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Pycnodysostosis with a Cathepsin K Mutation: A Case Report

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Summary. **Pycnodysostosis (PKND) is a type of osteosclerosing bone disease. Recent studies have demonstrated that several cathepsin K mutations can be identified in PKND families. A fifty-three-year-old Japanese woman diagnosed with PKND with typical features suffered from the nonunion of the right tibial shaft. We did open reduction and performed a vascularized fibular graft using an external fixator. A low-intensity pulsed ultrasound device was used three months after surgery. Union of the tibia was completed about one year after surgery. Histomorphometric analysis of the iliac bone revealed that the patient had low turnover bone with increased bone volume. Analysis of the cathepsin K coding region from the genomic DNA of the patient and her family (consanguineous parents and three sisters who were all of normal stature) revealed that the patient had a deletion of genomic DNA nucleotide 426 T in exon 5. Her parents and two sisters had a heterozygous mutation, while one sister had a normal sequence. In summary, a mutation in the cathepsin K gene was identified, providing further evidence that a deficiency in the activity of this enzyme causes PKND.**

Key words—cathepsin K, Pycnodysostosis, mutation, histomorphometric analysis, nonunion.

INTRODUCTION

Pycnodysostosis (PKND), an autosomal recessive sclerosing skeletal dysplasia, has recently been shown by a positional candidacy approach to result from a deficient activity of the lysosomal cysteine protease cathepsin K[1,2,3]. The disease is characterized by dwarfism, osteosclerosis, acro-osteolysis of the distal phalanges, frequent fractures, and skull deformities with delayed suture closure[4,5]. The cathepsin K gene, which wa cloned originally from rabbit osteoclasts[6], was highly expressed in osteoclasts. This molecule plays an important role in bone resorption and remodeling. Cathepsin K knockout mice show impaired osteoclastic bone resorption, which leads to osteopetrosis[7,8]. Recent studies have demonstrated several mutations in patients with PKND[1,9,10,11]. In this paper, we present a clinical, histomorphometric, and genomic study of a patient with the typical form of pycnodysostosis. Since parental consanguinity has been noted in more than 30% of cases, we also analyzed her consanguineous parents and three sisters. A cathepsin K gene mutation was detected by direct DNA sequencing analysis.

CASE REPORT

A fifty-three-year-old Japanese woman was referred to Niigata University Hospital with nonunion of the right tibial shaft. Eight years prior to admission, she had injured her tibia and had it operated on using a plate, because closed reduction and casting were not successful. The plate broke and fracture union did not ensue. Six and three years prior to admission, nonunion of the tibia was fixed each time using an intramedullary nail with iliac bone graft, resulting in failure of the nail three years after re-fixation. She was referred to our hospital for investigation and treatment.

Physical examination of the patient revealed that she was obese (133 cm tall and 53 kg in weight) (Fig. 1a). She had a saddle nose, hypoplasia of the face, and an open cranial suture. She has used dental prosthesis due to dental caries and periodontal disease following malformed teeth since the age of twenty. She appear-

