Vitamin D Dependent Rickets Type 1A Caused by CYP27B1 Mutation

Vitamin D dependent rickets type 1A (VDDR1A) is an autosomal recessive disorder caused by mutations in CYP27B1. Clinical findings are growth retardation, hypotonia, muscle weakness, hypocalcemic seizures, and radiological features of rickets. We aimed to present the VDDR1A case with a genetic study of CYP27B1. The 14-month-old boy was admitted to the hospital due to a seizure. Serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone (PTH), 25(OH) vitamin D, and 1,25(OH)2 vitamin D values were 5.1 mg/dL, 3.7 mg/dL, 705 IU/L, 429 pg/mL, 24.9 ng/mL, and 8.8 pg/mL, respectively. Radiological study showed cupping and fraying of the distal ulna and radius. The molecular genetic study revealed that the patient had a compound heterozygous mutation, Phe443Profs*24 and c.589+1G>A, in CYP27B1. Genetic analysis of the family members presented that the mother was heterozygous for the mutation c.589+1G>A, and that the father was heterozygous for Phe443Profs*24. The patient was treated with calcium lactate and calcitriol. Until now, six Korean patients with VDDR1A have been studied. Including this case, Korean patients with VDDR1A were found to have only three different mutations in 14 alleles, indicating that the mutation in the CYP27B1 gene is homogeneous in the Korean population.

Key words: Rickets, CYP27B1 gene, Vitamin D dependent rickets type 1A

Introduction

There are two types of vitamin D: ergocalciferol (D2) and cholecalciferol (D3). Two types of vitamin D are biologically inactive prohormones, and it is necessary to undergo two enzymatic conversions in the liver and kidney. CYP2R1 catalyzes the initial hydroxylation step in the liver converting vitamin D to 25(OH) vitamin D (25OHD). Albeit 25OHD is the most plentiful type of vitamin D in circulation, it has minimal ability bind to the vitamin D receptor (VDR). The 25OHD 1α-hydroxylase gene (CYP27B1) encodes the 1α-hydroxylase enzyme, which catalyzes 25OHD to 1,25(OH)2 vitamin D [1,25(OH)2D]. The 1,25(OH)2D is the active type of vitamin D that acts via the VDR to increase the expression of genes. It is stimulated by parathyroid hormone (PTH), hypocalcemia, and hypophosphatemia.

Vitamin D dependent rickets type 1A (VDDR1A), also known as vitamin D 1α-hydroxylase deficiency or pseudovitamin D deficiency rickets, is an autosomal recessively inherited disorder resulting from defects in the CYP27B1. The associated clinical findings are growth retardation, hypotonia, muscle...
weakness, hypocalcemic seizures, and radiological features of rickets. Biochemical findings show hypocalcemia, elevated PTH, and decreased values of 1,25 (OH)2D in spite of normal or elevated concentrations of 25OHD. Generally, a good response to medication with alfacalcidol or calcitriol is estimated for patients with VDDR1A. Calcitriol and calcium treatment was modified based on the laboratory and clinical features. Treatment results in the normalization of laboratory parameters and radiographic abnormalities within 3 months.

So far, six Korean patients with VDDR1A have been investigated at a genetic study. We now report the results of clinical and molecular genetic studies of CYP27B1 mutations in one additional patient and review the findings in all reported Korean patients with CYP27B1 defects.

Case report

A 14-month-old boy was admitted in our hospital due to a generalized tonic seizure lasting for two minutes. He was born to non-consanguineous Korean parents as their first baby at 40 weeks of gestation. The boy was healthy at birth and weighed 4,220 g. The parents were healthy and they do not have any hereditary disorders. At the age of 9 months, he had the ability to crawl, and at 13 months, he was able to walk alone. At a physical examination, he was 77.0 cm tall (25–50 percentile) and weighed 9.5 kg (10–25 percentile).

Laboratory profile showed as follows; serum total calcium of 4.8 mg/dL (normal range, 8.4–10.2 mg/dL); ionized calcium of 1.4 mEq/L (normal range, 2.2–2.6 mEq/L); phosphorus of 3.7 mg/dL (normal range, 3.8–6.5 mg/dL), alkaline phosphatase of 705 U/L (normal range, 145–420 U/L); and PTH of 429 pg/mL (normal range, 10–65 pg/mL). 25OHD level was 24.9 ng/mL (normal range, 30–90 pg/mL) and 1,25(OH)2D was 8.8 pg/mL (normal range, 20–55 pg/mL). Radiological study showed cupping and fraying of the distal ulna and radius (Fig. 1). The above laboratory and radiological signs were compatible with a diagnosis of rickets. A decreased 1,25(OH)2D level with relatively normal 25OHD and very high PTH concentrations indicated a diagnosis of a genetic type of rickets, precisely 1α-hydroxylase deficiency.

