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Case Report

Effect of calcium supplementation on bone deformity and histopathological findings of skin papules in a pediatric patient with vitamin D–dependent rickets type 2A: A case report

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Highlights

- Calcium supplementation can be used to treat rickets in VDDR2A.
- Multiple skin papules comprise one skin phenotype in patients with VDDR2A.
- Altered DNA-binding domain of the *VDR* gene usually leads to skin symptoms.

Abstract. Vitamin D–dependent rickets type 2A (VDDR2A) is an autosomal recessive disease caused by pathogenic variants of the vitamin D receptor (*VDR*) gene. VDDR2A rickets are usually resistant to native or active vitamin D treatment because of impaired active calcium absorption against the calcium concentration gradient, which is a ligand-dependent VDR action in the small intestine. Alopecia due to an impaired skin follicular cycle is occasionally observed in patients with VDDR2A. Among the pathogenic VDR variants, most in the DNA-binding domain and some in the ligand-binding domain, which affect the dimerization of VDR with the retinoic X receptor, are associated with alopecia. Herein, we report a case of VDDR2A caused by compound heterozygous pathogenic variants of the DNA-binding domain of *VDR*. Active vitamin D treatment did not ameliorate genu varum, rachitic changes in the roentgenogram, or abnormal laboratory findings. However, oral administration of calcium lactate dramatically improved these findings. The patient also experienced hair loss at two months of age and multiple papules on the skin at two yr of age, which did not improve with vitamin D or calcium supplementation. We also report the histopathological findings of skin papules in this patient.

Key words: rickets, receptor, alopecia, papules, calcium

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Introduction

Vitamin D–dependent rickets type 2A (VDDR2A) is an autosomal recessive disease caused by pathogenic variants of the vitamin D receptor (*VDR*) gene (1). VDDR2A rickets are usually resistant to native or active vitamin D treatment. Vitamin D acts via the vitamin D receptor (VDR) in the small intestine, which increases active calcium absorption against the calcium concentration gradient by increasing the expression of *TRPV6*, *calbindin9k*, and *PMCA1b* and is impaired in patients with VDDR2A (2). Alopecia, owing to an impaired skin follicular cycle, also occurs in some patients with VDDR2A; alopecia in VDDR2A is related to pathogenic VDR variants located in the DNA-binding domain (DBD) or ligand-binding domain (LBD), and shows impaired dimerization of the retinoic X receptor (1).

Herein, we report a case of VDDR2A caused by compound heterozygous pathogenic variants in the DBD of the *VDR* gene. We also report skin phenotypes, such as alopecia and skin papules, as well as the histopathological findings of the skin papules, in this patient.

Case Report

A 2-yr, 3-mo-old male pediatric patient with suspected rickets was referred to the pediatric department from the orthopedics department of Okayama University Hospital. He was born to non-consanguineous parents via cephalic delivery at 41 wk gestation. His height and body weight at birth were 51 cm (standard deviation [SD], 0.95) and 4190 g (SD, 2.57), respectively. His 5-min Apgar score was 9. The patient was fed artificial milk, had no food allergies, and was often outdoor without sunscreen. The patient was weaned at 11 mo of age and follow-up milk was not used. His motor development was unremarkable and he was able to walk alone at 11 mo of age. During a health checkup at 1 yr and 6 mo of age, genu varum was noted, and the patient was referred to our hospital's orthopedic department. Radiological examination revealed no rachitic changes (**Figs. 1A and B**), and physiological bow leg or Blount's disease was suspected. However, at the follow-up at 2 yr and 3 mo of age, genu varum and rachitic changes were obvious on a roentgenogram (**Figs. 1C–E**).