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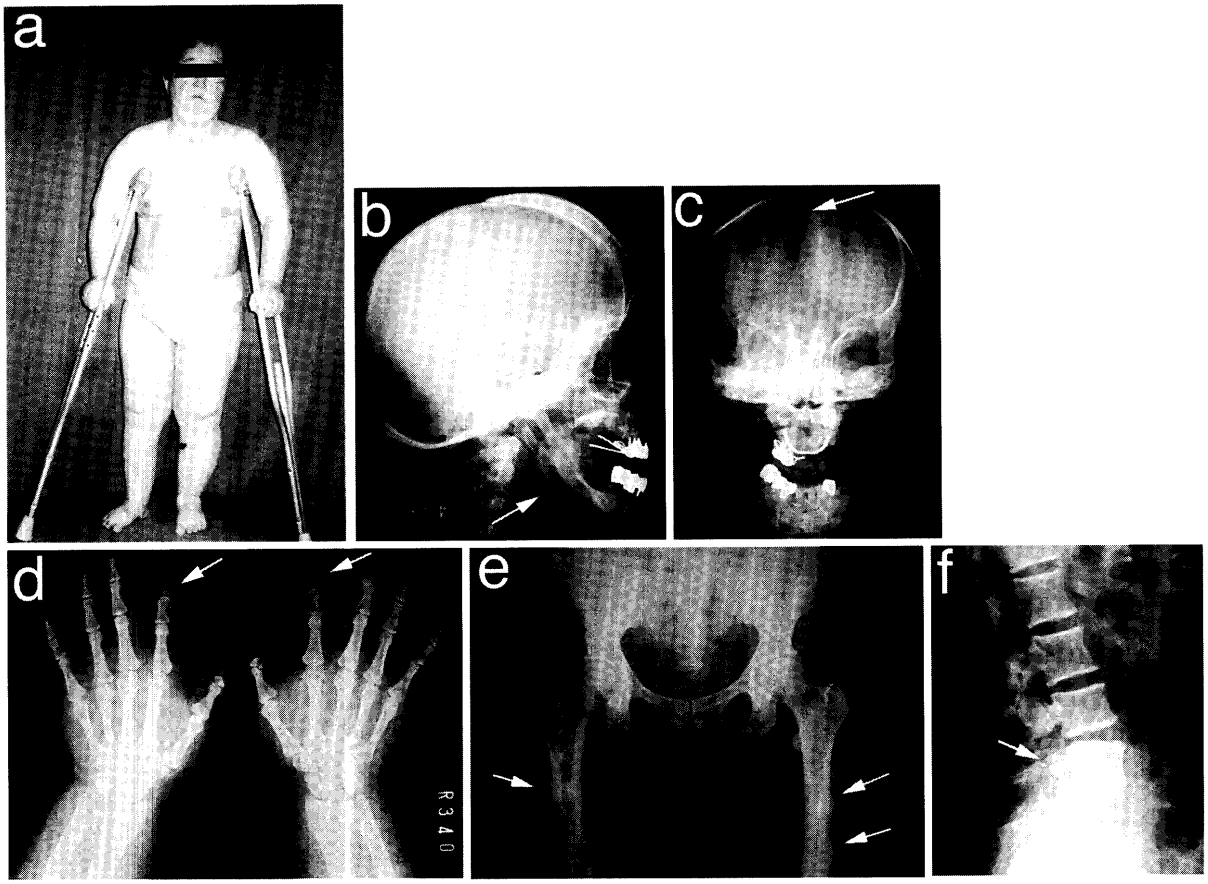

Fig. 1. Clinical features of the patient. **a.** Photograph showing short stature and obesity. **b, c.** Radiograph of the skull showing no fusion of either saggital or coronal cranial sutures (*arrows*). The angle of the mandible is obtuse (*arrow*). **d.** Radiograph demonstrating acro-osteolysis of the hands (*arrows*) and ulnar minus variant. **e.** Radiograph illustrating increased bone density of the pelvis and scleroses of the femoral shaft due to stress fracture (*arrows*). **f.** lumbosacral radiograph showing spondylolisthesis at the L5 level (*arrow*).

ed to have normal intelligence. Extensive laboratory procedures including the study of serum electrolyte concentrations, renal function, liver function, and counts of blood cells were within normal limits. Past medical history revealed that the patient had been diagnosed as PKND with typical features since adolescence. She had multiple fractures in both femora and tibiae. No severely-displaced fracture had occurred. In all cases, therefore, closed reduction was effective, and open reduction was not necessary. Her menses were regular for about forty years and stopped at fifty-two years of age. She had consanguineous parents and three sisters, all of normal stature. Six female and two male siblings of her mother and the mother's parents were also unaffected. Her father's brother and parents were also unaffected.

