)C (Hangzhou Bioer Co., Ltd.). PCR reaction conditions for these primers were as follows: 95˚C for 5 min (once only), followed by 25 cycles consisting of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 40 sec, followed by 20 cycles at 95˚C for 30 sec, 50˚C for 30 sec and 72˚C for 40 sec, then a final 10 min extension step was performed at 72˚C, and maintained at 4˚C. The PCR products were further purified with 2% agarose gel electrophoresis, stained with Gelstain Red (cat. no. S2009L; Shanghai Bioscience Technology Co., Ltd.), and then sequenced by a ABI 3730XL DNA Sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sanger sequencing results were analyzed by Chromas Lite v2.01 (Technelysium Pty Ltd.).

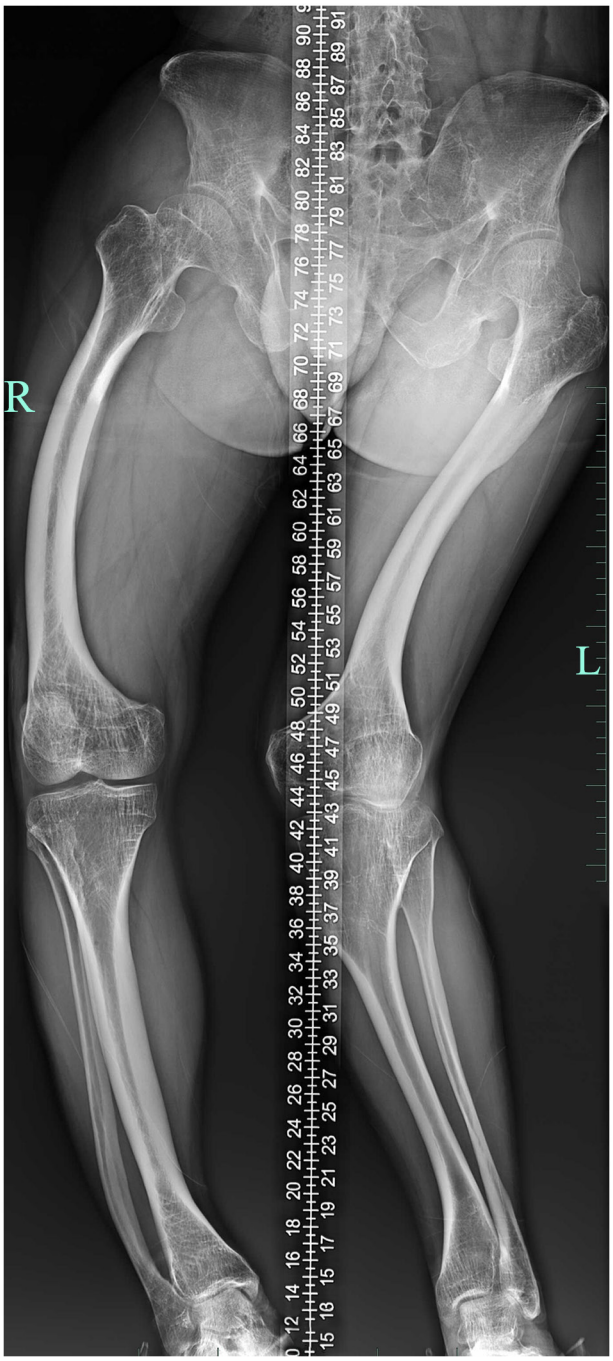

Figure 1. X-ray image demonstrating deformities in both lower limbs. The R means right side, and the L means left side.

