Transforming growth factor-β1 gene mutations and phenotypes in pediatric patients with Camurati-Engelmann disease

CHUN WANG1*, BAO-HONG ZHANG2*, YU-JUAN LIU1, YUN-QIU HU1, JIN-WEI HE1 and ZHEN-LIN ZHANG1

1Department of Osteoporosis and Bone Diseases, Metabolic Bone Disease and Genetics Research Unit, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, Shanghai 200233; 2Capital Institute of Pediatrics, Beijing 100020, P.R. China

Received November 23, 2012; Accepted March 6, 2013

DOI: 10.3892/mmr.2013.1367

Abstract. The aim of the present study was to investigate the clinical characteristics and major causative gene in pediatric patients with Camurati-Engelmann disease (CED). Biochemical and radiographic examinations, bone scintigraphy and genetic analyses were performed in two affected males and their parents. The two patients experienced waddling gait, muscular weakness and growth developmental delay. X-ray radiography revealed typical fusiform thickening of the diaphyseal portions of the long bones. The abnormal uptake of tracer Tc-99m was visualized in the skull and both sides of the upper humeri, ulnas, radii, femurs and tibias using bone scintigraphy. Serum levels of the bone formation marker procollagen type I N-terminal propeptide (PINP) and the bone resorption marker β1-isoemerized C-terminal cross-linked telopeptide of type I collagen (β1-CTX) in the 6-year-old patient were significantly increased compared with the normal value range, while only the β1-CTX levels were elevated in the 16-year-old patient. A heterozygous missense mutation p.Arg218Cys in exon 4 of the TGFβ1 gene was detected in the affected patient. The two patients, while their parents had normal wild-type genotypes. In conclusion, the p.Arg218Cys mutation was shown to contribute to the clinical phenotypes in two pediatric patients with CED. The results of this study suggest that abnormal bone turnover marker levels, typical radiological findings and mutations in the TGFβ1 gene are three important factors in the diagnosis of sporadic CED cases.

Introduction

Camurati-Engelmann disease (CED, OMIM 131300) is a rare autosomal dominant disease with variable clinical manifestations. The main clinical and laboratory manifestations include painful limbs, waddling gait, muscular weakness, joint contracture, cranial nerve impingement, delayed pubertal development and increased serum alkaline phosphatase (ALP) levels. Fusiform thickening of the diaphyseal and metaphyseal cortex of the long bones is the typical radiographic signature. Occasionally, a number of patients experience blindness or deafness due to thickened skull bones. The incidence of this disease is ~1:1 million births. The primary causative gene in CED is the transforming growth factor β1 (TGFβ1) gene (1), which contains seven exons. TGFβ1, encoded by the TGFβ1 gene, is a member of the TGFβ1 signaling pathway and regulates cell proliferation, migration, differentiation and apoptosis. TGFβ1 is particularly abundant in the bone matrix, where it is involved in the regulation of bone formation and resorption. The inactive form of the TGFβ1 protein (pre-pro-TGFβ1) is composed of three subunits; the signal peptide, the latency-associated peptide (LAP) and the mature peptide. The activation procedure includes the cleavage of the signal peptide followed by cleavage of the LAP from the mature peptide. Mutations in different domains of the TGFβ1 gene lead to inherited sclerosing bone disorder or osteoporosis (2). To date, >40 CED families from Europe, Australia, Israel, Japan, Korea, South America, the USA and China with ~10 mutations have been reported. The majority of these mutations are located in the LAP region (3). The first Chinese family with CED was reported in 2006 (4); the heterozygous missense mutation p.Arg218His (R281H) in exon 4 of the TGFβ1 gene was detected in the affected patient.

The present study aimed to investigate the cases of two Chinese males diagnosed with CED, using clinical and X-ray examinations, bone scintigraphy and TGFβ1 gene mutation screening.

Patients and methods

Patients. Patient one (P1) was a 6-year-old male (height, 114 cm; weight, 19 kg) who had been born at full term and was the third

Correspondence to: Professor Zhen-Lin Zhang, Department of Osteoporosis and Bone Diseases, Metabolic Bone Disease and Genetics Research Unit, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, 600 Yishan Road, Shanghai 200233, P.R. China
E-mail: zzl2002@medmail.com.cn

*Contributed equally

Key words: Camurati-Engelmann disease, transforming growth factor β1, gene mutation, bone turnover marker
child born to the mother. P1 was unable to walk independently until 18 months of age and experienced waddling gait, muscular weakness and left pelvic limb pain during the last 4 years.

Patient two (P2) was a 16-year-old male (height, 147 cm; weight, 21 kg). The parents of P2 noted that the patient was short and underweight at the age of 6, compared with others of the same age. P2 had a waddling gait, mild muscular weakness and no signs of sexual development.

