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DATA REPORT

Osteogenesis imperfecta IIC caused by a novel heterozygous mutation in the C-propeptide region of *COL1A1*

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Osteogenesis imperfecta IIC (OI IIC), which is a rare variant of lethal OI that has been considered to be an autosomal recessive trait, is characterized by twisted, slender long bones with dense metaphyseal margins. Here, we report a typical case of OI IIC caused by a novel heterozygous mutation in the C-propeptide region of *COL1A1*. OI IIC seems to be caused by a dominant mutation of *COL1A1*.

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Osteogenesis imperfecta (OI) comprises a heterogeneous group of connective tissue disorders characterized by fragile bones with susceptibility to fractures. Currently, a molecular genetic classification of OI contains 15 types which display either autosomal-dominant or autosomal-recessive patterns of inheritance and exhibit broad variations in clinical severity (OI type I-XV; MIM #166200, #166210, #259420, #166220, #610967, #610968, #610682, #610915, #259440, #613848, #613849, #613982, #614856, #615066 and #615220).[1]

Most cases of OI are caused by heterozygous mutations in *COL1A1* or *COL1A2* (OI type I–IV), the genes encoding the two type I procollagen alpha chains, proα1 (I) and proα2 (I). Each chain contains a core triple helical domain, composed of uninterrupted repeats of the Gly–Xaa–Yaa tripeptide, flanked by propeptides at both the amino- and carboxyl-terminal ends. From the practical viewpoint, OI is clinically diagnosed using the Sillence classification,[2] according to which lethal OI due to *COL1A1/A2* mutations are classified as types IIA, IIB and IIC. Among these three type II OI, OI IIC is extremely rare, with distinct phenotypical and radiological manifestations, such as slender, twisted long bones with fractures and normal height of the vertebral bodies.[3–5] Slender, twisted long bones contrast with thick, crumpled ones seen in other forms of lethal OI, whereas normal height of the vertebral bodies differs from multiple compression fractures of the vertebral bodies in other lethal OI. Another hallmark of OI IIC is osteosclerosis in the metaphyseal ends, outer margins of the flat bones and fracture surfaces.

OI IIC had been believed to be inherited as an autosomal recessive trait, based on affected individuals in the same kindred.[4,6] Pace *et al.*[7] reported a case of lethal OI caused by a heterozygous mutation (D1441Y) in the C-propeptide region of *COL1A1* They described the phenotype as a lethal variant of OI with features of dense bone diseases. However, radiological examination showed that the phenotype was consistent with that of OI IIC. Moreover, we have previously reported that heterozygous C-propeptide mutations of *COL1A1* are responsible for OI IIC (c.4247delC), with a phenotype similar to but slightly milder than OI IIC (A1387V).[8] The heterozygosity of these cases contradicted the initial hypothesis that OI IIC was inherited as an autosomal recessive trait. However, the conclusion was not decisive because of the limited number of cases. Here, we report another case with a typical OI IIC phenotype caused by a novel heterozygous frameshift mutation in the C-propeptide region of *COL1A1*. This observation enhances the hypothesis of the pathogenic link between OI IIC and the C-propeptide heterozygous mutation in *COL1A1*.

The patient was a male child with healthy parents. Prenatal ultrasonography at 20 weeks of gestation showed short limbs and a hypoplastic bell-shaped thorax. The pregnancy was electively interrupted at 21 weeks' gestation. Disproportionately short and bent limbs, a hypoplastic thoracic cage and caput membranaceum were noted. The findings of post-mortem radiography fulfilled the radiological criteria of OI IIC, including slender ribs with multiple fractures, erratic ossifications of the flat bones, slender and twisted long bones with sclerosis of the metaphyseal ends and fracture surfaces and normal height of the vertebral bodies (Figure 1).

After genetic counseling and with written informed consent, we obtained genomic DNA from the umbilical cord blood (patient) and peripheral blood (parents) by using a standard technique. This study was approved from the institutional review board of the Keio University School of Medicine. We checked all the coding exons and flanking introns of *COL1A1* and *COL1A2* by PCR and direct sequencing. Deletion in and duplications of *COL1A1* and *COL1A2* were examined by multiplex ligation-dependent probe amplification (MLPA) analyses (SALSA MLPA KIT P271, P272; MRC-Holland, Amsterdam, The Netherlands). We found a novel heterozygous mutation in *COL1A1,* c.4309delC (p.L1437CfsX89), located in the C-propeptide region of pro α1 (I) (Figure 2). The mRNA with c.4309delC seems to escape the nonsense-mediated decay, because this frameshift occurrs in the last exon of *COL1A1*. The unaffected parents did not have this mutation. MLPA analysis did not detect exon-level deletion or duplication. The p.L1437CfsX89 was not detected in 150 healthy Japanese controls