A genetic analysis of this patient revealed two heterozygous mutations in *CYP27B1*, namely exon 8 c.1376G>A (p.R459H) and exon 1 c.182T>C (p.L61P) (Fig. 2). The c.1376G>A (p.R459H) mutation indicates that nucleotide 1,376 in the coding region was mutated from guanine to adenine, resulting in amino acid 459 being changed from arginine to histidine. The c.182T>C (p.L61P) mutation indicates that nucleotide 182 in the coding region was mutated from thymine to cytosine, resulting in amino acid 61 being changed from leucine to cytosine. To the best of our knowledge, to date there are no previous studies available on exon 1 c.182T>C causing VDDR-1A. The heterozygous mutation in exon 8 [c.1376G>A (p.R459H)] was inherited from the

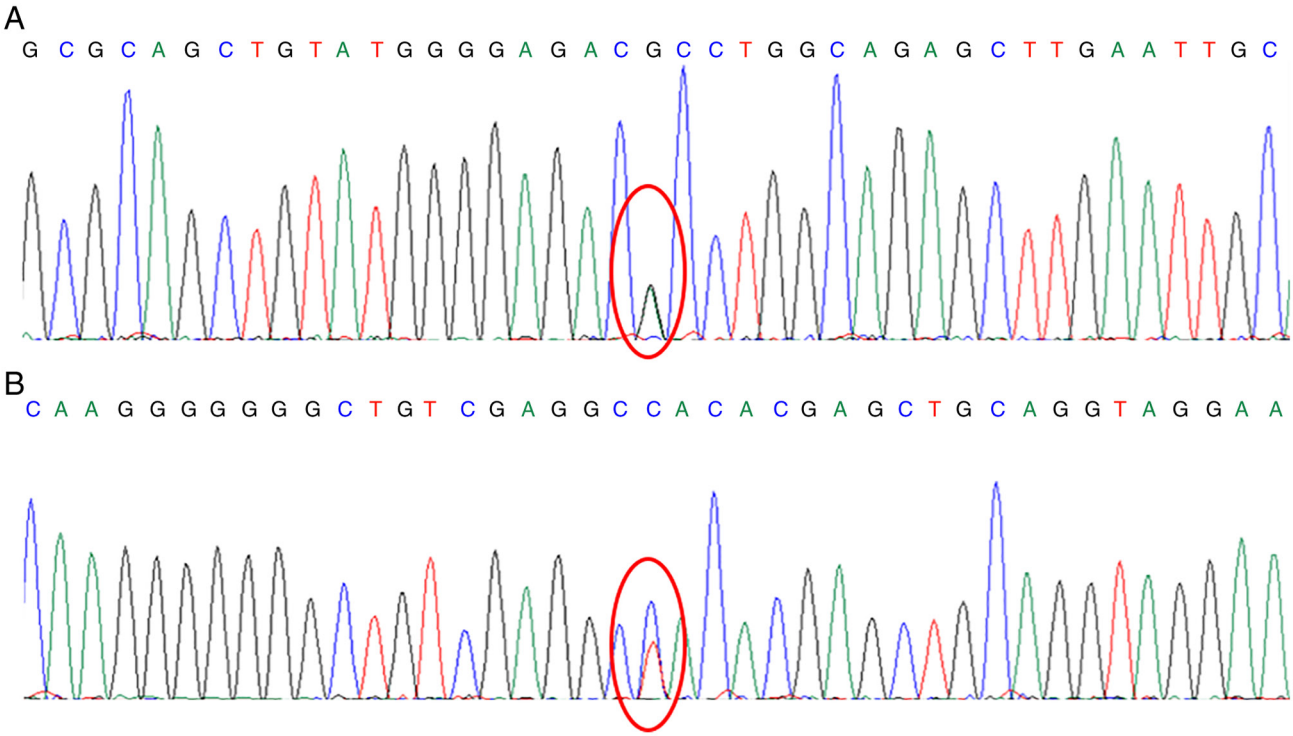

Figure 2. Whole-exome sequencing of the patient revealed the mutation in the *cytochrome P450 family 27 subfamily B member 1* gene. (A) Mutation in exon 1 (c.182T>C) is originated from the mother. (B) Mutation in exon 8 (c.1376G>A) is originated from the father.

