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Craniometaphyseal dysplasia with obvious biochemical abnormality and rickets-like features

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ABSTRACT

Background: Craniometaphyseal dysplasia (CMD) is a rare genetic disorder that is characterized by progressive sclerosis of the craniofacial bones and metaphyseal widening of long bones, and biochemical indexes were mostly normal. To further the understanding of the disease from a biochemical perspective, we reported a CMD case with obviously abnormal biochemical indexes.

Case report: A 1-year-old boy was referred to our clinic. Biochemical test showed obviously increased alkaline phosphatase (ALP) and parathyroid hormone (PTH), mild hypocalcemia and hypophosphatemia. Moreover, significant elevated receptor activator of nuclear factor kappa-B ligand (RANKL) level, but normal β-C-terminal telopeptide of type I collagen (β-CTX) concentration were revealed. He was initially suspected of rickets, because the radiological examination also showed broadened epiphysis in his long bones. Supplementation with calcium and calcitriol alleviated biochemical abnormality. However, the patient gradually developed osteosclerosis which was inconformity with rickets. Considering that he was also presented with facial paralysis and nasal obstruction symptom, the diagnosis of craniometaphyseal dysplasia was suspected, and then was confirmed by the mutation analysis of ANKH of the proband and his family, which showed a de novo heterozygous mutation (C1124-1126delCCT) on exon 9.

Conclusions: Our study revealed that obvious biochemical abnormality and rickets-like features might present as uncommon characteristics in CMD patients, and the calcium and calcitriol supplementation could alleviate biochemical abnormalities. Furthermore, although early osteoclast differentiation factor was excited in CMD patient, activity of osteoclast was still inert.

1. Introduction

Craniometaphyseal dysplasia (CMD) is a rare genetic skeletal disorder characterized by early progressive hyperostosis, sclerosis of the craniofacial bones and metaphyseal widening of long bones [1]. The pathogenesis of the disease is excessive skeletal mineralization mostly induced by decreased mineralization inhibitor pyrophosphate(PPi). The term craniotubular dysplasias and the variants were defined in 1954 [2]. CMD can be inherited in an autosomal dominant (AD, OMIM 605145) or autosomal recessive (AR, OMIM 121014) trait, and AD is more common. *ANKH* was identified as responsible gene for CMD (AD) [3]. *ANKH* codes ANK protein, serves as a transporter to channel intracellular PPi to extracellular matrix, regulating mineralization and preventing ectopic calcification [4]. AR CMD has been mapped on chromosome 6q21-22 [5], and gap junction protein, alpha 1 (GJA1) was considered linked to CMD (AR). *GJA1* codes for connexin 43, a major component of gap junctions in bone cells [6].

Frontonasal hyperostosis and sclerosis lead to prominence of the forehead. Excessive bone formation also results in recognizable facial features, like prognathism, a flattened nasal bridge and frontonasal bossing. Additional characters of CMD include hyperostosis of the cranial base and narrowing of the foramina, which may cause facial paralysis, even blindness or hearing loss [7]. Leontiasis ossia was used to describe severe facial abnormality in early literature, majority of defined patients with *ANKH* mutations present with lighter facial abnormality, but severe craniosclerosis [5,8–10]. Metaphyses of long bones are widened in CMD patients, they were described as erlenmeyer flask-shaped deformity in childhood and club-shaped in adulthood on metaphysis of long bones. These widening change on metaphysis of long bone in children were easily confused with rickets [11]. Previous literatures usually focused on radiographic findings and clinical manifestations, and only a few reports mentioned temporarily increased PTH and ALP in CMD patients

Abbreviations: CMD, Craniometaphyseal dysplasia; ANKH, ANKH inorganic pyrophosphate transport, regulator; PPi, Pyrophosphatase; GJA1, Gap junction protein, α 1; PUMCH, Peking Union Medical College Hospital; β-CTX, β C-terminal telopeptide of type I collagen; FGF23, Fibroblast growth factor 23; RANKL, Receptor activator for nuclear factor-κ B ligand; OPG, Osteoprotegerin; BLAST, Basic local alignment search tool; ENPP1, Ectonucleotide pyrophosphatase/phosphodiesterase 1.

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[3,12]. In this study, we report a patient who presented with slight skull malformations, wheeze and carpopedal spasm, obviously increased alkaline phosphatase (ALP) and parathyroid hormone (PTH), mild hypocalcemia, hypophosphatemia, broadened metaphysis and rickets liked changes on his long bone. A heterozygote deletion mutation on exon 9 of *ANKH* was identified by mutational analysis in this Chinese family.

2. Subjects and methods

2.1. Human rights and informed consent

Approval of the study was obtained from the local ethic committee of Department of Scientific Research at Peking Union Medical College Hospital (PUMCH). Patient and his family participate in this study with informed consents.

2.2. Subjects

In this study, a 7-month-old boy was initially evaluated clinically, biochemically, and radiographically in the Department of Endocrinology, PUMCH. He was the second child of a non-consanguineous couple, had a normal 2 years older sister, without any craniofacial or skeletal abnormalities recorded in his families.

2.3. Biochemical parameters

Fasting blood samples were collected, and the serum was kept at −80 °C for measurement. The concentration of serum calcium (Ca), serum phosphate (Pi), serum creatinine (Scr), serum alkaline phosphatase (ALP), 24-h urinary phosphate, 24-h urinary calcium were measured using routine method available at the central laboratory of PUMCH, and accept the quality supervision of PUMCH.

