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Research Article

A CLINICAL, GENETIC, AND TREATMENT ANALYSIS OF TWO VIETNAMESE PATIENTS WITH VITAMIN D-DEPENDENT RICKETS TYPE 1A

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ABSTRACT

This study examined the clinical and genetic features of two unrelated Vietnamese families exhibiting the characteristic symptoms of vitamin D-dependent rickets type 1A (VDDR-1A). The affected patients presented with prominent skeletal deformities, severe hypocalcemia, and markedly elevated serum levels of alkaline phosphatase (ALP) and parathyroid hormone (PTH), despite having normal or elevated concentrations of 25-hydroxyvitamin D [25(OH)D]. Genetic analysis revealed a homozygous frameshift mutation (c.1319_1325dupCCCACCC; p.Phe443Profs*24) in both patients. This previously reported pathogenic variant follows an autosomal recessive inheritance pattern. In both families, the asymptomatic parents were confirmed to be heterozygous carriers, and the patients' siblings carried the mutation in a heterozygous state but exhibited no clinical manifestations. This mutation appears to result in early-onset and severe skeletal abnormalities, consistent with the phenotype observed in our patients. Treatment with calcitriol led to significant clinical and biochemical improvement over time, including normalization of calcium and PTH levels and a reduction in skeletal symptoms. Continued follow-up has demonstrated sustained improvement, highlighting the efficacy of targeted therapy in genetically confirmed cases. Our findings underscore the importance of incorporating genetic testing into the diagnostic pathway for children presenting with unexplained rickets-like symptoms. Early identification of causative mutations facilitates timely intervention, the development of

optimized treatment strategies, and the prevention of long-term complications. Moreover, molecular diagnosis provides a basis for targeted genetic counselling, enabling families to better understand the condition and its implications. This approach supports the assessment of recurrence risk, identification of asymptomatic carriers among relatives, and informed reproductive decision-making. As such, genetic screening plays a pivotal role not only in individual patient management but also in shaping broader public health strategies, particularly in populations where inherited disorders may be underrecognized or underdiagnosed.

Keywords: 25-hydroxyvitamin D-1α-hydroxylase deficiency, autosomal recessive inheritance, calcitriol treatment, *CYP27B1,* vitamin D-dependent rickets type 1A.

INTRODUCTION

Vitamin D-dependent rickets type 1A (VDDR-1A; OMIM #264700) is a rare, inherited disorder of vitamin D metabolism caused by autosomal recessive mutations in the cytochrome P450 family 27 subfamily B member 1 gene (*CYP27B1*; OMIM #609506) (NM_000785.3). The disorder is defined by a deficiency of the 1α-hydroxylase enzyme, a crucial catalyst responsible for converting 25-hydroxyvitamin D [25(OH)D] into its biologically active form, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], also known as calcitriol. Despite having normal or elevated levels of 25(OH)D in their serum, individuals with this condition develop severe rickets due to an impaired synthesis of calcitriol (Fraser *et al.*, 1973; Labuda *et al.*, 1990).

The *CYP27B1* gene, situated on chromosome 12q13.1–q13.3, comprises nine exons encoding the 1α-hydroxylase enzyme. Mutations in this gene can disrupt the hydroxylation of 25(OH)D partially or completely, resulting in a deficiency of functional calcitriol. To date, approximately 100 mutations in the *CYP27B1* gene have been documented in patients with VDDR-1A, encompassing missense, nonsense, frameshift, point, and splicing variants. These genetic alterations generally lead to complete or severe loss of enzymatic activity (Babiker *et al.*, 2014; Dhull *et al.*, 2020; Dodamani *et al.*, 2021).

One notable mutation is a 7-nucleotide duplication (c.1319-1325dupCCCACCC) in exon 8 of the *CYP27B1* gene. This duplication induces a frameshift beginning at codon 443, leading to a modified amino acid sequence and the introduction of a premature stop codon. The result is a truncated, nonfunctional protein that lacks 1α-hydroxylase activity. This mutation has been documented in clinical studies from Japan, China, Korea, and several Southeast Asian countries, suggesting a potential founder effect in certain populations. It is considered one of the most common pathogenic variants of *CYP27B1* in Asia (Edouard *et al.*, 2011; Velásquez-Jones *et al.*, 2015; Tahir *et al.*, 2016; Li *et al.*, 2020; Yamazaki and Michigami, 2022).

