III. 研究成果の刊行に関する一覧表

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Heterozygous C-propeptide Mutations in *COL1A1*: Osteogenesis Imperfecta Type IIC and Dense Bone Variant

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Heterozygous C-propeptide Mutations in *COL1A1*: Osteogenesis Imperfecta Type IIC and Dense Bone Variant

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ABSTRACT

Osteogenesis imperfecta type IIC (OI IIC) is a rare variant of lethal OI that has been considered to be an autosomal recessive trait. Twisted, slender long bones with dense metaphyseal margins and normal vertebral bodies in OI IIC contrast with crumpled, thick long bones and multiple vertebral compression fractures in OI IIA. Here, we report on two sporadic patients with classical OI IIC and a pair of siblings, with features of OI IIC but less distortion of the tubular bones (OI dense bone variant). One case with OI IIC and the sibs had novel heterozygous mutations in the C-propeptide region of *COL1A1*, while the second patient with clear-cut OI IIC had no mutation in this region. Histological examination in the two sporadic cases showed a network of broad, interconnected cartilaginous trabeculae with thin osseous seams in the metaphyses. These changes differed from the narrow and short metaphyseal trabeculae found in other lethal or severe cases of OI. Our experience sheds light on the genetics and etiology of OI IIC and on its phenotypic spectrum.

Key words: Osteogenesis imperfecta; type IIC; dense bone; *COL1A1*; C-propeptide

INTRODUCTION

Osteogenesis imperfecta (OI; MIM 166200, 166210, 259420, and 166220) comprises a heterogeneous group of connective tissue disorders characterized by fragile bones with susceptibility to fractures. Most cases of OI are caused by heterozygous mutations in *COL1A1* or *COL1A2*, the genes encoding the two type I procollagen alpha chains, pro α 1 (I) and pro α 2 (I). Each chain consists of three domains including a core triple helical domain composed of uninterrupted repeats of the Gly-Xaa-Yaa tripeptide, and N and C propeptide domains flanking the triple helical domain at both the amino- and carboxyl-terminal ends. Germ cell mosaicism and intrafamilial recurrence have been reported in *COL1A1* and *COL1A2* associated OI. The most common mutation pattern is a single nucleotide transition that substitutes a glycine residue in the triple helical domain. Although uncommon, mutations in the C-propeptide domain of *COL1A1* and *COL1A2* have also been observed [Chessler et al., 1993; Lamandé et al., 1995; Pace et al., 2001; Pace et al., 2002].

The Sillence clinical/radiological classification divides OI into a non-lethal type I with blue sclera, lethal types IIA, IIB, and IIC, progressively deforming type III, and non-lethal type IV with white sclera [Sillence et al., 1979]. Among the lethal forms, OI IIC is especially rare. It manifests with severe calvarial demineralization, discontinuous beading of the ribs, normal vertebral bodies, misshapen scapulae and ischia, and slender, twisted long bones with fractures [Sillence et al., 1984; Spranger et al., 1984; Thompson et al., 1987; van der Harten et al., 1988]. OI IIC is hallmarked by absence of vertebral compression fractures and slender long bones despite multiple fractures, which contrast with the compressed vertebral bodies and thick, crumpled long bones seen in other lethal or severe types of OI (OI IIA, IIB, and III). Chondroosseous histology is also distinctive in OI IIC exhibiting a network of relatively broad and irregularly arranged cartilaginous trabeculae with many interconnections and thin osseous seams in the metaphyseal spongiosa, which differs from the narrow and short metaphyseal trabeculae seen in other types [van der Harten et al., 1988]. It has been thought that OI IIC, unlike other forms, is inherited as an autosomal recessive trait [Sillence et al., 1984]. The etiology of OI IIC has not

been elucidated.

Here, we report on two sporadic patients with OI IIC and a pair of siblings, who had features of OI IIC but less distortion of the tubular bones. One case with OI IIC and the sibs had novel heterozygous mutations in the C-propeptide region of *COL1A1*, while the second patient with clear-cut OI IIC had no mutation in this region. Our experience sheds light on the genetics and etiology of OI IIC and on its phenotypic spectrum.

PATIENT REPORTS

The four patients were unrelated and of Japanese origin. No consanguinity was reported in their parents. Family history was unremarkable in all families.