patient's father (Fig. S1), who has no mutation in exon 1 at nucleotide 182. By contrast, the mutation in exon 1 [c.182T>C (p.L61P)] was inherited from the mother, who has no mutation in exon 8 at nucleotide 1376 (Fig. S2). The parents only carry the mutations and do not have VDDR-1A. The patient carried both mutations. Based on the genetic sequencing results, the patient was diagnosed with VDDR-1A. The standard clinical treatment, 0.5 μg 1,25$(OH)_2$D twice daily and 0.6 g calcium carbonate once daily (6), were administered to improve the patient's serum marker levels and BMD. Following 1 month of treatment, follow-up examinations revealed a PTH concentration of 38.4 pg/ml and a serum calcium level of 2.36 mmol/l. After 1 year of treatment, the serum PTH and serum calcium concentration remained at normal levels, and BMI were -1.6 at the left femoral neck and -1.8 at the left hip, which suggested that it was improved compared with the previous year. However, since the diagnosis was established in adulthood, the patient had already suffered irreversible deformities.

Discussion

The patient described in the present case report had exhibited symptoms of rickets since his childhood and had visited the orthopedic department several times. However, the treatment and diagnosis have not changed over time. The patient was diagnosed with rickets induced by calcium deficiency. Following irregular treatments with oral calcium carbonate and vitamin D3, the patient developed severe deformities in his lumbar spine and lower limb, in addition to osteoporosis inconsistent with his age. VDDR-1A was confirmed through physical examination, laboratory tests, imaging and genetic testing. The patient was then supplemented with calcium and 1,25$(OH)_2$D based on the cause.

Sanger sequencing in the present case revealed a mutation in the *CYP27B1* gene. *CYP27B1* is located on chromosome 12q13.3, spans 4,859 bases and is 5 kb in length (9). It encodes 1α-hydroxylase (10), which regulates calcium metabolism by synthesizing 1,25$(OH)_2$D in the kidneys. Homozygous or compound heterozygous alterations of this gene have been previously associated with VDDR-1A (11). The mutations in the present patient are located in the *CYP27B1* gene and the clinical manifestations are consistent with the symptoms of VDDR-1A. Therefore, it can be speculated that these mutations are meaningful. Due to the rarity of this disease and the fact that the mutation sites in each patient differs, it is highly difficult to study this relationship. The patient in the present report carried two heterozygous mutations in this gene, namely exon 8 [c.1376G>A(p.R459H)] and exon 1 [c.182T>C (p.L61P)]. The mutation site inherited from the patient's father was consistent with the amino acid position of the mutation site reported in the literature (12). This pathogenic variant previously reported is c.1375C>T (p.R459C), which results in the substitution of cysteine into arginine (12). However, this nucleic acid variant site was not consistent with that of the patient in the present report. Another previous study reported mutations at position R459 in a Chinese patient, suggesting that this genetic region may be a mutation hotspot within the Chinese population (13). By contrast, the exon 1 [c.182T>C (p.L61P)] mutation originating from the patient's mother has not been previously recorded in the Human Gene Mutation Database (HGMD; https://www.hgmd.cf.ac.uk/ac/index.php). Therefore, this suggests that novel mutations were discovered in the present report, which expanded the knowledge on existing mutation sites.

