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Case Report

Hypophosphatemic rickets developed after treatment with etidronate disodium in a patient with generalized arterial calcification in infancy

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ABSTRACT

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) was originally reported as a responsible gene for generalized arterial calcification in infancy (GACI). Though the prognosis of GACI patients is poor because of myocardial infarction and heart failure in relation to medial calcification of the coronary arteries, some patients rescued by bisphosphonate treatment have been reported. Recently, *ENPP1* is also reported as responsible for autosomal recessive hypophosphatemic rickets type 2. We show here a boy with homozygous *ENPP1* mutations diagnosed as having GACI in early infancy. After the diagnosis, he was treated with etidronate disodium (EHDP) in combination with antihypertensive drugs. The calcification of major arteries was diminished and disappeared by the age of eight months. He also showed mild hypophosphatemia (2.6–3.7 mg/dl) from the age of one year. After the treatment with EHDP for five years, he showed genu valgum with hypophosphatemia (2.6 mg/dl). He was diagnosed as having hypophosphatemic rickets at the age of seven years. The findings that hyper-mineralization of the arteries and hypo-mineralization of the bone observed in the same patient are noteworthy. *ENPP1* could be regarded as a controller of the calcification of the whole body at least in part.

1. Introduction

Generalized arterial calcification of infancy (GACI; OMIM #208000) is a disorder characterized by medial calcification of elastic fibers in major arteries, such as aorta, renal arteries and coronary arteries, leading to angiostenosis throughout the body. Patients suffered from GACI show fetal distress, poor sucking and hypertension, and are often fatal within the first 6 months due to myocardial infarction and heart failure (Rutsch et al., 2001; Cheng et al., 2005).

ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1) is reported as one of the responsible genes for GACI (Rutsch et al., 2001). ENPP1 has nucleotide pyrophosphohydrolase (NPPH) activity in the extracellular fluid generating inorganic pyrophosphate (PPi) and nucleotide monophosphate from nucleotide triphosphate. Accumulated PPi inhibits alkaline phosphatase (ALP) activity and mineralization through binding to hydroxyapatite crystals (Fleisch et al., 1966; Addison et al., 2007; Anderson et al., 2005). Therefore, loss-of-function mutations of *ENPP1* gene cause lack of PPi and up-regulating ALP activity, leading to promotion of mineralization in the vascular smooth muscle cells (VSMCs) (Villa-Bellosta et al., 2011; Zhu et al., 2011).

Recently, loss-of-function mutations of *ENPP1* gene were also found in patients with autosomal recessive hypophosphatemic rickets type 2 (ARHR2; OMIM #613312) by linkage analyses (Lorenz-Depiereux et al., 2010; Levy-Litan et al., 2010). To date, the mechanisms that loss-of-function mutations of *ENPP1* cause hyper-mineralization in the extra bone tissues and hypo-mineralization in the bone are still unclear.

Etidronate disodium (ethane 1-hydroxy-1, 1-diphosphonate; EHDP) is one of the first-generation bisphosphonates and its structure resembles that of pyrophosphate. It has been reported that treatment with EHDP improved the overall survival of patients with GACI by diminishing arterial calcification (Otero et al., 2013; Edouard et al., 2011; Galletti et al., 2011; Rutsch et al., 2008). Because the dose of EHDP required to inhibit bone resorption is near the one that impairs mineralization, EHDP could also serve as an inhibitor of mineralization in the bone and in the extra bone tissues (Fleisch, 2002).

We previously reported a boy who was diagnosed as having GACI with homozygous *ENPP1* gene mutations. He was treated with EHDP

Abbreviations: GACI, Generalized arterial calcification of infancy; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; NPPH, nucleotide pyrophosphohydrolase; PPi, inorganic pyrophosphate; VSMCs, vascular smooth muscle cells; ARHR2, autosomal recessive hypophosphatemic rickets type 2; EHDP, etidronate disodium.

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and antihypertensive drugs from the age of two months, and calcification of the arteries was disappeared by the age of eight months (Numakura et al., 2006). Afterwards he showed genu valgum with hypophosphatemia (2.7 – 3.7 mg/dl) at the age of five years and diagnosed as having hypophosphatemic rickets at the age of seven. Here we report his clinical course and discuss the role of ENPP1 in the mineralization in the bone and extra bone tissues.

2. Materials & methods

Written informed consent was obtained from the parents of our patient, and the study was approved by local ethical review board of our hospital.

2.1. Biochemical measurements

Serum calcium (Ca), phosphate (Pi), ALP levels were measured by standard colorimetric methods (SRL, Inc., Japan). Serum FGF23 level was measured by an ELISA kit (Kainos, Japan) which can only recognize the intact FGF23 (Yamazaki et al., 2002). Tubular reabsorption of phosphate (%TRP) was calculated by $100 \times \{1 - (\text{urine Pi/serum Pi}) / (\text{urine Cr/serum Cr})\}(\%)$. Maximal tubular reabsorption of phosphate per GFR (TmP/GFR) was calculated by TRP × serum Pi.