Genetic sequencing that identifies the c.1319-1325dupCCCACCC mutation provides a definitive molecular diagnosis. This diagnosis enables carrier screening, family-based genetic counseling, and prenatal or preimplantation genetic diagnosis (PGD). Molecular epidemiological studies are essential for identifying population-specific mutations and developing targeted screening

panels. Due to the relatively high frequency of the c.1319-1325dupCCCACCC mutation in Asian populations, early screening for this mutation should be prioritized in suspected cases of VDDR-1A (Fraser *et al.*, 1973; Edouard *et al.*, 2011; Velásquez-Jones *et al.*, 2015; Tahir *et al.*, 2016).

VDDR-1A is a rare disorder that is treatable with appropriate supplementation if diagnosed early. Understanding the molecular basis of the disease aids in diagnosis and prognosis, informs treatment strategies, and supports public health measures for prevention (Li *et al.*, 2020; Kaygusuz *et al.*, 2021). The c.1319-1325dupCCCACCC mutation has been reported in people of various ethnic backgrounds, including Filipinos, Poles, Chinese, Caucasians, African Americans, and Hispanics. This widespread occurrence supports the hypothesis of a potential common ancestral origin, particularly among populations with shared ethnic or familial backgrounds. The exact prevalence of this mutation across different populations is unclear, but it is frequently identified in patients with a family history of rickets or unexplained vitamin D deficiency when no other mutations are detected (Durmaz *et al.*, 2012; Ito *et al.*, 2014).

This study presents the clinical features, biochemical data, and genetic findings of two unrelated Vietnamese patients diagnosed with VDDR-1A. Both individuals were identified as homozygous carriers of the c.1319_1325dupCCCACCC mutation in the *CYP27B1* gene. Furthermore, we assessed their therapeutic response to calcitriol and highlight the critical role of early genetic testing for effective treatment planning and genetic counseling.

MATERIALS AND METHODS

DNA sequence analysis of the *CYP27B1* gene

This study was conducted in accordance with the principles set out in the Declaration of Helsinki. Ethical approval for the study protocol was granted by the Ethics Committee of the Institute of Genome Research under the Vietnam Academy of Science and Technology (Approval no. 01-2024/NCHG-HĐĐĐ, dated 18 January 2024 in Hanoi, Vietnam). The patients' parents were informed about the study and gave consent for their children to participate.

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Whole exome sequencing (WES) was performed, followed by subsequent bioinformatics analysis according to the protocol described by Nguyen *et al.* (2020). Variants showing a minor allele frequency (MAF) above 1% were filtered out using reference data from the 1000 Genomes Project (http://browser.1000genomes.org/). Pathogenic variants were prioritized within a curated list of 196 genes associated with hereditary rickets (Gu *et al.,* 2018).

Candidate variants were confirmed through Sanger sequencing in the patients as well as their family members. Primers targeting exon 8 of the *CYP27B1* gene were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) with the following sequences: a forward primer (5′-GGCCAGTAGGGGACTTCTTG-3′) and a reverse primer (5′-ATGCCTGCCCTATT-CTGAGC-3′). PCR amplification was carried out under the following conditions: initial denaturation at 95°C for 12 minutes, followed by 35 cycles of 95°C for 45

seconds, 56°C for 45 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 8 minutes. The 380-bp PCR product was visualized on a 1.5% agarose gel.

Sanger sequencing was performed using an ABI Big Dye Terminator Kit on an ABI 3100 Genetic Analyzer (Applied Biosystems, USA). The obtained sequences were aligned against the *CYP27B1* reference gene (NM_000785), which was retrieved from the NCBI database (Lien *et al.*, 2024; Nguyen *et al.*, 2025). The potential impact of the detected variants was evaluated using in silico prediction tools, including MutationTaster (Steinhaus *et al.*, 2021) and PolyPhen-2 (Adzhubei *et al.*, 2013). Variant interpretation was conducted in accordance with the guidelines issued by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (Richards *et al.*, 2015).

Biochemical tests

Total blood calcium concentration was measured using the optical density method on the AU5800 Atellica analyzer. Blood phosphorus concentrations were measured using the optical absorbance technique on the AU5800 Atellica analyzer. Serum 25-hydroxyvitamin D [(25(OH)D] levels were quantified using the electrochemiluminescence immunoassay method, conducted on the Cobas Pro integrated system in conjunction with the Cobas Infinity platforms. Alkaline phosphatase (ALP) activity was measured using the enzyme kinetic method on the AU5800 Atellica analyzer. Parathyroid hormone (PTH) levels were quantified using the electrochemiluminescence method on the Cobas Pro and Cobas Infinity platforms in the Department of Biochemistry at the National Children's Hospital.