Patient 1 was the newborn female and was the first child of healthy parents. Prenatal ultrasonography at 26 weeks of gestation revealed short limbs, multiple fractures of the long bones and a hypoplastic thorax. She was vaginally delivered at 36 weeks' gestation after breech presentation. She succumbed shortly after birth. Birth weight was 1638 g (below 3rd percentile), length 40.0 cm (below 3rd percentile), and OFC 30.5 cm (10th percentile). Physical findings included disproportionately short, bent limbs with relatively long fingers and toes, a hypoplastic thoracic cage, relative macrocephaly with caput membraneceum, and triangular face with hypertelorism, protruding eyes, and low set, malformed ears. Postmortem radiographs demonstrated severe calvarial demineralization and small, dense facial bones (Fig. 1A). The skull base was also dense. The clavicles and ribs showed discontinuous beading with multiple fractures. The diaphyses of the long bones appeared slender and twisted despite many fractures. Broad gap and sclerosis of the fracture surfaces resembled those of pseudoarthrosis. The metaphyses of the long bones were flared with dense transverse bands. The scapular, ischial, and iliac margins were irregular and sclerotic. Despite conspicuous deformities of the tubular bones and flat bones, the spine appeared unremarkable.

Patient 2-1 (older sister of Patient 2-2) was born to healthy parents who had one healthy child. Fetal

ultrasound at 34 weeks' gestation showed bowing of lower limbs and a hypoplastic thorax. She was delivered by caesarian section at 37 week's gestation. Birth weight was 2523 g (3^{rd}-10^{th} percentile), length 38.5 cm (below 3^{rd} percentile), and OFC 33.4 cm (50-75^{th} percentile). She had dysmorphic facial features including micrognathia and a triangular face. Radiographs showed wormian bones of the skull, and slender long bones and ribs with multiple fractures (Fig. 1B). Dense transverse bands were found in the metaphyses and fracture surfaces of the long bones. No vertebral compression fracture was found. She had respiratory distress requiring a ventilation support, and died at age 4 months.

Patient 2-2 (younger brother of Patient 2-1) is the third child of the couple and currently 10 years old. Fetal ultrasound at 24 weeks' gestation showed deformity of lower limbs and ribs. He was delivered by caesarian section at 37 weeks' gestation. Birth weight was 2776g (10-25^{th} percentile), length 47.0cm (3^{rd}-10^{th} percentile), and OFC 36.0cm (97^{th} percentile). He had micrognathia and a triangular face. Radiographs showed wormian bones of the skull and slender bones with multiple fractures in the long bones and ribs. Unlike his sister, he showed very mild metaphyseal sclerosis (Fig. 1C). The metadiaphyseal junction was radiolucent, and the diaphyses were relatively dense. He required mechanical ventilation followed by nasal continuous positive airway pressure for several months. After discharge from the hospital at age 18 months, he still necessitated respiratory support (oxygen and biphasic positive airway pressure). He had chronic lung disease and was susceptible to pulmonary infections. Pamidronate treatment was started at age 11 months. No new fracture was recorded for latest three years. Gross motor development was delayed and he never sat without support.

Patient 3 was the newborn female and delivered at 29 weeks' gestation. She died soon after birth. Polyhydroamnios was noted. Birth weight was 1170g (10-50^{th} percentile), length 36.0cm (10-50^{th} percentile), and OFC 27.0cm (50-90^{th} percentile). Physical findings included caput membranaceum, a small face with mild hypertelorism, white sclerae, and bent limbs. On radiographs, discontinuous beading of the ribs and irregular clavicles, scapulae, pubic and ischial bones were similar to those of Patient 1. The long bones were twisted and slender with incomplete fractures. The metaphyses were

significantly dense (Fig. 1D). The diaphyses presented with alternating radiodense and radiolucent bands.

Histological findings

The samples from Patient 1 and 3 were processed according to the standard procedure, and the paraffin-embedded sections were stained with hematoxylin and eosin. In Patient 1 (FIG. 2A) and 3 (FIG. 2B), chondroosseous junction of the proximal femur showed a network of relatively broad, interconnected cartilaginous trabeculae with thin osseous seams in the metaphyseal spongiosa. Thick, cartilaginous trabeculae (cartilaginous cores) were also found in the diaphyseal spongiosa (data not shown). Chondrocyte columnization appeared somewhat irregular.

MATERIALS AND METHODS

PCR-Based Mutation Screening

Approval for this study was obtained from the Institutional Review Board of Keio University School of Medicine. We obtained written informed consent for molecular studies from the parents.

