Maternal–fetal transport of calcium (Ca\(^{2+}\)) is important for bone mineralization in fetal development. Insufficient Ca\(^{2+}\) transport causes transient neonatal hyperparathyroidism (TNHP). Transient receptor potential cation channel, subfamily V, member 6 (TRPV6), has been found to play an important role in the active transport of Ca\(^{2+}\) through the placenta. Recently, TRPV6 gene was found to be the gene responsible for TNHP with severe skeletal undermineralization. To date, only seven cases of TNHP caused by TRPV6 recessive mutations have been reported. We present a case of TNHP caused by TRPV6 gene mutations. A female newborn was hospitalized because of respiratory distress. Marked undermineralization of the skeleton was observed in X-ray imaging. Laboratory examination revealed markedly high PTH and absence of hypercalcemia along with vitamin D deficiency. Her twin brother presented with almost no symptoms. Maternal laboratory findings indicated normocalcemia, but vitamin D deficiency with a high PTH level for the lactation period was observed. We initially diagnosed the patient as having secondary hyperparathyroidism because of maternal vitamin D deficiency. Nevertheless, the reasons underlying the discordant clinical manifestations between the twin siblings remained unclear. Our analysis of TRPV6 gene clarified that the patient had compound heterozygote mutations, which were reported previously (p.Ile223Thr and p.Gly428Arg). Pathologic mutations in TRPV6 gene were not detected in the other sibling. The clinical symptoms in the patient were transient: they resolved during infancy. TNHP caused by TRPV6 gene mutations is a unique disease in terms of its transient pathology in utero and relief after birth.

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**Freeform/Key Words:** maternal–fetal calcium transport, transient neonatal hyperparathyroidism, TRPV6

The etiologies of transient neonatal hyperparathyroidism (TNHP) are heterogeneous. The major cause of TNHP is insufficient maternal–fetal calcium (Ca\(^{2+}\)) transport. Transient receptor potential cation channel, subfamily V, member 6 (TRPV6), has been identified as one of the components of the apical Ca\(^{2+}\) entry pathway of intestine and placenta [1, 2]. In 2008, Suzuki et al. presented the first *in vivo* evidence that TRPV6 plays an important role in the active transport of Ca\(^{2+}\) through the placenta in an animal model [1]. Recently, Suzuki et al. [3] reported six patients with TNHP and severe skeletal undermineralization caused by...
TRPV6 gene mutations. Subsequently, Burren et al. [4] reported an additional case. To date, only seven cases of TNHP caused by TRPV6 mutations have been reported.

1. Case Report

A female baby was born at 37 weeks’ gestational age to a 29-year-old healthy Japanese woman by cesarean section because of pelvic position and twin pregnancy. Her birth weight was 2140 g (−1.6 SD) with birth length of 45.0 cm (−1.3 SD). Apgar scores were 4 at 1 minute and 8 at 5 minutes. Shortly after birth, the infant was hospitalized in the neonatal intensive care unit at our hospital because of respiratory distress. Among her physical findings on admission, tachypneic and subcostal retractions were observed. Chest-abdominal X-ray images revealed thoracic deformity and skeletal osteopenia (Fig. 1). Subperiosteal resorption of cortical bones and coarse trabecular bones were observed, the appearance of which matches the skeletal findings of hyperparathyroidism. Her twin brother’s perinatal course was uneventful. His birth weight was 2345g (−1.2 SD). He showed mild PTH elevation and low 25-hydroxy vitamin D, but no abnormal skeletal finding. Maternal calcium and phosphate were within normal ranges, but vitamin D deficiency was found, along with a high PTH level for the lactation period (Table 1). Initially, we diagnosed the patient as having secondary hyperparathyroidism caused by maternal vitamin D deficiency; however, reasons for the discordance in clinical symptoms between the twin siblings were unclear at that time. We administered vitamin D analog (alfacalcidol) and calcium lactate to the patient. After treatment, the PTH level decreased gradually. It had normalized by 6 weeks of age (Table 2). Bone mineral content (BMC) and areal bone mineral density (BMD) of her lumbar spine (L2-L4) were measured using dual X-ray absorptiometry (Lunar Prodigy, GE Healthcare) at 2 months of age. Her BMC was 0.50 g; BMD was 0.101 g/cm² (z score, −6.97 SD). Those values

Figure 1. X-ray findings of the patient. (A, B) Neonate. Osteopenia of the skeleton is observed. The ribs are thin and wavy, the thorax is bell shaped and narrow, and the cortex of the rims is coarse, thin, and markedly demineralized. The femora are mildly bowed, and metaphyseal constriction resulting from fracture is observed (arrow). (C, D) Six months of age. The skeletal lesions almost completely resolved.
were remarkably lower than those of age-matched Canadian children [5]. Respiratory support was required up to 2 months of age. By 4 months, her osteopenia had gradually improved: BMC and BMD were 1.05 g and 0.164 g/cm², respectively. She was discharged from the neonatal intensive care unit with enteral tube feeding. By 6 months of age, X-ray images showed that her skeletal deformity had resolved almost completely (Fig. 1). At 11 months of age, she completed her treatment with alfacalcidol. On review at age 18 months, she showed catch-up in growth and developmental milestones, with no recurrence of skeletal deformity occurred after she completed the treatment.

2. Subjects and Methods

The study was approved by the Institutional Ethical Review Board of the University of Miyazaki. Written informed consent was obtained from legal guardians. Mutation analysis of the TPRV6 gene in peripheral blood lymphocyte by Sanger sequencing was conducted on the patient, her twin brother, and their parents. Information related to PCR primers and conditions is available upon request.