25-Hydroxyvitamin D (25OHD), serum intact parathyroid hormone (PTH) and β C-terminal telopeptide of type I collagen (β-CTX) concentration were measured by an automated electrochemiluminescence system (Roche Diagnostics, Switzerland). Serum 1,25-dihydroxyvitamin D_3 (1, 25$(OH)_2D_3$) was measured by 1,25-dihydroxyvitamin D ^{125}I RIA Kit (Diasorin). Intact fibroblast growth factor 23 (FGF23) (Kainos Laboratories Inc.), sclerostin (Biomedica Medizinprodukte GMBH), receptor activator for nuclear factor-κ B ligand (RANKL) (Biomedica Medizinprodukte GMBH) and osteoprotegerin (OPG) (Biomedica Medizinprodukte GMBH) were measured by 2-site enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's operating instructions.

2.4. Radiography

Radiographic studies were performed in the Department of Radiology of PUMCH. Plain X-ray of the cranium, pelvis, tibia and fibula, lateral thoracolumbar spine was performed.

2.5. Mutation analysis

Genomic DNA of the patient, his parents, and his elder sister were extracted from peripheral leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Germany) and analyzed for mutations in the *ANKH* by Sanger sequencing. Polymerase chain reaction was to cover all exons and 50 base pairs on both sides of the exon–intron boundaries of gene. The samples were sequenced by an automated sequencer (ABI3730XL) and sequence alignment was performed using the basic local alignment search tool (BLAST) on the National Center for Biotechnology Information database.

3. Results

3.1. Clinical features

The proband of this family was born at full term by spontaneous vaginal delivery, without prenatal or perinatal complications except mouth breathing. His birth weight was 3.35 kg, and birth length was about 50 cm. He was vulnerable to respiratory infections, always cried and wheezed. When he was 37 days old, he presented with right facial paralysis. At 4 months of age, he was found slight skull malformations, containing a larger head, thinning hair, a small nose and a wide bony nasal bridge, as well as depressed paranasal bones.

When referred to our hospital, he was 7 months old, presented with sign of rickets, the physical examination revealed cephalus quadratus, rachitic rosary, widening of the wrist and ankle bilaterally. His head circumference was 46.5 cm (98th percentile), body length was 66 cm. Laboratory studies in local hospital and our hospital both showed hypocalcemia, serum concentration of total calcium was 1.85 and 1.96 mmol/l respectively. 25OHD level was low at 13.7 ng/ml, hyperparathyroidism was found with a serum PTH level of 604 and 832 pg/ml. Serum concentration of ALP was 2417 and 3002 U/l respectively, serum 1, 25$(OH)_2D_3$ level was reported obviously increased, up to 248.59 pg/ml (Table 1 and Fig. 1).

Plain radiographs of the boy's tibia and fibula revealed broadened metaphysis, but without disorganization of the growth plate (Fig. 2). With the markedly biochemical changes and radiological feature, as well as cephalus quadratus, rachitic rosary, he was suspected of vitamin D dependent rickets type II firstly.

The bone turnover markers were also assessed, RANKL concentration was significantly increased, up to 1.16 pmol/l, β-CTX was normal. However, serum OPG and sclerostin concentrations were decreased, reach 1.3 and 7.1 pmol/l respectively. Moreover, FGF23 level was below the lower limit of measurement (Table 1 and Fig. 1).

3.2. Treatment and dynamic changes of biochemical parameters in CMD patient

Calcium (500 mg/day) and calcitriol (Rocaltrol 0.75 μg/day) supplementation were started for the hypocalcemia and carpopedal spasm. Four weeks later, a repeat total calcium level was 2.19 mmol/l, while ALP 2417 U/l, PTH 604 pg/ml, 25-hydroxyvitamin D level was 13.9 ng/ml. Then vitamin D_3 (approximately 3000–4000 IU/day) supplement was administered for 2 months. After nearly 1 year of calcium (500 mg/day) and vitamin D supplement, as well as calcitriol (0.5 μg/day) therapy, he exhibited a 13 cm height gain and did not show carpopedal spasm any more. He could walk and presented alleviated wheeze. Repeat total calcium level was 2.53 mmol/l, as well as increased ALP (1279 U/l), normalized PTH (21.5–42.8 pg/ml), 25-OH D level was 45.1 ng/ml, and 1, 25$(OH)_2D_3$ was 87.36 pg/ml (Table 1 and Fig. 1). His craniofacial features like large head, prognathism, flatted nasal bridge and frontonasal bossing was noticed. X-ray radiograph of skull indicated the mineral density and thickness of the endosteal bony plate was significantly increased in the basicranial skull and orbital bone (Fig. 2). He also presented with neurological impairment such as facial paralysis, nasal obstruction symptom like wheeze and mouth breathing. The diagnosis of craniometaphyseal dysplasia was suspected. Therefore, his calcium and vitamin D supplements, as well as calcitriol treatment were discontinued.

3.3. Mutation analysis of ANKH gene

The *ANKH* sequences for the patient and his families were performed, and the sequence alignment used reference sequence (NM_054027.4). The sequencing data from PCR-amplified genomic DNA showed heterozygous alleles on exon 9 (c.1124_1126delCCT,