Imaging techniques

Radiographic evaluations were conducted using the Carestream DRX1 System (Carestream, USA) at the Diagnostic Imaging Department of the Vietnam National Children's Hospital. Standard X-rays of the lower limbs were obtained to identify skeletal deformities.

Long-term treatment with calcitriol and calcium

Children were treated with an initial dose of calcitriol of 50 ng/kg/day, divided into two to three doses per day, and elemental calcium at a dose of 50-100 mg/kg/day. Clinical information was recorded both prior to and following the administration of calcitriol and calcium in patients 1 and 2. Following the initiation of calcitriol therapy, serum concentrations of calcium, phosphate, ALP, PTH, 25-hydroxyvitamin D_3 [$25(OH)D_3$], and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] were evaluated at each clinical visit.

RESULTS

Patient clinical profiles

Patient 1

The patient was a 15-month-old female, the second offspring of healthy, nonconsanguineous parents. There was no family history of VDDR-1A or skeletal abnormalities. According to the mother, the pregnancy and delivery were uneventful. The patient was referred at 15 months old for evaluation due to the inability to stand or walk. The family reported delayed motor milestones, poor linear growth, and limb shortening despite the early initiation of vitamin D supplementation at a dose of 800 IU per day.

The patient achieved head control at two months, sat unaided at six months, and began crawling at nine months. However, her motor development subsequently plateaued, and by 15 months, she was still unable to stand or walk. She exhibited mild hypotonia. Anthropometric measurements revealed a height of 74 cm (Z-score: −0.9 SD, per WHO standards) and a weight of 8.4 kg (Z-score: −0.9 SD). Physical examination revealed no rachitic rosary or chest wall deformities; however, the patient's wrists and ankles were noted to be thickened and enlarged. There was no spinal curvature.

Biochemical evaluation demonstrated profound hypocalcemia and hypophosphatemia, accompanied by significantly elevated PTH levels (796 ng/L; reference range: 11-69 ng/L). Additionally, there was extremely high ALP activity (3003.7 IU/L; reference range: 108-317 IU/L) and serum 25(OH)D levels fell within reference limits.

Skeletal radiographs revealed pronounced metaphyseal abnormalities located at the distal regions of the long bones, such as fraying, cupping, and impaired mineralization, consistent with severe rickets (Figure 1A).

Patient 2

The patient was a 71-month-old female, nearly six years of age, and the second child of healthy, non-consanguineous parents, with an older brother. There was no reported family history of VDDR-1A or congenital skeletal abnormalities. The mother denied any exposure to teratogenic agents or illnesses during pregnancy. The patient was delivered at term via spontaneous vaginal delivery, with a birth weight of 2.8 kg.

Although there was no history of fractures, the family reported early-onset skeletal abnormalities. At presentation, her height was severely stunted (Z-score: –7.3 SD, WHO standards) and her weight was 15 kg. Notable clinical features included significant long bone bowing, enlarged wrists and ankles, a prominent rachitic rosary, a pigeon chest, scoliosis, and a complete loss of ambulation; she was entirely dependent on others for mobility.

Biochemical tests revealed hypocalcemia, hypophosphatemia, markedly elevated PTH levels (727.4 ng/L; reference range: 11-69 ng/L), elevated ALP levels (1791.9 IU/L; reference range: 108-317 IU/L), and serum 25-hydroxyvitamin D [25(OH)D] levels above the normal range (259.5 nmol/L). Bone mineral density (BMD) testing showed severely reduced values: a lumbar spine BMD of 0.45 g/cm^2 (Z-score: −3.5 SD) and a femoral neck BMD of 0.357 g/cm^2 (Z-score: −4.4 SD).

Radiographic evaluation revealed generalized osteopenia, severe skeletal deformities, and Loose's zones, which are pseudofractures characteristic of rickets due to metabolic bone disease (Figure 1B).

Based on the clinical manifestations and biochemical results, both patients were diagnosed with 25-hydroxyvitamin D-1α-hydroxylase deficiency, confirming the diagnosis of VDDR-1A.