We did open reduction and external fixation using an external fixator (Orthofix, Oxford, UK) with iliac-bone and vascularized fibular graft. Intraoperatively, a biopsy specimen from the iliac crest was obtained for histomorphometric study. Three months after surgery, a specifically programmed, pulsed, low-intensity ultrasound device (SAFHS: Sonic Accelerated Fracture Healing System, Exogen, Inc., Piscataway, NJ) was used for 20 min. per day. Six months after the operation, the patient was able to bear weight partially. The external fixator was rigidly stabilized without dynamization of the fracture site until the removal. Nine months after surgery, the external fixator was removed, and a short leg brace was applied to bear weight fully. Union of the tibia was completed about one year after surgery. No tenderness was observed, and no movement was

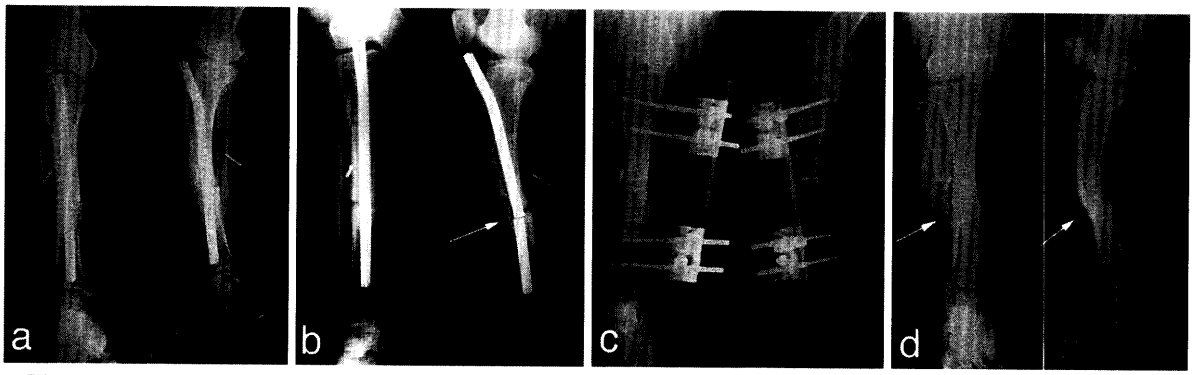

Fig. 2. Clinical course of the right tibial shaft fracture. **a.** Anteroposterior and lateral roentgenograms of the right lower extremity after the third operation with intramedullary nailing and bone graft. **b.** Three years later, the nail has broken (*arrow*). **c.** External fixation with vascularized fibular and iliac bone graft was performed (fourth operation). **d.** One year after the fourth operation, union has been achieved.

noted at the fracture site. Recent follow-up X-ray at two years after the operation showed the fracture site to be stable.

Roentgenographic findings

A roentgenogram showed an obtuse mandible angle and no fusion of the cranial sutures (Fig. 1b, c), acro-ostelysis of the hands (Fig. 1d), increased bone density of the pelvis and sclerosis of femoral shaft due to stress fracture (Fig. 1e), and spondylolisthesis of fifth lumbar spine (Fig. 1f).

A roentgenogram of the right lower extremity made at the second nailing showed the alignment of the fragments to be satisfactory (Fig. 2a). A roentgenogram at admission showed non-union of the tibia and the breakage of the intramedullary nail (Fig. 2b), A post-operative roentgenogram showed that the fracture was fixed was an external fixator (Fig. 2c). A fracture callus appeared about ten months after the operation, and the union of the tibia was completed about one year after surgery (Fig. 2d).

Histomorphometric analysis

Methods

A biopsy specimen of the iliac crest was obtained during surgery for the nonunion treatment (labeling schedule 2-5-2-6). Informed consent was obtained. The specimen was fixed in 70% ethanol, prestained with Villanueva bone stain, embedded in methylmethacrylate, and cut to a thickness of 5 micrometers. Histomorphometric analysis was performed as previously described using a semi-automatic digitizing system (Sytem Supply, Nagano, Japan). Histomorphometric terminology was used according to Parfitt et al[12].

Results

The bone volume (BV/TV) and wall thickness (W. Th) were significantly increased compared with age- and sex-matched Japanese women[13]. The osteoid volume (OS/BS) and osteoid thickness (O. Th) were decreased. Osteoblasts were rarely observed. The eroded surface (ES/BS) was increased although no osteoclasts were observed. A double-labeled surface was slightly detected. The mineral apposition rate (MAR) was normal, but bone formation rate (BFR/BS) was at a lower level (Table. 1 and Fig. 3).