At the patient's first visit to the pediatric department (a in **Fig. 2**), his height was 84.2 cm (SD,

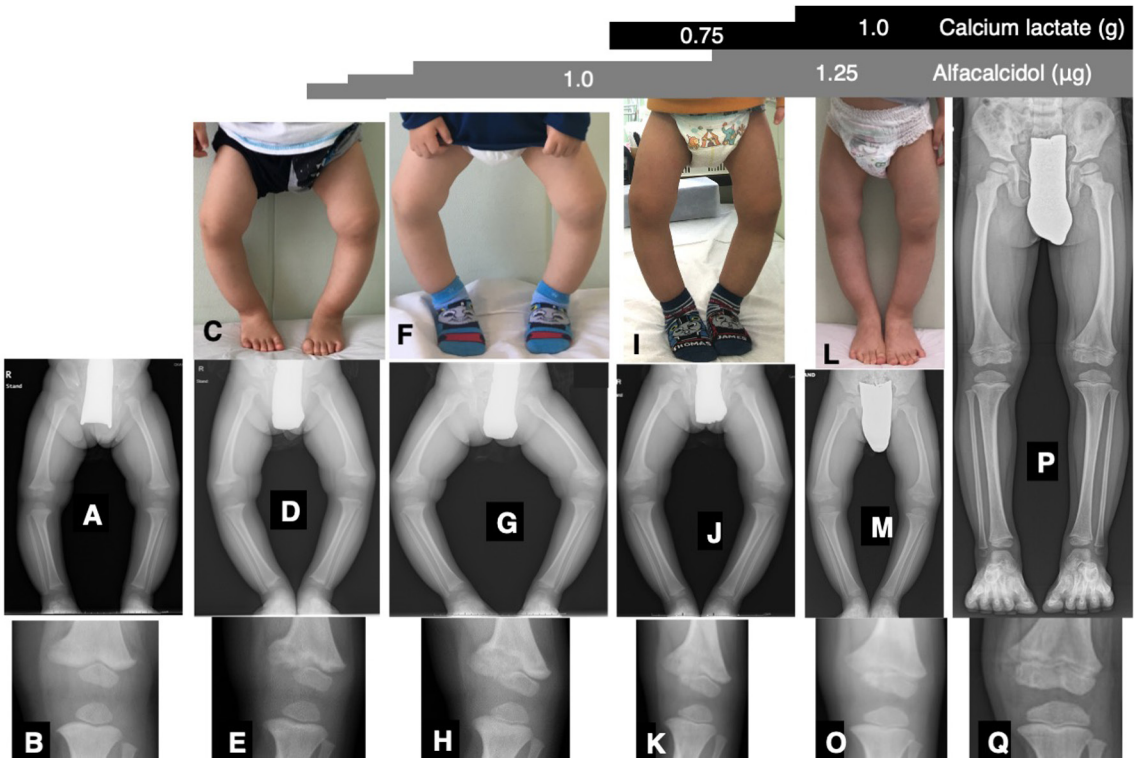

Fig. 1. Radiological (lower leg: A, D, G, J, M, and P; distant metaphysis of the left femur: B, E, H, K, O, and Q) and bow leg (C, F, I, and L) changes during treatment. (A and B) The 21-mo-old patient at the first visit to the orthopedic department. Bow leg was observed, but rachitic changes were not apparent. (C–E) The 27-mo-old patient at the first visit to the pediatric department. The bow leg worsened and rachitic changes were visible in the metaphysis of the femur. (F–H) The 30-mo-old patient. Three months after initiating active vitamin D treatment, the bow leg did not improve, and the rachitic changes worsened. (I–K) The 33-mo-old patient. Three months after adding calcium lactate in addition to active vitamin D, the bow leg and rachitic changes improved. (L–O) The 39-mo-old patient at 1 yr after initiating treatment. (P and Q) The 57-mo-old patient. The femoral deformity persisted, but the alignment improved. Treatment during this period is shown in the upper part of the figure.