3. Case report

A boy from the first-cousin parents was born by emergency cesarean section at 36 weeks gestation because of fetal distress. He showed systemic edema, hepatomegaly and hypertension up to 120 mmHg of systolic blood pressure. Calcification of the major arteries including aorta, carotid artery, renal artery and pulmonary artery was detected on whole body computed tomography (CT). He was diagnosed as having GACI. DNA analyses from the peripheral blood leukocytes showed that he had homozygous nonsense mutations of *ENPP1* gene (c.2188C > T, p.R730*) and his parents were heterozygous for the same mutation. NPPH activity of mutated ENPP1 was 4% compared to control (Numakura et al., 2006). A blood examination showed normal Ca (10.0 mg/dl), slightly decreased Pi (4.3 mg/dl), and high ALP levels (2683 IU/l) at the age of two months, when the treatment with EHDP at a dose of 18 mg/kg was started. To treat hypertension, antihypertensive drugs (amlodipine, lisinopril hydrate and varsartan) were also started, and then his systolic blood pressure was maintained below 100 mmHg. The calcification of the arteries was diminished and disappeared on CT scanning by the age of eight months. Because hypertension was improved, treatment with amlodipine and lisinopril hydrate was stopped at the age of four years. Treatment with EHDP was stopped by the age of five years, when he showed genu valgum of the both legs. At the age of seven years, X-ray of his knee and ankle showed flaying of metaphyseal bone (Fig. 1). A routine blood examination showed normocalcemia (8.8–10.4 mg/dl), hypophosphatemia (2.6–3.7 mg/dl), and high ALP (2591–3836 IU/l) continuously since he was at the age of one year (Fig. 2). Based on these examinations, he was diagnosed as having hypophosphatemic rickets. At the age of ten years, serum Pi, %TRP and TmP/GFR were 2.8 mg/dl, 90.3% and 2.5 mg/dl, respectively. No ectopic calcification was observed including major arteries, kidneys, joints, and spinal ligaments by the age of ten years, and his systolic blood pressure was maintained below 120 mmHg with varsartan. At the age of twelve years, high serum FGF23 level (84 pg/ml) was observed with low serum Pi level (2.8 mg/dl).

4. Discussion

We reported here a case with GACI subsequently developed hypophosphatemic rickets. To our knowledge, there are only three reports about GACI patients similar to our patient (Otero et al., 2013; Edouard et al., 2011; Rutsch et al., 2008).

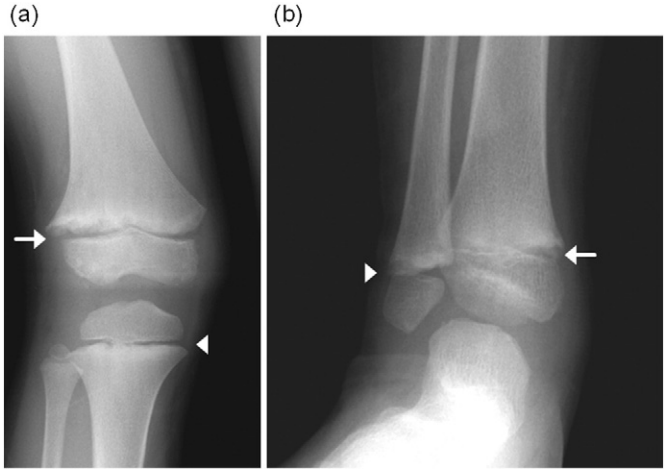

Fig. 1. X-ray pictures of the knee (a) and the ankle (b) of the patient at the age of seven. (a) Metaphyseal fraying and flaring of the distal end of the femur (arrow) and metaphyseal fraying of proximal end of the tibia (arrowhead). (b) Metaphyseal fraying and flaring in the distal end of the tibia (arrow) and the fibula (arrowhead).

One can easily question why GACI patients showed hypomineralization in the bone after hyper-mineralization in the extra bone tissues. The signs of GACI are usually evident in early infancy, even in prenatal period (Eronen et al., 2001; Crade et al., 1991; Nagar et al., 2003). On the other hand, hypophosphatemia or rickets in GACI patients were noted first at the age between two and three years (Edouard et al., 2011; Rutsch et al., 2008). To explain these phenotypic differences depending on age, we speculate possibilities of the change in the level of serum phosphate, production of FGF23 and treatment with EHDP.

Age dependent change in serum phosphate levels might explain the hyper-mineralization in the arteries observed in GACI patients. A previous report showed that high phosphorus diet exaggerated aortic calcification in *Enpp1* −/− mice by the age of two months. In addition, calcium content of aorta two months after transplantation from two-month-old wild type mice to *Enpp1* −/− littermates was significantly greater than that from wild type to wild type, but far lower than that from *Enpp1* −/− to *Enpp1* −/− (Lomashvili et al., 2014). These data suggest that high Pi/PPi ratio is responsible for arterial calcification in early infancy in mice. Circulating Pi is relatively higher in infancy than

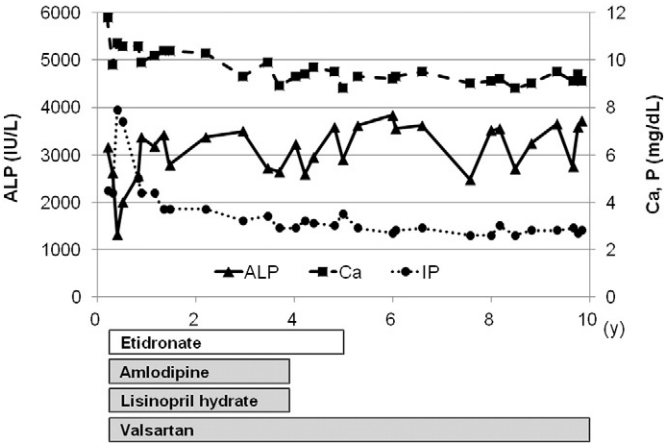

Fig. 2. Biochemical parameters and the treatment regimen of the patient. After diagnosis, the patient was treated with EHDP and antihypertensive drugs. From infantile period, serum ALP remains high and serum phosphate remains low compared to age-related references.