DNA sequencing analysis

Methods

Blood samples were obtained with informed consent from the patient and her family (parents and three sisters). Her family was not phenotypically affected with PKND. Genomic DNA was extracted from to peripheral blood. Exons 1-8 of the cathepsin K gene[14] were amplified from the genomic DNA of the PKND patient and her family by polymerase chain reaction (PCR), isolated, and sequenced via cycle sequencing with an AB1377 Sequencer (Perkin-Elmer Corp., Norwalk, Connecticut, USA). PCR primers were designed from the sequence of Cathepsin K mRNA (S79895 in the GenBank) and the genomic structure[14] as folllows:

HCK 1F, 5'-gcactcacagtcgcaacct-3'
HCK 1R, 5'-ctgctgatggaaatctgttgt-3'

Table 1. Results of histomorphometric analysis

Parameter	Abbreviation	Value	Reference range* (Mean±SD)
Bone volume(%)	BV/TV	33.8	23.19 ± 4.37
Trabecular thickness(mcm)	Tb. Th	129.6	133.0 ± 22.0
Wall thickness(mcm)	W. Th	45.0	34.16 ± 2.32
Osteoid volume(%)	OV/TV	0.2	0.32 ± 0.19
Osteoid Vol./Bone Vol.(%)	OV/BV	0.5	1.48 ± 0.93
Osteoid surface(%)	OS/BS	5.4	12.1 ± 4.64
Osteoid thickness(mdm)	O. Th	5.2	10.34 ± 2.05
Eroded surface(%)	ES/BS	12.3	4.09 ± 2.33
Mineral appos. rate(mcm/day)	MAR	0.587	0.589 ± 0.082
Double labeled surface/BS(%)	dLS/BS	0.73	
Single labeled surface/BS(%)	sLS/BS	0	
Bone formation rate(mm^3/mm^2/year)	BFR/BS	0.0015	0.016 ± 0.008
Bone formation rate(%/year)	BFR/BV"	2.42	24.6 ± 13.3

*Konno T.: J Jpn Orthop Assoc, 61: 1081-1091, 1987.

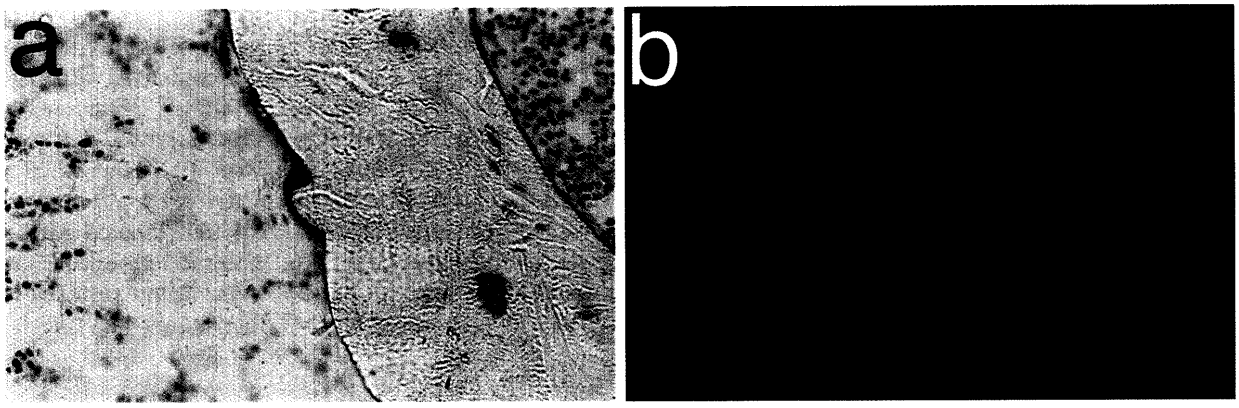

Fig. 3. Microphotographs of trabecula of iliac bone under light **a.** and fluorescent **b.** microscopy. No tetracycline label is detected. (Villanueva bone stain, original magnification: × 40)