Genomic DNA was extracted from blood of the umbilical cord (Patient 1), Epstein-Barr virus-transformed lymphoblasts (Patient 2-1), and peripheral blood of Patient 2-2 together with the unaffected parents and older sister of the Patient 2-1 and 2-2 by a standard technique. In Patient 3, only formalin-fixed liver was available for the analysis, and thus we extracted genomic DNA by using TaKaRa DEXPAT (Takara Shuzo, Otsu, Japan) according to the manufacture's instructions. In Patient 1, 2-1, and 2-2, we analyzed all coding exons and flanking introns of *COL1A1, COL1A2, LEPRE1, CRTAP, PPIB* by PCR and direct sequencing. Deletion/duplication involving *COL1A1* and *COL1A2* were checked by multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA KIT P271, P272; MRC-Holland, Amsterdam, The Netherlands). In Patient 3, PCR amplification was difficult; thus, we could examine only the C-propeptide region of *COL1A1*.

Subcloning

To demonstrate paternal somatic mosaicism for the unaffected father of the Patient 2-1 and 2-2, we subcloned PCR products of DNA from his peripheral blood and nail, took 100 colonies of each, screened the mutation, and calculated the ratio of mutant to wild type alleles.

RESULTS

Molecular analysis

The sequence analysis revealed novel heterozygous *COL1A1* mutations, c.4247delC (p.T1416RfsX10) in Patient 1 and c.4160C>T (p.A1387V) in Patient 2-1 and 2-2 (Fig. 3A and 3B). These mutations are located in the C-propeptide region of pro α 1(I). The A1387 is evolutionarily highly conserved in all vertebrate pro α 1(I) and in all human fibrillar procollagens (Fig. 3C). T1416RfsX10 and A1387V were not found in 100 control individuals. In Patient 1, 2-1, and 2-2, no sequence variation was found in *COL1A2*, *LEPRE1*, *CRTAP*, and *PPIB*, and neither exon-level deletion nor duplication was detected by the MLPA analyses. Familial gene analysis of Patient 2-1 and 2-2 revealed that clinically unaffected father also had the A1387V. Mother and healthy older sister did not have the mutation. We did not found any sequence variation in the C-propeptide region of *COL1A1* in Patient 3.

Subcloning

The subcloning analysis revealed somatic mosaicism of A1387V in the father of the Patient 2-1 and 2-2. The ratio of mutant to wild type alleles was 35/65 in the peripheral blood and 7/93 in the nail (Fig. 3D).

DISCUSSION

We found novel heterozygous mutations in the C-propeptide region of *COL1A1* in three of four cases. The heterozygosity of the mutations disputed against an autosomal recessive trait that has been

assumed in OI IIC. The sib case was caused by germinal mosaicism of the unaffected father. We could not find a *COL1A1* C-propeptide mutation in Patient 3 suggesting genetic heterogeneity of OI IIC. Unfortunately, poor quality of available DNA precluded analyses for other known OI genes.

Radiological variation and consistency of OI IIC spectrum are illustrated in FIG. 4. Patient 1 and 3 fulfilled the previously reported radiological and histological criteria of OI IIC, as outlined above. Patient 2-1 exhibited radiological changes similar to those in the 'OI dense bone variant' previously reported in a patient with a C-propeptide mutation in *COL1A1* [Pace et al., 2002]. This phenotype differs from classic OI IIC by the better preservation of the long bone shape. Skeletal changes were even less severe in the second sib with only discrete metaphyseal hyperdensity.

The dense bone segments and absence of vertebral compression fracture in OI IIC deserve comment. Mild metaphyseal hyperdensity is seen in many newborns with severe OI. However, they are more prominent in classic OI IIC and in the so-called 'OI dense bone variant' (Pace et al 2002, our patient 2-1 and 2-2). Histologic sections showed a network of broad and irregularly arranged trabeculae with retained cartilage cores in the metaphyseal spongiosa. It contrasts with the narrow and short metaphyseal trabeculae in other lethal or severe cases of OI [van der Harten et al., 1988]. Hypothetically, the cartilaginous trabeculae may be resistant to compression forces but susceptible to bending forces explaining the long bone distortion and absence of vertebral compression fractures in OI IIC.

The pathogenesis of metaphyseal hyperdensity is more difficult to explain. Previous reports on C-propeptide mutations of *COL1A1* showed that trimer assembly was delayed, secretion was diminished, and a total amount of procollagen production was reduced [Chessler et al., 1993; Lamandé et al., 1995]. Pace et al. claimed that the dense bones in their reported case reflected both diminished amounts of secreted type I procollagen and the presence of overmodified, yet stable, molecules [Pace et al., 2002]. C-propeptide modulation of TGF-beta and collagen synthesis of osteoblastic cells at the early stage of differentiation [Mizuno et al., 2000] may also contribute to the extra bone formed in the metaphyses of patients with OI IIC (with or without twisted long bones). However, in what way C-propeptide mutations

influence that mechanism and stimulate metaphysesal bone formation, remains to be established.