A mutation analysis of the CYP27B1 gene was performed. Genomic DNA was extracted from leukocytes. All coding exons and intron in flanking regions of CYP27B1 were amplified by polymerase chain reaction and directly sequenced. The primers used for DNA sequencing of patients were as previously described. Molecular genetic study revealed that the patient had a compound heterozygous mutation for the 7-bp duplication 1319-1325dupCCCACCC (Phe443Profs*24) and c.589+1G>A in the CYP27B1 gene (Fig. 2). The family members revealed that the mother was heterozygous for the mutation c.589+1G>A, and the father was heterozygous for Phe443Profs*24. G to A substitution in the first nucleotide of intron 3 (c.589+1G>A) was reported previously in Japanese and Korean patients. The Phe443Profs*24 mutation was reported in multiple ethnic groups including Korean population.

The boy was treated with calcitriol and calcium lactate. Compliance with the supplementation was good, resulting in quick improvement of the abnormal clinical, laboratory, and radiographic findings. The patient showed normocalcemia without recurrent hypocalcemic seizures. Radiologic study showed marked improvement after 5 months of treatment (Fig. I).

Discussion

There are four types of genetic errors in vitamin D me-
tabolism, and it can cause rickets. The first type is the VDDR1A, which is caused by defects in the CYP27B1 gene. A mutation in the CYP27B1 gene that results in 25-hydroxylation deficiency causes vitamin D dependent rickets type 1B (VDDR1B). Vitamin D dependent rickets type 2A (VDDR2A), also referred to as vitamin D resistant rickets (VDDR), is due to a defect in the VDR. VDDR2B is a rare type of rickets owing to abnormal expression of a hormone response element-binding protein that disrupts the normal function of VDR13,14).

The clinical and laboratory findings in patients with VDDR1A are similar to those seen in patients with nutritional rickets or hypophosphatemic rickets. However, VDDR1A can be discriminated from hypophosphatemic rickets by increased PTH and decreased 1,25(OH)2D concentrations and nutritional rickets by a normal 25OHD value. The hypophosphatemia in VDDR1A is an effect of high PTH level and renal excretion of phosphate. PTH promotes calcium reabsorption in the renal proximal tubules, and also promotes serum calcium through bone resorption15). It also increases the alkaline phosphatase level15). Most of the reported patients with VDDR1A showed low or undetectable 1,25(OH)2D levels. But there have been a few reported cases of patients with VDDR1A with normal 1,25(OH)2D levels6,16,17). Nishikawa et al.18) presented that liver mitochondrial CYP27A1 can catalyse 1α-hydroxylation of 25OHD. A little bit increment of 1,25(OH)2D concentrations has been reported in CYP27B1 knockout mice after high vitamin D supplementation, suggesting the conversion from 25OHD to 1,25(OH)2D by a non-CYP27B1 enzyme14). Wang et al.17) reported two patients with VDDR1A who had normal 1,25(OH)2D values. Both exhibited a mild phenotype, and their mutations had partial enzyme function in vitro. So, VDDR1A should be suspected in cases with a mild or severe phenotype of rickets and normal or elevated values of 25OHD albeit 1,25(OH)2D concentrations are within the normal values.

The CYP27B1 gene is constituted of 9 exons spanning 5 kb19). Until now, more than 70 mutations have been reported in the CYP27B1 gene in multiple ethnic groups10,14). Several mutations in the CYP27B1 gene are more common in specific populations8,12). Deletion of guanine 958 (958delG) was reported on 20 French Canadian allele, and a microsatellite haplotype study revealed that these arose from a single ancestor20). A seven nucleotide duplication in exon 8 (Phe443Profs*24) was reported in 14 affected alleles, and these arose from different populations with diverse microsatellite haplotypes12).

To date, six Korean patients with VDDR1A have been investigated at a genetic study, and three different mutations have been reported8-10). There are two frequent mutation points; c.589+1G>A in seven alleles and Phe443Profs*24 in four alleles. Our patient was also found to have two mutations common in the Korean population: c.589+1G>A and Phe443Profs*24. Thus, Korean patients with VDDR1A have been found to have only three different mutations in

![Fig. 2. Mutation analysis of the proband revealed a compound heterozygous mutation for c.589+1G>A and Phe443Profs*24 in the CYP27B1 gene.](image-url)
14 alleles.