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–1.10); however, his growth rate decreased after 1 yr of age (**Fig. 2A**). Hair loss began at 2 mo of age and the patient developed incomplete alopecia (**Fig. 3A**), and sparse eyebrows. Multiple 1–3-mm papules were also observed on his skin from the age of 2 yr (**Figs. 3B–D**). Laboratory examinations revealed hypocalcemia (8.7 mg/dL) and hypophosphatemia (4.0 mg/dL) with an elevated alkaline phosphatase (ALP) level (512 U/L [IFCC]; Japan Society of Clinical Chemistry [JSCC] measurement values exchanged with the International Federation of Clinical Chemistry and Laboratory Medicine [IFCC] measurement values using the following formula: ALP [IFCC] = 0.35 × ALP [JSCC]) and an intact parathyroid hormone (PTH) level (386 pg/mL). Decreased 25(OH) vitamin D (10 ng/mL) and increased $1,25(OH)_2$ vitamin D (192 pg/mL) levels were observed.

Based on the clinical features and laboratory examination results, VDDR2A was considered. The patient's mother was hesitant about the diagnosis of VDDR2A and subjected her son to genetic analysis for *VDR*. A nutritionist suggested a deficiency in vitamin D and calcium intake after weaning, which became sufficient after the first visit to the pediatric department. Therefore, the patient was initially treated with alfacalcidol (because native vitamin D cannot be prescribed in Japan), considering vitamin D–deficient rickets. However, rickets and laboratory data did not improve with increasing alfacalcidol dose (**Figs. 1F–H**, b in **Figs. 2A and B**). Therefore, oral administration of calcium lactate 0.75 g/d (97.5 mg of calcium) was initiated and improvements were observed in the clinical and radiological phenotypes of ricket (**Fig. 1I–K**, c in **Figs. 2A and B**). To check whether the further increase in alfacalcidol under calcium supplementation

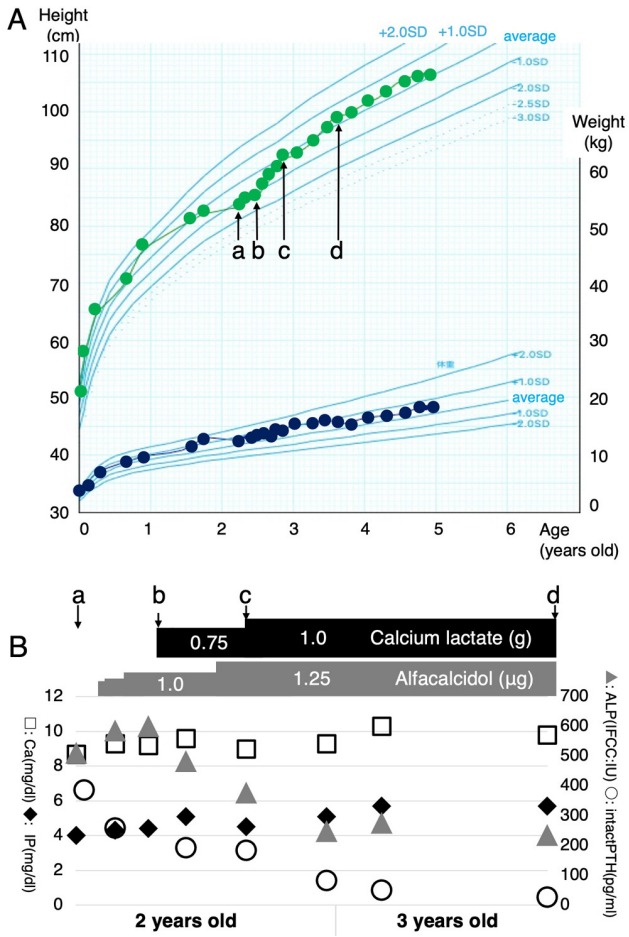

Fig. 2. Clinical course before and after visiting the pediatric department. (A) Growth chart of the patient. Specific points in time are marked as follows: a, first visit to the pediatric department and alfacalcidol treatment initiation; b, calcium lactate initiation; c, 3 mo after starting calcium lactate; d, 1 yr after starting treatment. (B) Changes in laboratory examinations between a and d in **Fig. 2A**. Specific points a–d show the same points a–d in **Fig. 2A**. Alkaline phosphatase (ALP) measurement values by the Japan Society of Clinical Chemistry (JSCC) were exchanged with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) measurement values using the following formula: ALP (IFCC) = 0.35 × ALP (JSCC).