No fractures, auditory or visual impairments or family history have been reported in either patient. The two patients and their healthy non-consanguineous parents were enrolled in this study by the Department of Osteoporosis and Bone Diseases Outpatient Clinic (Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China). The present study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Informed consent was obtained from the parents of the two patients. Two hundred age- and gender-matched healthy donors were used as controls for the mutation analysis after being recruited for a previous study (5).

Methods. Biochemical parameters, including the complete blood count and levels of serum calcium (Ca), phosphonium (P), ALP, blood urea nitrogen (BUN), serum creatinine (Scr), β-isomerized C-terminal cross-linked telopeptide of type I collagen (β-CTX), procollagen type I N-terminal propeptide (PINP), 25-hydroxy D [25(OH)D] and parathyroid hormone (PTH) were determined in the two patients and their parents. X-ray radiography of the thoracic and lumbar vertebrae, limbs, hips and skull was performed individually. A lunar prodigy dual-energy X-ray absorptiometry (DXA) densitometer (Lunar Corporation, Madison, WI, USA) was used to measure the bone mineral density (BMD) values of the left proximal femur, including the femoral neck and total hip, and the anteroposterior lumbar spine 1-4 (L1-4). The machine was calibrated daily. Prodigy enCORE version 6.70 software was used to analyze the data (standard-array mode; GE Healthcare, Madison, WI, USA). DXA measurements were obtained from triplicate measurements at L1-4 and the total hip, femoral neck and trochanter in 15 individuals; the coefficient of variability (CV) values for the DXA measurements were 1.39, 0.70, 2.22 and 1.41%, respectively (6). Weekly repeated phantom measurements were carried out; these determined that the long-term reproducibility of the DXA data during the study was 99.55% (7). Standardized equipment were used to determine the body weight and height of subjects. The body mass index (BMI) was calculated as the weight/height$^2$ (kg/m$^2$). Bone scintigraphy with Tc-99m hydroxymethylene diphosphonate (HMDP) was performed to detect abnormal bone metabolism.

For the mutation analysis, genomic DNA was isolated from peripheral blood leukocytes using the conventional phenol-chloroform extraction method. The entire coding region and adjacent splice sites of the TGFβ1 gene were amplified and sequenced directly from the two patients, their parents and 200 healthy donors.

Results

| Biochemical parameters, bone turnover markers and bone mineral density (BMD) values of lumbar spine 1-4 (L1-4) and hip sites in patients one (P1) and two (P2). |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Height (cm) | Weight (kg) | BMI (kg/m$^2$) | ALP (U/l) | Ca (mmol/l) | P (mmol/l) | PTH (ng/ml) | 25(OH)D (ng/ml) | β-CTX (ng/ml) | PINP (ng/ml) | L1-4 (g/cm$^2$) | Femoral neck (g/cm$^2$) | Trochanter (g/cm$^2$) | Total hip (g/cm$^2$) |
| P1 | 114 | 19 | 14.62 | 435 | 2.33 | 1.57 | 56.19 | 10.66 | >1200 | 0.591 | 0.596 | 0.413 | 0.413 |
| P2 | 147.5 | 21 | 9.65 | 157 | 2.16 | 1.34 | 21.42 | 12.13 | 70.99 | 0.512 | 0.512 | 0.407 | 0.407 |

Biochemical and bone turnover markers. Serum levels of ALP, Ca, P, PTH, 25(OH)D, PINP and β-CTX in P1 and P2...
are shown in Table I. Notably, levels of the bone resorption marker β-CTX were markedly elevated 7-fold compared with the upper limit of normal (ULN) in P1, while only a slight increase was observed in P2. The results of two bone formation markers, ALP and PINP, were inconsistent in the two patients. In P1, the serum PINP level exceeded the measurement range, while the serum ALP level was only mildly increased (normal range in childhood, 85-400 U/l). However, in P2, serum ALP and PINP levels were within the normal range. Furthermore, the complete blood count and renal function markers in the two patients were normal (data not shown).

**Radiographic examinations.** The X-ray examinations revealed typical fusiform thickening of the diaphysis of the long bones among the tibias, humeri, femurs, ulnas and radii (Fig. 1). The skulls of the two patients exhibited cortical thickening located at the base (Fig. 2).

Abnormal tracer uptake was observed in the skull and both sides of upper humeri, ulnas, radii, femurs and tibias using bone scintigraphy in the two patients (Fig. 3).

**BMD.** The BMD values of the two patients at L1-4 and hip sites are shown in Table I. The Z scores of P1 were imponderable due to the lack of reference BMD values for Chinese children aged <10 years. The BMD values of L1-4 and femoral neck in P1 were similar to those in a study by Wu et al (8). For P2, the BMD values at each site were lower compared with the age- and gender-matched mean reference values (8,9).