Including our case, the clinical characteristics of seven patients with vitamin D dependent rickets type 1A were summarized in Table 1. The most prevalent mutation is the c.589+1G>A splice donor site mutation, affecting 8 out of 14 alleles in Korean patients with VDDR1A. This mutation also have been reported in Japanese patients\(^{11}\). G to A change in the first nucleotide of an intron is a relatively frequent mutation and generally results in the skipping of the exon or initiation of potential splice sites\(^{17}\). The c.589+1G>A mutation introduces a premature stop codon, and it causes disruption of 1α-hydroxylase activity.

The mutation of Phe443Profs*24 had been found in 5 of 14 alleles in Korean patients. This seven nucleotide duplication (CCCCACCC) causes a premature TGA stop signal. It eliminates the heme-binding domain of 1α-hydroxylase and thus inactivate enzyme activity. Wang et al.\(^{12}\) presented that six families of different ethnic groups had this mutation in association with four diverse microsatellite haplotypes. Kitanaka et al.\(^{11}\) reported 10 different \(CYP27B1\) gene mutations in 20 Japanese alleles, indicating that there was no single ancestor effect in Japanese population.

In conclusion, we present a case study of VDDR1A in which the patient was found to have a compound heterozygous mutation, Phe443Profs*24 and c.589+1G>A, in the \(CYP27B1\) gene. Korean patients have been found to have only three different mutations in 14 alleles. This suggests that the mutation of the \(CYP27B1\) gene is relatively homogeneous in the Korean population.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

**Patient consent**

This study was approved by the institutional review board (IRB), and the consent was waived due to the nature of the retrospective study [IRB number CNUH-EXP-2018-295].

**References**


**Table 1. Clinical Characteristics of Seven Patients with Vitamin D Dependent Rickets Type 1A**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis (months)</th>
<th>Presentation</th>
<th>Serum Ca (mg/dL)</th>
<th>Serum P (mg/dL)</th>
<th>Serum ALP (IU/L)</th>
<th>Serum PTH (pg/mL)</th>
<th>Serum 25OHD (ng/mL)</th>
<th>Serum 1,25-(OH)2D (pg/mL)</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>rachitic rosary</td>
<td>6.4</td>
<td>4.0</td>
<td>2,043</td>
<td>1,320</td>
<td>27</td>
<td>9.2</td>
<td>Phe443Profs<em>24/Phe443Profs</em>24</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>rachitic rosary</td>
<td>5.9</td>
<td>3.6</td>
<td>1,326</td>
<td>750</td>
<td>77</td>
<td>&lt;4</td>
<td>c.589+1G&gt;A / 2561G&gt;A</td>
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<tr>
<td>3</td>
<td>7</td>
<td>seizure</td>
<td>5.2</td>
<td>6.2</td>
<td>665</td>
<td>750</td>
<td>38</td>
<td>&lt;5</td>
<td>c.589+1G&gt;A/Phe443Profs*24</td>
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<tr>
<td>4</td>
<td>12</td>
<td>bowed legs</td>
<td>6.0</td>
<td>3.8</td>
<td>1,050</td>
<td>560</td>
<td>N/A</td>
<td>N/A</td>
<td>c.589+1G&gt;A / c.589+1G&gt;A</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>femur fracture</td>
<td>7.3</td>
<td>5.0</td>
<td>1,437</td>
<td>16</td>
<td>N/A</td>
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<tr>
<td>6</td>
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<td>4.3</td>
<td>1,665</td>
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<tr>
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<td>3.7</td>
<td>705</td>
<td>429</td>
<td>24.9</td>
<td>8.8</td>
<td>c.589+1G&gt;A / Phe443Profs*24</td>
</tr>
</tbody>
</table>

Abbreviations; ALP, Alkaline phosphatase; N/A, Not available. Data of Patients 1 to 5 are adopted from reference 9. Patient 6 is adopted from reference 10. Patient 7 is in this study.

*Nucleotide numbers refer to genomic DNA and are numbered from the transcription start site\(^{20}\). The reference sequence is available on the NCBI, Entrez, Nucleotide database: http://www.ncbi.nlm.nih.gov/Entrez; accession number AF 027152.*