3. Results

We identified compound-heterozygous mutations in the TPRV6 gene; the patient had c.668T>C, (p.Ile223Thr) and c.1282G>A (p.Gly428Arg). The allele with c.668T>C was inherited from her father; c.1282G>A was from her mother. These missense mutations were reported earlier as pathological mutations [3]. Neither mutation was detected in her twin sibling.

Table 1. Laboratory Findings for the Patient, Twin Sibling, and Mother

|                     | Patient (Day 0) | Twin Sibling (Day 4) | Mother (Day 5 Postpartum) | Normal Range |
|---------------------|-----------------|----------------------|---------------------------|--------------|
| Intact PTH, pg/mL   | 2700            | 176                  | 56                        | 14–72        |
| Alkaline phosphatase, U/L | 998            | 887                  | 409                       | Adult: 106–322 |
| Calcium, mg/dL      | 7.7             | 8.3                  | 8.2                       | Neonate: 1.05–0.164 g/cm² |
| Phosphate, mg/dL    | 5.6             | 6.8                  | 3.3                       | Adult: 2.7–4.6 |
| 25(OH)D, ng/mL      | 9               | 5                    | 6                         | Neonate: 4.6–8.0 |
| Albumin, mg/dL      | 3.1             | 3.7                  | 3.0                       | Adult: 3.8–5.3 |
| Creatinine, mg/dL   | 0.59            | 0.53                 | 0.44                      | Neonate: 0.46–0.79 |
| Urinary calcium/creatinine ratio | 0.045       | Undetectable      | Undetectable              | <0.14        |

Abbreviation: 25(OH)D, 25-hydroxy vitamin D.
4. Discussion

One of the dizygous twin siblings presented with TNHP with severe skeletal undermineralization and respiratory distress. The twin sibling in the same uterine environment showed no pathological symptoms. Genetic analysis clarified the \textit{TRPV6} gene variant in this patient with severe clinical manifestation. The twin sibling demonstrated that maternal vitamin D deficiency does not affect the fetal phenotype. Calcium metabolism of the fetus is regulated differently than that of a neonate. A human fetus typically accumulates 30 g of calcium by term through the placenta. The \( \text{Ca}^{2+} \) concentration in the fetus is set markedly higher than the maternal level to sustain adequate mineralization of the fetal skeleton \([6, 7]\). Multiple animal and human studies have shown that maternal vitamin D deficiency and genetic absence of the vitamin D receptor or calcitriol do not affect fetal calcium \([6]\). The existence of maternal–fetal active \( \text{Ca}^{2+} \) transport through the placenta has been suggested to maintain higher \( \text{Ca}^{2+} \) concentration in the fetus than in maternal circulation, but details of the related molecular mechanisms have remained unclear until recently. In fact, TRPV6 plays an important role in the apical \( \text{Ca}^{2+} \) entry pathway for intestinal \( \text{Ca}^{2+} \) absorption \([2]\). TRPV6 is expressed mainly in intestines, but it is also expressed in placental trophoblast \([1, 8]\). In \textit{Trpv6} knockout (KO) mice, the transport activity of radioactive \( \text{Ca}^{2+} \) from mother to fetus was found to be 40% lower than in wild-type mice. The ash weight in \textit{Trpv6} KO mice is also lower than in wild-type mice \([1]\). Despite the strong proof of important roles in calcium homeostasis, TRPV6 was not implicated in human disease in any study reported during the past decade. Recently, Suzuki \textit{et al.} \([3]\) reported that inactivating \textit{TRPV6} gene mutation resulted in TNHP with severe undermineralization. They found that TRPV6 variants interfered with the placental maternal–fetal \( \text{Ca}^{2+} \) transport from functional analysis. The \textit{TRPV6} variants engender fetal hypocalcemia, secondary hyperparathyroidism, and skeletal demineralization because of the combined effects of impaired primary mineralization and increased bone resorption. To date, seven cases of TNHP caused by \textit{TRPV6} recessive mutations have been reported. All subjects of those studies presented hypoplasic thorax and postnatal respiratory distress \([3, 4]\). It is particularly interesting that vitamin D deficiency was coincident in most reported cases with \textit{TRPV6} variant, as it was in our case. TRPV6 expression is regulated strongly by 1,25-dihydroxy vitamin D \([9]\). We hypothesized that TNHP caused by the \textit{TRPV6} variant develops with the existence of both \textit{TRPV6} impairment as a genetic factor and vitamin D deficiency as an environmental factor.

After delivery, the main calcium source changes dramatically from the placenta to intestinal absorption. In general, epithelial \( \text{Ca}^{2+} \) transport in intestines has two main pathways: transcellular and paracellular \([2]\). The process of transcellular pathway is \( \text{Ca}^{2+} \) transport by crossing of both apical and basolateral membranes of the epithelial cells. By contrast, the paracellular pathway is simple diffusion and passive transport through the tight junctions. The paracellular pathway is the predominant form of \( \text{Ca}^{2+} \) absorption in intestines under physical conditions \([10]\). Despite insufficient \( \text{Ca}^{2+} \) supply \textit{in utero} because of impaired \textit{TRPV6}, when adequate nutrition is provided from the diet after birth, transport of \( \text{Ca}^{2+} \) from the intestines is secured by the paracellular pathway; therefore, clinical symptoms of \textit{TRPV6} variant resolved during infancy.

In summary, we describe a case of TNHP caused by \textit{TRPV6} gene mutations. TNHP with \textit{TRPV6} gene variants is a unique disease in terms of its transient pathology \textit{in utero} and relief after birth.

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