Genetic results

WES data analysis identified a homozygous frameshift duplication mutation in exon 8 of the *CYP27B1* gene in both patients. This mutation is designated c.1319_1325dup-CCCACCC/p.Phe443Profs*24. It involves the duplication of a seven-nucleotide segment ("CCCACCC"), resulting in a frameshift that alters the amino acid sequence beginning at codon 443 and

introduces a premature stop codon 24 amino acids downstream. The asterisk (*24) indicates termination after 24 aberrant amino acids. Consequently, the mutation results in the production of a truncated protein devoid of 1α-hydroxylase activity, causing enzymatic deficiency and impairing normal vitamin D metabolism.

This aberrant protein is incapable of catalyzing the 1α-hydroxylation of 25-hydroxyvitamin D [25(OH)D] to generate 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the biologically active form of vitamin D. Insufficient levels of 1,25$(OH)_2$D led to reduced intestinal absorption of calcium and phosphate. This leads to the clinical manifestations of rickets, including delayed bone growth, skeletal deformities, and symptoms related to hypocalcemia and hypophosphatemia.

This mutation has been documented in pathogenic variant databases, including ClinVar (accessed in May 2025), as a disease-causing variant. Both patients carried this mutation in a homozygous state, in line with the autosomal recessive mode of inheritance characteristic of the disorder.

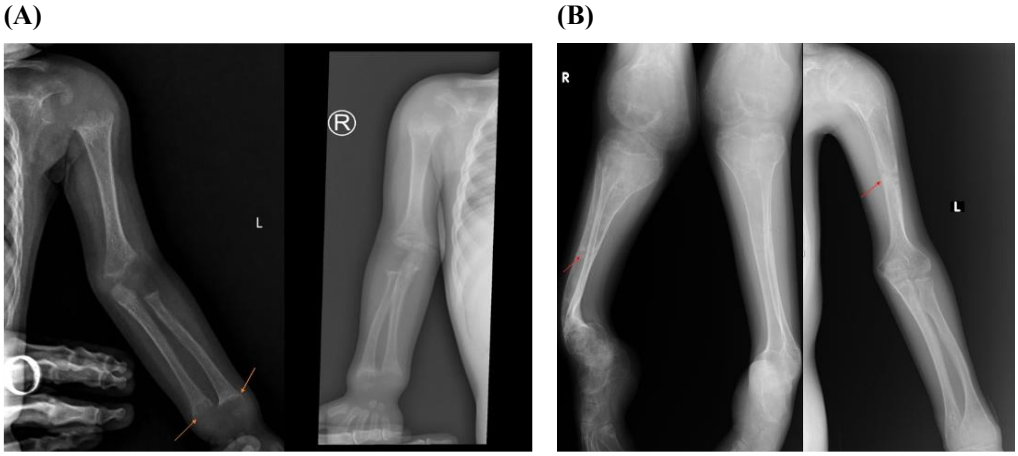

Figure 1. Radiographic images of two patients. (A) Patient 1: The arrow indicates concavity and irregularity at the metaphyseal ends of the long bones. (B) Patient 2: Marked bone deformities, decreased BMD, and the presence of pseudofractures (Looser's zones).

Confirmation and genetic analysis by Sanger sequencing

To confirm the WES findings and analyze the pattern of inheritance within the families, Sanger sequencing was performed on family members of the patients. In Patient 1's family, the father, mother, and older brother were found to be heterozygous carriers of the c.1319_1325dupCCCACCC mutation. Each family member possessed one mutated allele but did not exhibit any clinical symptoms (Figure 2).

Similarly, Patient 2 carried the mutation in a homozygous state, which was inherited from both of her heterozygous carrier parents. The father, mother, and sister of Patient 2 were confirmed to be heterozygous carriers without any clinical manifestations.

These results confirm the autosomal recessive inheritance pattern of VDDR-1A, showing that affected patients inherit one mutant allele from each parent, while heterozygous family members remain asymptomatic carriers (Figure 3).