In conclusion, heterozygous C-propeptide mutations in *COL1A1* may result in OI IIC with or without twisting of the long bones. Absence of vertebral compression fractures and presence of conspicuous metaphyseal sclerosis in these patients seem to be related to a distinctive histology with broad, cartilaginous, rather than thin osseous trabeculae. OI IIC appears to be inherited as an autosomal dominant trait. Failure to detect *COL1A1* propeptide mutations in one patient points to genetic heterogeneity of OI IIC.

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Figure legends

FIG. 1.

Radiographs of Patient 1(A), 2-1(B), 2-2(C) and 3(D)

A: There were severe calvarial demineralization with small, dense facial bones, discontinuous beading of the ribs, and twisted long bones with multiple fractures. The metaphyses of the long bones were flared with dense transverse bands. The scapular, ischial, and iliac margins appeared irregular and sclerotic. The height of vertebrae was normal.

B: Dense sclerotic bands were found in the metaphyses and fracture sites of the long bones (arrows). No vertebral compression fracture was found. The manifestation of the long bones was milder than that seen in Patient 1.

C: Note mild metaphyseal sclerosis (arrow) and adjacent radiolucency (arrowhead).

D: Alternating bands of radiodensity and radiolucemcy were found in the long bones. Osteosclerosis was particularly conspicuous in Patient 3. Discontinuous beading of the ribs and twisted long bones were noted.

FIG. 2.

Histological findings of Patient 1 (A) and 3 (B)

The paraffin-embedded sections were stained with hematoxylin and eosin.

Histological findings of Patient 1 and 3 were almost identical.

A network of broad, interconnected cartilaginous trabeculae in the metaphyseal spongiosa were shown. Chondrocyte columnization appeared somewhat irregular.

FIG. 3.

Identification of mutations in the C-propeptide region of *COL1A1*

A, B: Partial sequences of PCR products of Patient 1, 2-1, and 2-2 are shown. Heterozygous single base

pair deletion (c.4247delC) in Patient 1 and heterozygous missense mutation (c.4160C>T; p. A1387V) in Patient 2-1 and 2-2 are indicated by arrows.

C: Alanine at codon 1387 is conserved among all human fibrillar procollagens and several other species.

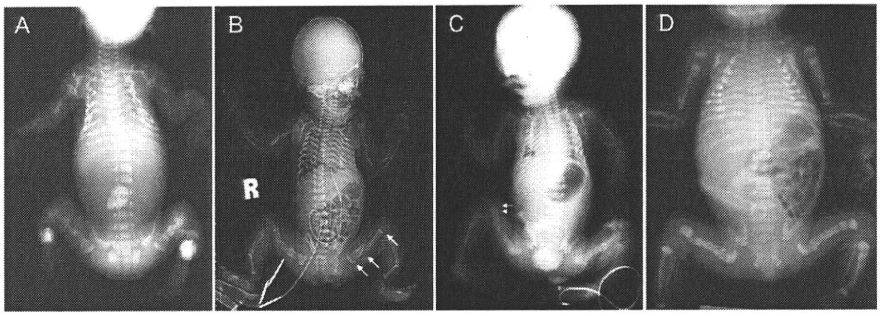
D: Subcloning of the PCR products from the father of Patient 2-1 and 2-2

The ratio of mutant to wild type alleles was 35/65 in the peripheral blood and 7/93 in the nail, suggesting somatic mosaicism.

FIG. 4.

Radiological variation and consistency of OI IIC spectrum

OI dense bone variant differs from classic OI IIC by the better preservation of the long bone shape (twisted or straight). Normal vertebral bodies, thin tubular bones and metaphyseal hyper density are consistent in OI IIC spectrum.