In total, ~100 *CYP27B1* gene mutations have been reported in the ClinVar (www.ncbi.nlm.nih.gov/clinvar/), the most common of which are missense mutations. However, they also occasionally include nonsense, deletion and splicing mutations. Different mutations result in varying levels of disease severity. The patient's genetic sequencing results were consistent missense mutations. Partial mutations in the *CYP27B1* gene, such as Q65H, R107H and P112L, can cause loss of enzyme activity, resulting in severe phenotypes (14). Other mutations, such as G57V, G73W and R459C, are associated with moderate to severe phenotypes (14). VDDR-1A was first reported internationally in 1961 (15), in which children carrying mutations exhibited symptoms from 6 months to 2 years of age. This disease is rarely reported in the Chinese population. A previous study in 2020 (16) cited 11 reports of cases involving Chinese patients, in which all had heterozygous compound mutations. This is consistent with the patient in the present study. This suggests that Chinese patients with VDDR-1A are predominantly heterozygous for this mutation. A previous report in 2020 (14) described two brothers from India with VDDR-1A diagnosed at 30 and 15 months, respectively. They presented with complaints of slow growth, inability to stand, hypotonia and rickets, where genetic testing revealed a homozygous c.1294C>T (p.R432C) mutation. This mutation is associated with severe enzyme inactivation, which in turn results in a severe phenotype. A previous study (11) reported that 38 of the 63 CYP27B1 mutations in the database were missense. This same study also reported that a 13-month-old Saudi Arabian girl homozygous for the nonsense c.1510C>T (p.Q504X) mutation developed a premature stop codon, leading to a severe phenotype. Kaygusuz *et al* (4) analyzed 183 patients with VDDR-1, reporting a median age at diagnosis of 2.55 (min, 1.0; max, 12) years. They also found that the c.195+2T > G and p.K192E mutations resulted in the most and least severe phenotypes, respectively. Li *et al* (16) analyzed two Chinese children with VDDR-1A with the same homozygous mutations in exon 8 (c.1319_1325dupCCCACCC, p.Phe443Profs * 24) who were diagnosed at 33 and 22 months of age, where both patients presented with severe growth retardation. In particular, one patient experienced a fracture, which is a severe clinical phenotype. Therefore, there is highly likely to be a clear genotype-phenotype association in patients with VDDR-IA. However, another study on nine patients observed differences in the severity of the clinical presentations among patients with the same mutation (17). Therefore, larger sample sizes are required to verify this genotype-phenotype association.

Unlike the patient reported in the present report, the cases in the aforementioned literature revealed that the majority of patients were diagnosed during childhood. Results from previous studies showed that early diagnosis and appropriate treatment will allow significant improvements in the skeletal symptoms and serum indicators (4,18). Owing to a lack of awareness of rare diseases among physicians in China and a lack of widespread access to genetic testing, the patient described in the present case report was diagnosed with VDDR-1A late in adulthood. As a result, the patient experienced severe physical ailments that negatively affected his quality of life and caused him significant psychological damage. This case report may improve awareness of this disease among clinicians and increase their vigilance. Although the present report is limited to only one case and therefore the inferred results are not universal, it remains to be recommended that the early genetic screening of children with calcium deficiencies and the early diagnosis of rare diseases are performed to reduce the burden of these disorders on these individuals, families and society.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CY, JHe, JX, XiaoFZ, XianFZ and JHu contributed to the acquisition, analysis and interpretation of the data. All authors read and approved the final manuscript. CY, JHe, JX, XiaoFZ, XianFZ and JHu confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the Institutional Ethics Board of the 'Hangzhou First People's Hospital, Zhejiang University School of Medicine'. The patient and the parents of the patient provided written informed consent for participation in the present report.

Patient consent for publication

The patient and the parents of the patient provided written consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Pang X, Yang Z, Wang J, Duan Y, Zhao L, Yu D and Lai J: Relationship between serum 25OH-vitamin D2 level and vitamin D status of children aged 3-5 years in China. Nutrients 13: 4135, 2021.
2. Goyal A, Anastasopoulou C, Ngu M, *et al*: Hypocalcemia. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL, 2022.
3. Giannakopoulos A, Efthymiadou A and Chrysis D: A case of vitamin-D-dependent rickets type 1A with normal 1,25-dihydroxyvitamin D caused by two novel mutations of the CYP27B1 gene. Horm Res Paediatr 87: 58-63, 2017.