**TGFβ1 gene mutation.** A heterozygous missense mutation p.Arg218Cys (R218C) in exon 4 was detected in the two patients, while their parents and the 200 healthy donors had normal wild-type genotypes (5).

**Discussion**

CED, also termed progressive diaphyseal dysplasia (PDD), is a type of inherited sclerosing bone disorder characterized by hyperostosis on the periosteal and endosteal surface of the long bones. The age of onset varies greatly; however,
the majority of patients initially exhibit symptoms, including pain and weakness, by adolescence. The typical radiological characteristic is fusiform thickening of the diaphyseal portions of the long bones. Vanhovenacker et al (10) reported that the radiographic manifestations were typically detected before the age of 30 and were usually more extensive with increasing age. Concomitant broadening of the diaphyses of long bones and the narrowing of the medullary canal suggest that an excessive periosteal apposition of bone and a defective resorption of bone at the endosteal side of the long bones exist.

The TGF\(\beta\) gene has been identified as the causative gene of CED; numerous mutations in TGF\(\beta\) have been detected in CED patients worldwide. The majority of the mutations detected in CED are missense mutations, including the arginine residue at position 218 (R218C), R218H, H222D, C223S and C225R, located in exon 4 at the C-terminal region of LAP, close to or within the two cysteine residues (3). Additional mutations, including E169K and R156C in exon 2, X81H in exon 1 and L10-L12dup and LLLL12-13ins in the signal region, have been identified in CED family studies (3, 11-14). Among them, R218C is the most common mutation hotspot in CED patients, accounting for \(>60\%\) of the mutations (3, 11). However, to date, no CED cases caused by the TGF\(\beta\) gene R218C mutation have been reported in Chinese patients. Furthermore, the correlation between polymorphisms and clinical variability has not yet been elucidated (11).

Mutations located in the LAP region have not been demonstrated to lead to the overproduction of TGF\(\beta\) in functional studies; however, they are able to increase its activity. Two possible mechanisms may explain this; firstly, the destabilized disulphide bridging of the LAPs leads to premature activation of the mature peptide mediated by exon 4 mutations. Secondly, mutations in exon 1 lead to intracellular retention of the mutant protein, which affects secretion (15, 16). Overactive TGF\(\beta\) proteins lead to increased bone density and decreased fat and muscle tissue (15, 16); this contributes to the signs and symptoms of CED.

An animal study by Tang et al (17) showed that TGF\(\beta\) was involved in bone resorption and formation through an SMAD signaling pathway that mediates bone mesenchymal stem cell (BMSCs) migration. A high level of active TGF\(\beta\) was detected in the bone marrow of CED mice carrying TGF\(\beta\) gene mutations, and typical progressive diaphyseal dysplasia manifestations were observed.

Occasionally, individuals may possess the gene mutation that causes CED yet never develop the characteristic features of this condition, supporting the incomplete penetrance of CED (11). A number of individuals with clinical manifestations of CED with no identified mutations in the TGF\(\beta\) gene are diagnosed with CED type II (OMIM 606631) (18).

The two patients in the present study harbored the most frequently detected R218C mutation in exon 4. Their clinical manifestations, X-ray signatures and bone scintigraphy results were consistent with previously reported phenotypes. With regard to the 6-year-old patient (P1), the ALP level was slightly increased compared with the normal range in children aged 0-6 years. Generally, serum ALP level is higher in childhood and adolescence than in adults, due to the increased bone turnover associated with growth. However, the markedly elevated levels of the bone formation marker PINP indicated an upregulated bone formation process in CED. Notably, the bone absorption marker \(\beta\)-CTX was also significantly increased in the same patient. The 16-year-old patient (P2) also had increased serum \(\beta\)-CTX levels, while the bone formation markers ALP and PINP were within the normal ranges. To date, no studies have reported increased \(\beta\)-CTX levels in CED patients. This novel result requires further investigation in future studies.

In conclusion, the present study reported the cases of two Chinese pediatric patients with CED caused by the heterozygous missense mutation R218C in the TGF\(\beta\) gene. The results of this study suggest that abnormal bone turnover marker levels, typical radiological findings, bone scintigraphy results and mutations in the TGF\(\beta\) gene are important factors for diagnosis and appropriate genetic counseling in apparently sporadic CED cases.

Acknowledgements

The authors would like to thank the two patients and their family members for their cooperation. This study was supported by the National Natural Science Foundation of China (NSFC; nos. 30771019, 30800387 and 81070692), the Program of Shanghai Chief Scientist (project nos. 08XD1403000 and STCSM10DZ1950100) and the Shanghai Science and Technology Development Fund (project nos. 08411963100 and 11ZR1427300).

References