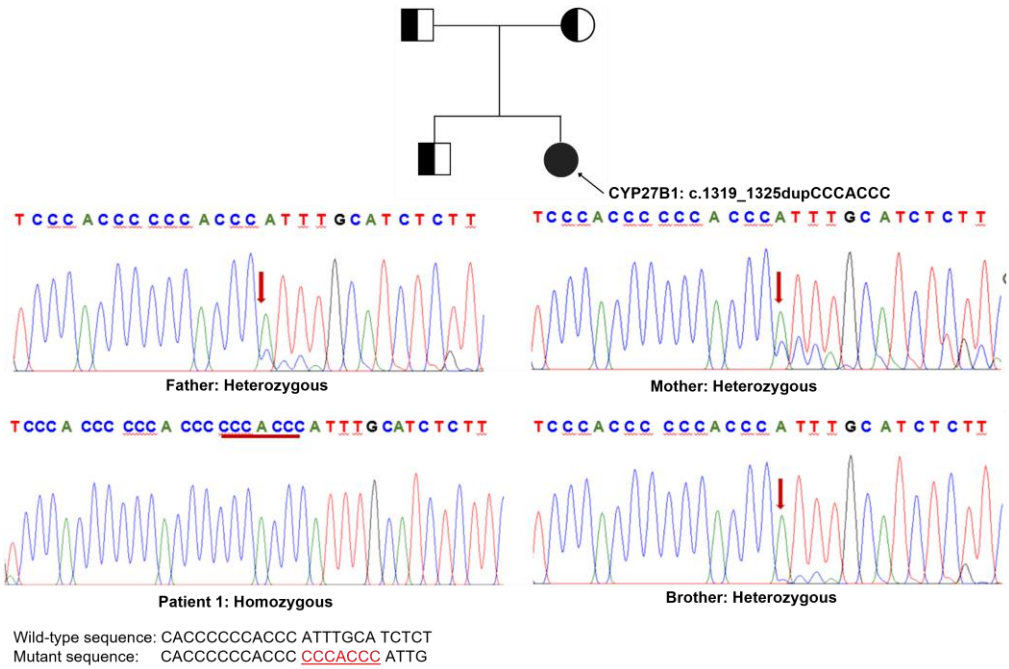

Figure 2. Sequencing electropherogram of family 1. A seven-nucleotide duplication (c.1319_1325dupCCCACCC, pPhe443Profs*24) was present in Patient 1, her father, mother, and brother. Patient 1 was homozygous and the others were heterozygous.

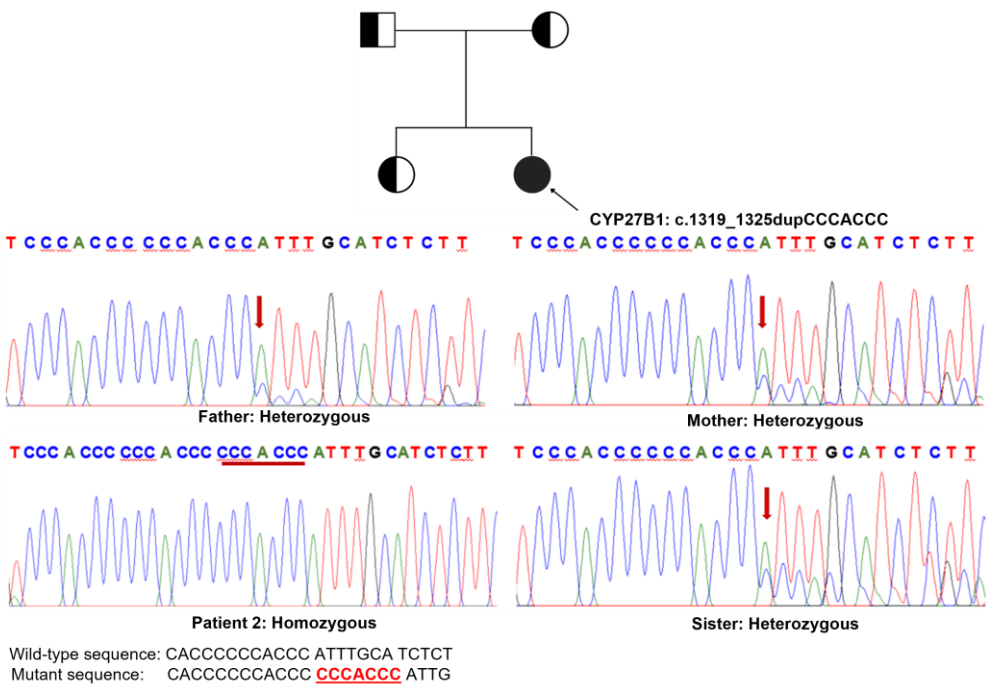

Figure 3. Sequencing electropherogram of family 2. A seven-nucleotide duplication in exon 8 (c.1319_1325dupCCCACCC, p.Phe443Profs*24) was present in Patient 2 and her father, mother, and sister. Patient 2 was homozygous and the others were heterozygous.