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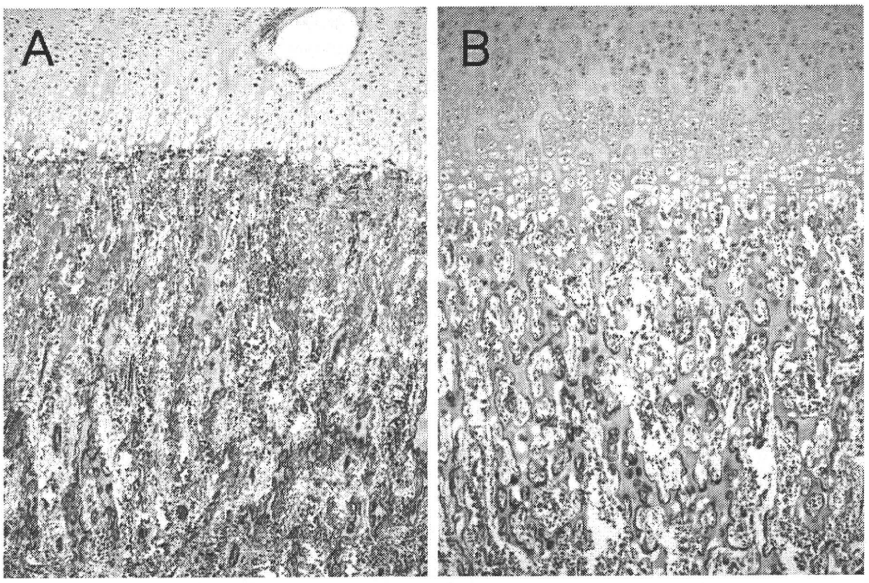

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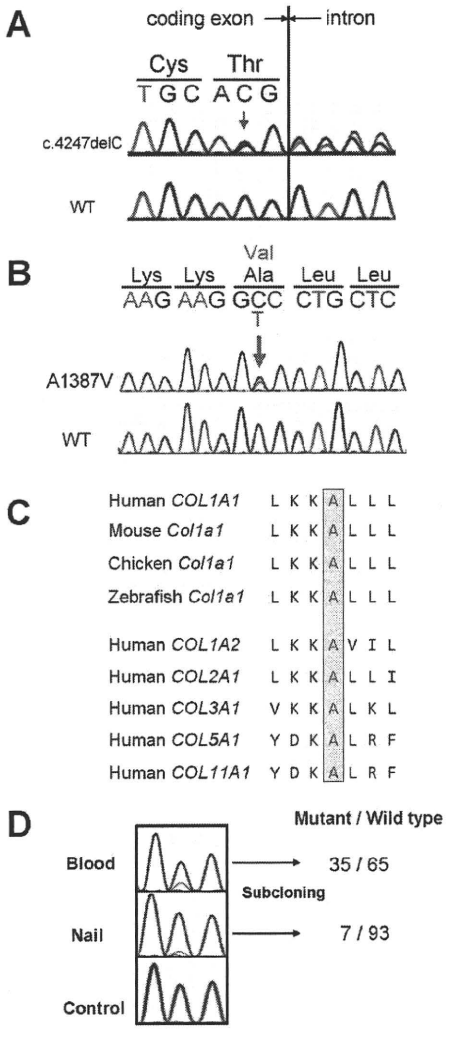

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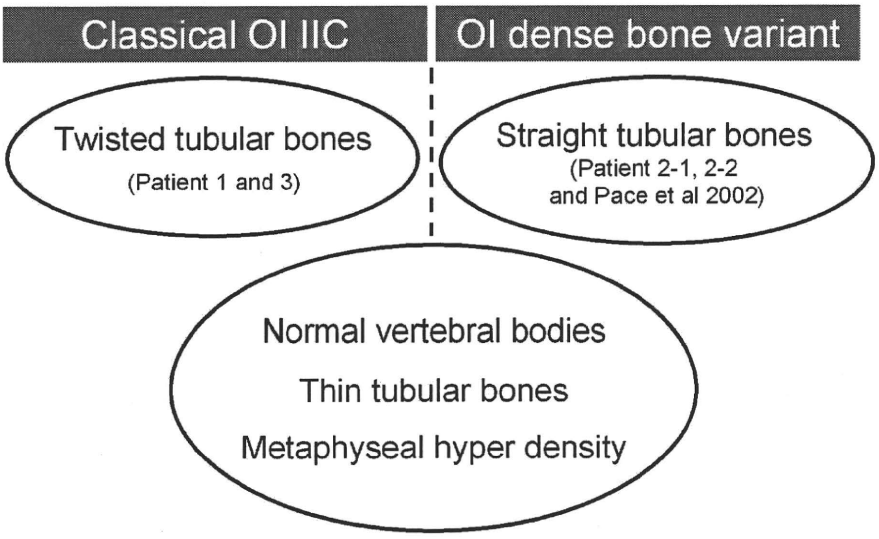

C-propeptide mutation in *COL1A1* or other causes

423x317mm (96 x 96 DPI)