HCK 2F, 5'-ctacattcttctgcaggatg-3'
HCK 2R, 5'-cttgttgttatattgcttcctg-3'
HCK 3F, 5'-tatgctttgtttaggtggatga-3'
HCK 3R, 5'-tgaagctatacttgccatgtc-3'
HCK 4F, 5'-accagtgaagaggtggttca-3'
HCK 4R, 5'-ctgatttttgacaggagtaaca-3'
HCK 5F, 5'-ggtcagtgtggttcctgttg-3'
HCK 5R, 5'-cctgtcccacatatgggtag-3'
HCK 6F, 5'-tccagccaggaagagagttg-3'
HCK 6R, 5'-gcagcttcttacctttgctg-3'
HCK 7F, 5'-tattctaggtgtattatgatg-3'
HCK 7R, 5'-tgttcccattacctgtttt-3'
HCK 8F, 5'-ttggtcttacagctggggag-3'
HCK 8R, 5'-caaagtgcatcgttacactgc-3'

Results

Analysis of the cathepsin K coding region from genomic DNA of the patient revealed a deletion of genomic DNA nucleotide 426 T in exon 5 (Fig. 4). This mutation was closely located at the active cysteine residule in a mature peptide, resulting in a frame shift (F142L) and stop codon (L160X), predicting premature termination of the mature cathepsin K polypeptide. Analysis of her parents' genomic DNA demonstrated that the parents and two sisters were heterozygous for the deletion mutation, while one sister had a normal sequence (Fig. 4). These results indicated Mendelian inheritance in this family.