DISCUSSION

Clinical and genetic findings

In this study, we identified the previously reported c.1319_1325dupCCCACCC duplication in two unrelated patients diagnosed with VDDR-1A. This variant introduces a frameshift leading to premature translational termination, resulting in a truncated protein devoid of enzymatic function (Yamamoto *et al.*, 2004; Durmaz *et al.*, 2012; Li *et al.*, 2020; Lin *et al.*, 2022). The patients exhibited clinical and genetic features consistent with vitamin D 1α-hydroxylase deficiency, caused by mutations in the *CYP27B1* gene, which is inherited in an autosomal recessive manner. Both patients had the seven-nucleotide duplication (CCCACCC) in exon 8 of the *CYP27B1* gene. Although these families are unrelated and originate from different cities in Vietnam, this mutation may represent a founder effect derived from a distant common ancestor. The duplication of the normal DNA sequence 5′-CCCACCC CCCACCC-3′ (Pro-Thr-Pro-His-Pro) causes a frameshift that introduces a premature stop codon at position 466 (Phe443Profs*24), resulting in a truncated protein without enzymatic activity (Durmaz *et al.*, 2012; Koek *et al.*, 2016; Li *et al.*, 2020; Yamazaki and Michigami, 2022).

Patient 1 is a 15-month-old girl who was diagnosed with VDDR-1A. She presented with delayed motor development, an inability to stand, hypocalcemia, hypophosphatemia, markedly elevated PTH, and highly increased ALP. Her Rickets Severity Score (RSS) was very high, as evidenced by typical radiographic changes at the metaphyses of her long bones, including cupping and fraying. The patient carries a homozygous c.1319_1325dupCCCACCC mutation in the *CYP27B1* gene. This mutation explains why the body cannot produce the active form of vitamin D despite normal (even elevated due to supplementation) serum vitamin D levels. This leads to severe bone metabolism disturbances with low serum calcium and phosphate levels and significantly elevated PTH. Family pedigree and genetic analysis revealed that the father, mother, and older brother are heterozygous carriers without clinical symptoms. The patient is the only affected individual because she is homozygous for the mutation.

Patient 2 has a similar genetic background and was also diagnosed with VDDR-1A. His clinical features include delayed motor development, long bone deformities, widened wrists and ankles, pigeon chest, scoliosis, and an inability to walk. The patient's height is severely below average for their age (–7.3 SD). Laboratory investigations showed reduced serum calcium and phosphate levels, accompanied by elevated concentrations of PTH and ALP. She also had elevated 25-hydroxyvitamin D [25(OH)D] levels, very low BMD, and radiographic evidence of Looser's zones (pseudofractures), indicative of severe osteomalacia. Genetic analysis showed that the patient was homozygous for the mutation and clinically affected, while the father, mother, and sister are heterozygous carriers without symptoms.

These findings are consistent with an autosomal recessive inheritance pattern, in which the disease manifests only in individuals who carry two mutated alleles - one inherited from each heterozygous carrier parent. The occurrence of the disease in children with asymptomatic parents is a typical feature of this model. VDDR-1A exhibits complete penetrance; however,

clinical variability may occur even among individuals carrying the identical CYP27B1 mutation (Cao *et al.*, 2011; Cui *et al.*, 2012; Sahay and Sahay, 2012).

The c.1319_1325dupCCCACCC mutation is one of the most commonly reported *CYP27B1* mutations in various studies from Vietnam and other Asian countries (Li *et al.*, 2020; Lin *et al.*, 2022; Tran *et al.*, 2025). A study by Vu *et al*. (2025) in Vietnam showed that over 90% of children with vitamin D deficiency rickets in their cohort carried this mutation, predominantly in the homozygous state (Tran *et al.*, 2025). Most were born to heterozygous carrier parents.

Treatment

Follow the treatment regimen recommended by the National Children's Hospital. Treatment for these patients includes supplementation with calcitriol (the active form of vitamin D) and calcium to compensate for deficiencies and to maintain serum calcium and phosphate levels within normal ranges. However, patients with this mutation often require lifelong calcitriol therapy, although some may improve with age. Treatment was initiated with calcitriol at a dosage of 0.5 µg per day, combined with phosphate supplementation at standard therapeutic levels (Haffner *et al.*, 2022).