4. Kaygusuz SB, Alavanda C, Kirkgoz T, Eltan M, Abali ZY, Helvacioglu D, Guran T, Ata P, Bereket A and Turan S: Does genotype-phenotype correlation exist in vitamin D-dependent rickets type IA: Report of 13 new cases and review of the literature. Calcif Tissue Int 108: 576-586, 2021.
5. Malloy PJ and Feldman D: Genetic disorders and defects in vitamin D action. Rheum Dis Clin North Am 38: 93-106, 2012.
6. Chanchlani R, Nemer P, Sinha R, Nemer L, Krishnappa V, Sochett E, Safadi F and Raina R: An overview of rickets in children. Kidney Int Rep 5: 980-990, 2020.
7. Kaddam IM, Al-Shaikh AM, Abaalkhail BA, Asseri KS, Al-Saleh YM, Al-Qarni AA, Al-Shuaibi AM, Tamimi WJ and Mukhtar AM: Prevalence of vitamin D deficiency and its associated factors in three regions of Saudi Arabia. Saudi Med J 38: 381-390, 2017.
8. Kim CJ, Kaplan LE, Perwad F, Huang N, Sharma A, Choi Y, Miller WL and Portale AA: Vitamin D 1alpha-hydroxylase gene mutations in patients with 1alpha-hydroxylase deficiency. J Clin Endocrinol Metab 92: 3177-3182, 2007.
9. Kitanaka S, Takeyama K, Murayama A and Kato S: The molecular basis of vitamin D-dependent rickets type I. Endocr J 48: 427-432, 2001.
10. Jansen GA, Wanders RJ, Watkins PA and Mihalik SJ: Phytanoyl-coenzyme A hydroxylase deficiency-the enzyme defect in Refsum's disease. N Engl J Med 337: 133-134, 1997.
11. Babiker AM, Al Gadi I, Al-Jurayyan NA, Al Nemri AMH, Al Haboob AAN, Al Boukai AA, Zahrani AA and Habib HA: A novel pathogenic mutation of the CYP27B1 gene in a patient with vitamin D-dependent rickets type 1: A case report. BMC Res Notes 7: 783, 2014.
12. Cui N, Xia W, Su H, Pang L, Jiang Y, Sun Y, Nie M, Xing X, Li M, Wang O, *et al*: Novel mutations of CYP27B1 gene lead to reduced activity of 1alpha-hydroxylase in Chinese patients. Bone 51: 563-569, 2012.
13. Hu WW, Ke YH, He JW, Fu WZ, Wang C, Zhang H, Yue H, Gu JM and Zhang ZL: A novel compound mutation of CYP27B1 in a Chinese family with vitamin D-dependent rickets type 1A. J Pediatr Endocrinol Metab 27: 335-341, 2014.
14. Dhull RS, Jain R, Deepthi B, Cheong HL, Saha A, Mehndiratta M and Basu S: Vitamin D-dependent rickets (VDDR) type 1: Case series of two siblings with a CYP27B1 mutation and review of the literature. J Bras Nefrol 42: 494-497, 2020 (In English, Portuguese).
15. Prader A, Illig R and Heierli E: An unusual form of primary vitamin D-resistant rickets with hypocalcemia and autosomal-dominant hereditary transmission: Hereditary pseudo-deficiency rickets. Helv Paediatr Acta 16: 452-468, 1961 (In German).
16. Li Y, Yuan X, Chen R, Lin X, Shangguan H, Yang X and Zhang Y: Clinical and genetic analysis of two Chinese families with vitamin D-dependent rickets type IA and follow-up. Orphanet J Rare Dis 15: 273, 2020.
17. Ozden A and Doneray H: The genetics and clinical manifestations of patients with vitamin D dependent rickets type 1A. J Pediatr Endocrinol Metab 34: 781-789, 2021.
18. Demir K, Kattan WE, Zou M, Durmaz E, BinEssa H, Nalbantoğlu O, Al-Rijjal RA, Meyer B, Özkan B and Shi Y. Novel CYP27B1 gene mutations in patients with vitamin D-dependent rickets type 1A. PLoS One 10: e0131376, 2015.