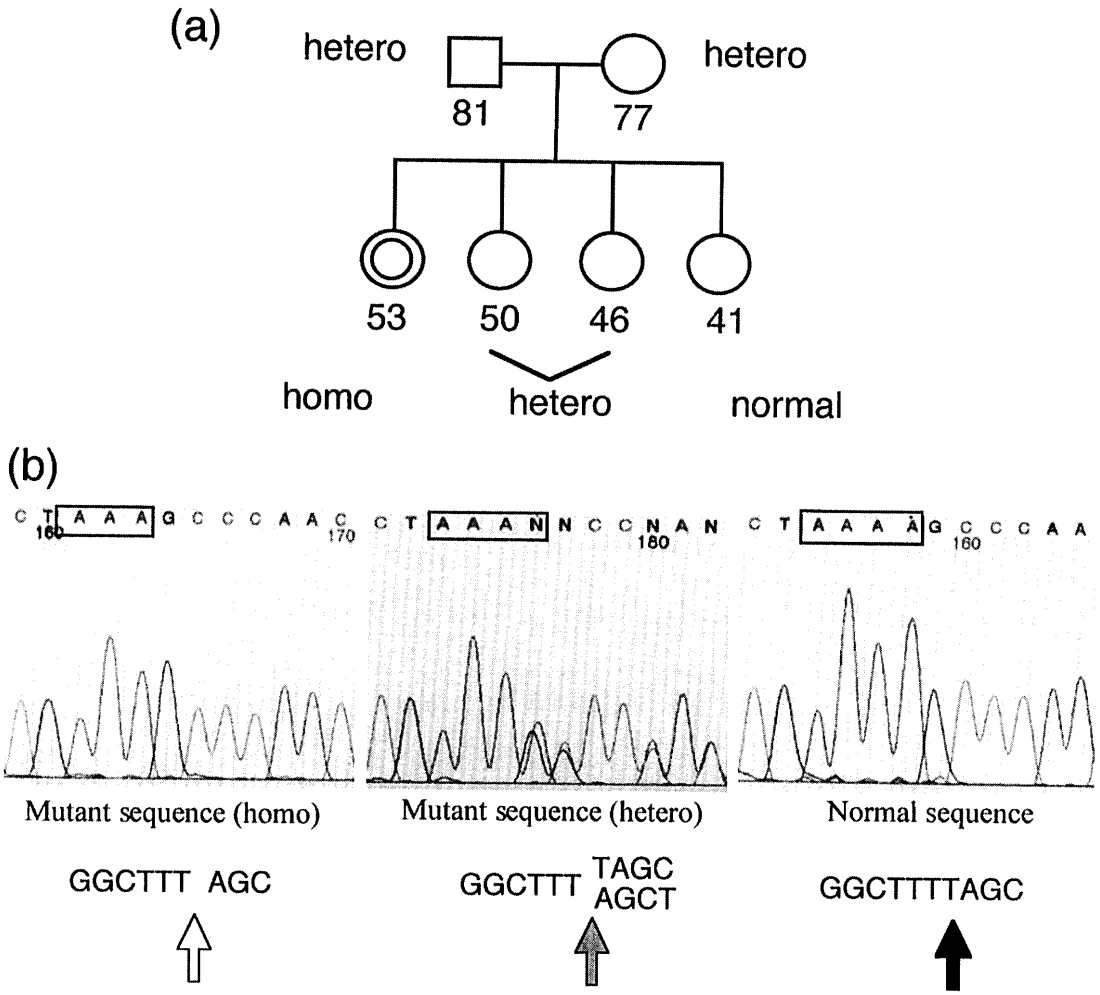

Fig. 4. Sequence analysis of exon 5 of the cathepsin K gene. **a.** Pedigree of the family. Numbers show their age. **b.** Homozygous (homo) deletion of genomic DNA nucleotide 426 T in the patient (*left panel*). Heterozygous (hetero) deletion in teh parents and two sisters (*center*). Normal sequence in a sister (*right*). Results of direct sequencing using reverse primers are shown because the forward primer is close to the deletion site and teh peak is weak. The sequence of genomic DNA in exon 5 is shown below the sequence analysis data.

DISCUSSION

Recent studies have demonstrated that several cathepsin K mutations can be identified in unrelated PKND families[1,9,10,11]. This further suggests that defects in the cathepsin K gene cause this skeletal dysplasia and emphasizes the degree of molecular genetic heterogeneity underlying this disease. In this case, we analyzed the genomic DNA of cathepsin K in the patient and her family using the appropriate PCR-based assays. A deletion mutation was homozygously detected in the patient. We did not analyze the cDNA or protein of the patient's cathepsin K; autosomal recessive inheritance in the family strongly suggested that a deletion in exon 5 of the patient's cathepsin K genome had resulted in PKND. The same finding of a frame shift and stop codon mutation was recently reported by Fuiita et al[15]., suggeting that this lesion may be related to Japanese PKND patients. Support for this concept would require population studies to determine the frequency of the genomic DNA nucleotide 426 T deletion in Japanese and other populations. Future development of intragenic cathepsin K polymorophic markers will allow further analysis of the ancestral background of this mutaion.

Histomorphometric analysis indicated that the

patient had low turnover bone with increased bone volume. ES/BS was increased although no osteoclasts were observed. This discrepancy strongly suggested disturbed bone resorption. A previous histomorphometric report on PKND patients demonstrated that low turnover bone was due to prolonged periods of resorption and the following formation[16]. Cathepsin K-deficient osteoclasts can resorb mineralized tissue, but not demineralized bone matrices[7]. In an osteoclast resorption assay, osteoclasts from cathepsin-K-deficient mice generated a low number of pits with larger areas and perimeters, but these areas were smaller in volume and shallower in depth compared with controls. These data suggest that a cathepsin K-deficiency induces insufficient bone resorption and a disturbed sequence from resorption into formation.

Like osteopetrosis, the osteosclerosis in PKND is accompanied by a predisposition to fracture and extremely poor callus elaboration. Our patient's past history revealed several fractures of both the femora and tibiae. In all fractures, closed reductions were effective. However, the fracture of the right tibia did not unite despite adequate immobilization when she was forty-five years old. The fracture was treated with a plate and an intramedullary nail, but union did not ensue. Thus, she was treated with a vascularized fibular graft and external fixation in our hospital. Operative findings revealed that the local condition of the fracture site was quite poor. Therefore, vascularized bone graft was essential for providing the osteoinductive capacity. The SAFHS was applied three months after surgery. It has been reported that low intensity ultrasound is effective for fracture repair[17]. The SAFHS may accelerate bone formation and incorporation of a bone graft.

The roentgenogram of the fracture showed callus formation about nine months after the operation. Finally, the fracture healed clinically twelve months later. In this case, these extensive fracture treatments were successful. In the future, gene transfer of cathepsin K into purified osteoclasts or a tissue engineering technique will be applied to obtain bony union without invasive bone grafts.

In summary, a mutation in the cathepsin K gene was identified, providing further evidence that a deficient activity of this enzyme causes PKND.

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