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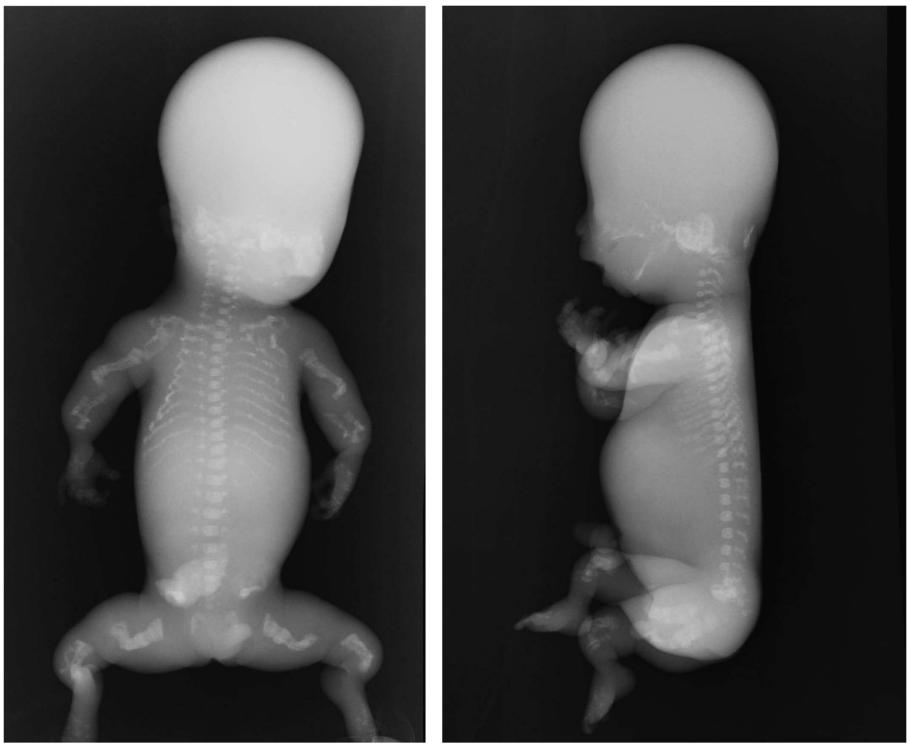

Figure 1. Post-mortem radiographs of patient. The findings of post-mortem radiography fulfilled the radiological criteria of osteogenesis imperfecta IIC, including slender ribs with multiple fractures, erratic ossifications of the flat bones, slender and twisted long bones with sclerosis of the metaphyseal ends and fracture surfaces and normal height of the vertebral bodies.

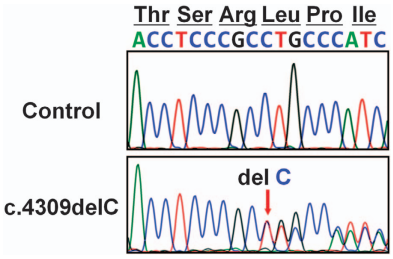

Figure 2. Identification of mutation in the C-propeptide region of *COL1A1*. Partial sequences of PCR products of the patient and control are shown. Heterozygous single base pair deletion (c.4309delC) in the patient is indicated by arrows.

and was absent from database, including dbSNP, the 1,000 Genomes Project, Exome Variant Server, NHLBI Exome Sequencing Project and the Human Genetic Variation Database (HGVD) in Japanese.

Here we describe the fourth observation of OI IIC with a C-propeptide mutation in *COL1A1*. The histological and radiological observations indicate that the distinctive skeletal changes in OI IIC or the 'dense bone phenotype' is related to the abundance of immature woven bone in the skeleton.[7,8] However, the phenotypic consequences of C-propeptide mutations of *COL1A1* reported thus far have ranged from mild OI type I to lethal OI type II.[7–12] Thus, the type of C-propeptide mutations responsible for the dense bone phenotype observed in OI IIC remain elusive. The absence of vertebral compression fracture in OI IIC deserve comment. Histologic sections showed a network of broad and irregularly arranged trabeculae with retained cartilage cores in the metaphyseal spongiosa.[8] It contrasts with the narrow and short metaphyseal trabeculae in other lethal or severe cases of OI.[5] 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 C-propeptide of type I collagen has a pivotal role in collagen assembly. Previous reports on C-propeptide mutations of *COL1A1* have reported delayed trimer assembly, diminished secretion and reduced production of the total amount of procollagen.[9,10,12] In contrast, it is known that the C-propeptide of type I collagen modulates TGF-beta and collagen synthesis in osteoblast cells at the early stage of differentiation.[13,14] Therefore, the C-propeptide of type I collagen acts as a signaling molecule.[7] This may be an explanation for the dense bone structure observed in OI IIC; however, the pathogenic mechanism remains largely unknown.

In conclusion, we report an additional case of OI IIC caused by a novel heterozygous frameshift mutation in the C-propeptide region of *COL1A1*. Patients with OI IIC are likely to harbor a dominant, *de novo* mutation of *COL1A1*.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.522.

COMPETING INTERESTS

The authors declare no conflict of interest.

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