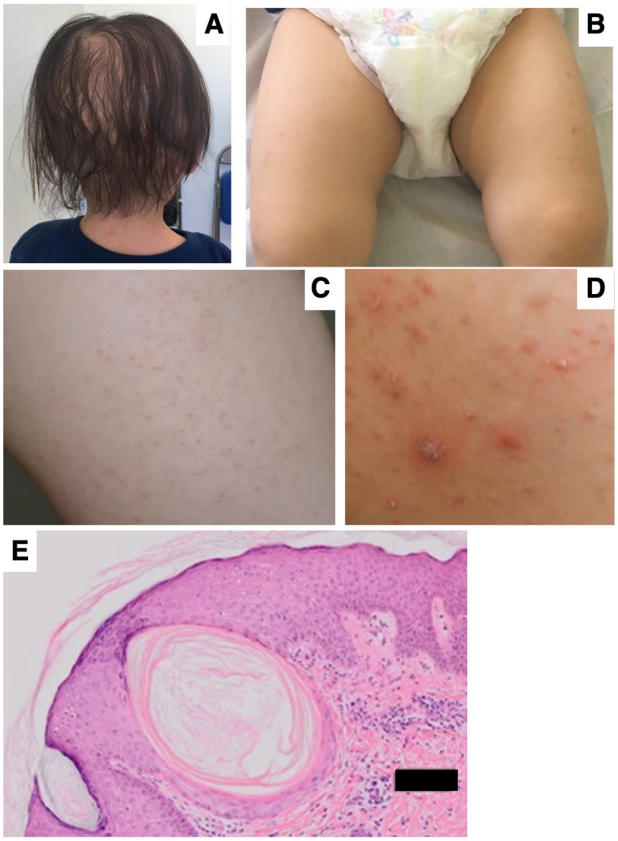

Fig. 3. Features of the patient's skin (A–D) and histopathological findings of the skin papule (E). (A) Hair loss. (B–D) Appearance of the skin of the thighs (B, whole thighs; C, low magnification; D, high magnification), where many 1–3-mm papules were observed. (E) A cyst was filled with stratum corneum and consisted of stratified squamous epithelium without a granular layer (hematoxylin and eosin [HE]; scale bar, 100 μm).

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affects abnormalities in laboratory examinations, such as elevated PTH levels, we increased the dose of alfacalcidol to 1.25 μg/d (between b and c in **Fig. 2B**). However, PTH levels did not decrease; therefore, we considered that calcium supplementation alone ameliorated the bone phenotype and abnormalities in laboratory examinations and decided to increase the dose of calcium supplementation. After increasing the oral administration of calcium lactate to 1.0 g/d (130 mg calcium), the laboratory abnormalities completely improved (d in **Fig. 2A**), and the patient's lower-limb alignment continued to improve (**Figs. 1L–Q**).

In contrast to the improved rickets phenotype, alopecia and small papules on the skin did not improve with vitamin D or calcium lactate supplementation. Pathological examination of the papular tissue revealed a cyst filled with stratum corneum consisting of stratified squamous epithelium, some areas of which lacked a granular layer (**Fig. 3E**).