After three months of combined treatment with calcitriol and oral calcium, Patient 1 demonstrated significant biochemical improvement, particularly the normalization of serum calcium (2.33 mmol/L), reflecting the efficacy of active hormone replacement in restoring intestinal calcium absorption and mineral homeostasis. PTH levels decreased from 796 pg/mL to 436 pg/mL, indicating partial control of secondary hyperparathyroidism, although still elevated, reflecting continued adaptation to incomplete recovery of mineralization. This phenomenon is common in prolonged metabolic rickets, where normalization of mineral levels does not immediately translate into bone tissue recovery (Thacher *et al.*, 2000; Goltzman *et al.*, 2018; Lin *et al.*, 2022).

ALP decreased from 3003.7 UI/L to 1661.3 IU/L, suggesting that active bone formation was beginning to normalize, consistent with early mineralization phases. Persistently high ALP is characteristic of early treatment stages in VDDR-1A when bone remodeling is still incomplete. Clinically, the patient showed significant improvement, gaining the ability to stand and walk, with reduced rachitic features such as rib beading and stabilized bone deformities, confirming the effectiveness of treatment (Cannalire *et al.*, 2023).

After six months of treatment, clear clinical improvements were noted for Patient 2, including the ability to stand and take short steps and a reduction in rib beading. Wrist and ankle widening persisted but were less pronounced. Biochemical tests after six months revealed that total calcium increased to 2.3 mmol/L and ionized calcium increased to 1.0 mmol/L. Phosphate increased mildly to 0.63 mmol/L. PTH remained elevated at 263 pg/mL, and although reduced, ALP remained elevated at 1583 U/L. These results indicate gradual improvement in metabolic bone disease but incomplete recovery. Continued long-term monitoring and therapy are necessary to achieve optimal bone mineralization and prevent permanent skeletal complications (Thacher *et al.*, 2000).

Assessment of early treatment effect – Comparison of two cases

Monitoring the clinical courses of these two children with VDDR-1A illustrates the

positive impact of early diagnosis and intervention on motor function and bone recovery. Patient 2, who presented with severe clinical features including pronounced long bone deformities, scoliosis, an inability to stand or walk, very low bone density, and Looser's zones on X-rays, was diagnosed and treated at 71 months of age. After six months of treatment, although the biochemical markers improved, the child could only stand and take a few tentative steps, with persistent bone deformities.

In contrast, Patient 1 was diagnosed and treated at 15 months of age before severe clinical manifestations developed, despite having similar biochemical abnormalities (hypocalcemia, hypophosphatemia, and elevated PTH). After three months of treatment, Patient 1 was able to walk independently, demonstrating more rapid and pronounced motor recovery compared to Patient 2.

The significant difference in functional recovery and skeletal deformity between the two patients highlights the critical importance of early diagnosis and treatment in VDDR-1A. Early detection before severe bone damage occurs accelerates the treatment response, reduces the risk of permanent skeletal sequelae, and significantly improves quality of life. Therefore, raising awareness of vitamin D-resistant rickets, especially type IA, at the primary healthcare level is essential for facilitating timely intervention and avoiding long-term adverse outcomes in children's development.

The c.1319_1325dupCCCACCC mutation in *CYP27B1* was the primary cause of VDDR-1A in both patients. The homozygous state of this mutation corresponds to the characteristic clinical presentation and recessive inheritance pattern. This further emphasizes the essential role of genetic testing in diagnosing and guiding prenatal counseling for rare genetic disorders such as VDDR-1A.

CONCLUSION

This study confirms that the c.1319_1325dupCCCACCC mutation in the *CYP27B1* gene is a predominant cause of VDDR-1A among Vietnamese patients. Both patients, who were unrelated and who carried this homozygous frameshift duplication exhibited the typical clinical features of VDDR-1A. These characteristics comprised marked hypocalcemia and hypophosphatemia, increased levels of PTH and ALP, as well as distinctive radiographic abnormalities. Early diagnosis and treatment with calcitriol and calcium supplementation resulted in significant biochemical and clinical improvement, particularly in the younger patient, who was diagnosed at 15 months of age. This patient demonstrated more rapid motor function recovery and fewer skeletal deformities compared to the older patient, who was diagnosed at 71 months of age. These findings underscore the critical importance of early genetic diagnosis and timely intervention in improving outcomes and preventing irreversible bone damage in patients with VDDR-1A. Furthermore, identification of this mutation highlights the value of genetic screening for optimizing disease management and providing accurate genetic counseling for affected families.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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