Soon after the initiation of calcium lactate administration (at 2 yr and 5 mo of age), the *VDR* gene was analyzed in the patient and his family after informed consent was obtained from the parents. Genetic analysis was approved by the Ethics Committee of Okayama University Hospital (1701-038; January 6, 2023). All procedures were performed in accordance with the 1964 Declaration of Helsinki and 2021 Japanese Ethical Guidelines for Clinical Research and their later amendments. All exon and exon–intron boundaries of the *VDR* gene were analyzed using genomic DNA extracted from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen Inc., Tokyo, Japan). The PCR amplicons were purified using a QIAquick PCR Purification Kit (Qiagen Inc.) and sequenced using a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and Genetic Analyzer (ABI Prism 310; Applied Biosystems, Foster City, CA, USA). The primer pairs used for PCR amplification and sequencing of each exon and exon–intron boundary are listed in Supplementary Table 1. The sequence reads were aligned with the reference sequences from GenBank (NG_008731.1). Two variants, p.Arg30Ter and p.Arg50Gln, derived from the father and mother, respectively, were identified in this patient (**Fig. 4**). These variants were previously reported to be pathogenic and located in the DBD of the VDR (3–5); therefore, VDDR2A was diagnosed.

Because the patient and his family moved to another prefecture in Japan before he reached 5 yr of age, we were unable to follow his clinical course.

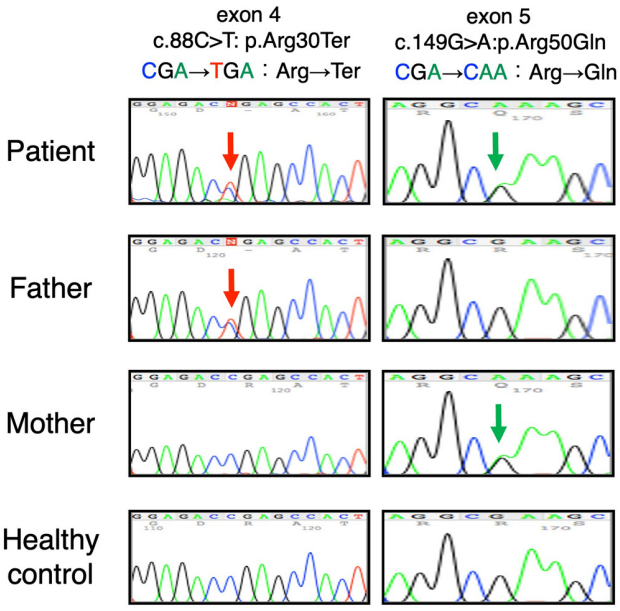

Fig. 4. Results of *VDR* analysis of the patient and his family. The red arrow indicates the basal change from cytosine to thymine at c.88 (c.88C>T) resulting in p.Arg30Ter. Green arrow indicates the basal change from guanine to adenine at c.149 (c.149G>A) resulting in p.Arg50Gln. The father harbored a heterozygous p.Arg30Ter variant, whereas the mother harbored a heterozygous p.Arg50Gln variant. The patient harbored both the variants in a compound heterozygous manner.

Discussion

Vitamin D and VDR are required for calcium homeostasis, mainly by accelerating the absorption of calcium and phosphorus in the intestine (6). In VDDR2A, calcium absorption in the small intestine is impaired owing to its resistance to active vitamin D, and secondary hyperparathyroidism leads to hypophosphatemia and rickets. In our patient, active vitamin D treatment did not ameliorate the clinical symptoms of rickets, and genu varum worsened. In the small intestine, calcium is absorbed in ligand-bound VDR-dependent and -independent manners. In a ligand-bound VDR-dependent manner, calcium is absorbed against a calcium concentration gradient from the intestinal lumen to the blood via the transcellular pathway (2). In contrast, calcium is absorbed through a calcium concentration gradient via the paracellular pathway, in a VDR-independent manner. Experiments using systemic VDR-ablated mice revealed that calcium could be absorbed without VDR and that the ratio of calcium to phosphorus in the diet affects the efficacy of calcium absorption in the small intestine (7–9). A previous report showed that high-dose calcium lactate supplementation alone improved laboratory parameters in an adult patient with VDDR2A and homozygous p.Arg30Ter, who exhibited high ALP levels and secondary hyperparathyroidism despite high-dose active vitamin D and calcium lactate treatments (10). Similar to this adult patient, calcium supplementation, which raises the calcium/phosphorus ratio in the small intestine, dramatically improved not only laboratory abnormalities such as high ALP and PTH levels, but also clinical phenotypes such as genu varum and growth disturbance in our patient. However, the treatment dose of calcium was relatively small (97.5–130 mg/d; approximately 1/4–1/3 of the estimated average requirement of daily calcium intake in Japanese

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boys aged 1–2 yr, 350 mg/d) (11). This finding may be partially attributed to the residual VDR function of the p.Arg50Gln variant. A previous report demonstrated that native vitamin D treatment improved laboratory abnormalities in a 3-yr-old brother and 1-yr-old sister with VDDR2A caused by homozygous p.Arg50Gln in the *VDR* gene, and treatment was not required for rickets after approximately 3 yr of native vitamin D treatment (4, 12). In contrast to our patient, Tamura et al. reported a 2-yr-old girl with VDDR2A caused by uniparental disomy of chromosome 12, who required high-dose calcium supplementation (300 mg/kg/d) in contrast to our patient (13). Based on these results, the required dose of calcium supplementation to treat the bone phenotype of VDDR2A has a broad spectrum that might be affected by various factors, such as residual VDR function, amount of calcium and phosphorus intake, and duration and status of bone mineralization before the start of calcium supplementation. Additional functional analysis or analysis of transgenic animals is required to confirm the residual VDR function

The patient presented with alopecia. The hair follicle cycle after birth comprises of three stages: catagen (follicular growth), anagen (follicular regression), and telogen (resting). Follicular development begins before birth and leads to a follicular cycle (14). Ligand-independent VDR action is necessary for a normal hair follicular cycle. Systemic or epidermis-specific VDR-ablated mice show normal first hair coating; however, hair loss after the first cycle and VDR itself act as regulators of the catagen stage (7, 14, 15). The VDR comprises an *N*-terminal DBD and a *C*-terminal LBD. Two zinc-finger motifs located in the DBD and the sequence from the *N*-terminus to the second zinc-finger motif play crucial roles in skin abnormalities in VDDR2A, although the detailed mechanisms have not been elucidated (14, 16). Two pathogenic *VDR* variants, p.Arg30Ter and p.Arg50Gln, have been identified. Located in the first zinc-finger motif, p.Arg30Ter is assumed to produce a truncated VDR protein by escaping nonsense-mediated RNA decay (1, 10, 17). Located between the first and second zinc-finger motifs, p.Arg50Gln affects the normal VDR protein structure after the first zinc-finger motif (1). Previously reported patients with VDDR2A accompanied by homozygous p.Arg30Ter or p.Arg50Gln variants show alopecia (3, 4, 10, 17). Normalization of calcium levels did not ameliorate alopecia in our patient, a finding consistent with systemic VDR-ablated mice (7) and previously reported patients with VDDR2A mutations (3, 12, 13, 18). In addition to the alopecia, multiple small skin papules were observed. Our results revealed that skin papules were observed in adult (19) and pediatric patients with VDDR2A, similar to young epidermis-specific VDR-ablated mice (15). The histopathological findings of the skin papules in our patient resembled those in systemic VDR-ablated mice and adult patients (7, 19). Furthermore, VDR-knockout mice exhibit defective epidermal differentiation, as shown by reduced levels of involucrin and loricrin and loss of keratohyalin granules (20). Similar to alopecia, normalization of the serum mineral status did not improve the skin papules. To treat skin lesions in patients with VDDR2A, the recovery or compensation of impaired ligand-independent VDR function in the hair follicle cycle and epidermal differentiation is necessary.

In conclusion, oral calcium supplementation ameliorated the rachitic phenotype in a pediatric patient with VDDR2A caused by compound heterozygous pathogenic variants located in the DBD of VDR. This case demonstrates that alopecia and multiple papules on the skin should be considered clinical phenotypes of VDDR2A.

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