American journal of medical genetics. Part A (2012.Aug) 158A 8:1953～1956.

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Okamoto Toshio, Nagaya Ken, Asai Hiroko, Tsuchida Etsushi, Nohara Fumikatsu, Hayashi Tokitsugi, Yamashita Akiko, Nishimura Gen, Azuma Hiroshi

Clinical Report

Platyspondylic Lethal Dysplasia Torrance Type with A Heterozygous Mutation in The Triple Helical Domain of *COL2A1* in Two Sibs from Phenotypically Normal Parents

Running Title: PLSD-T in sibs

Toshio Okamoto[a], Ken Nagaya[a], Hiroko Asai[a], Etsushi Tsuchida[a], Fumikatsu Nohara[a], Tokitsugi Hayashi[a], Akiko Yamashita[b], Gen Nishimura[c], and Hiroshi Azuma[a]

[a]*Department of Pediatrics, Asahikawa Medical University, Hokkaido, Japan;*

[b]*Department of Obstetrics and Gynecology, Asahikawa Medical University, Hokkaido, Japan;* [c]*Department of Pediatric Imaging, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan*

Corresponding author: Toshio Okamoto, MD, PhD

Department of Pediatrics, Asahikawa Medical University

2-1-1-1 Midorigaoka-Higashi, Asahikawa, Hokkaido 078-8510, Japan

Tel: +81-166-68-2481; Fax: +81-166-68-2489, E-mail; toshio5p@asahikawa-med.ac.jp

ABSTRACT

Heterozygous *COL2A1* mutations create a group of skeletal dysplasias collectively termed type II collagenopathies. Sporadic cases of type II collagenopathies are almost exclusively caused by *de novo* mutations. Very few cases with intrafamilial recurrence due to germinal mosaicism have been known. We report here on a family in which a severe form of skeletal dysplasia was recurrent in two sibs whose phenotype was most consistent with platyspondylic lethal skeletal dysplasia Torrance type (PLSD-T). A *COL2A1* analysis showed that the two sibs had a heterozygous mutation in the triple helical region of *COL2A1*, c.3545G>A (p.G1182A) in exon 50. The parents did not consent to a molecular analysis; however, the presence of the same mutation in the two sibs is proof of germinal mosaicism in one of the parents. PLSD-T has been believed to arise from a heterozygous dominant negative mutation in the C-propeptide region of *COL2A1*. However, our observation suggests that the phenotype is also caused by a mutation in the C-terminal triple helical region of *COL2A1*.

Key words: Skeletal dysplasia; *COL2A1*; platyspondylic lethal skeletal dysplasia, Torrance type; sib case; germline mosaicism

INTRODUCTION

Heterozygous *COL2A1* mutations create a family of skeletal dysplasias inherited as an autosomal dominant trait collectively termed type II collagenopathies [Spranger et al., 1994]. Sporadic cases of type II collagenopathies are almost exclusively due to *de novo* mutations. Affected sibs born to healthy parents can be attributed to germline mosaicism; however, few cases have been documented.

Patterns of *COL2A1* mutations correlate well with the clinical entities [Nishimura et al., 2005]. Stickler dysplasia type I (SDT-I; MIM 108300) is mostly attributed to haploinsufficiency [Freddi et al., 2000], while Kniest dysplasia (KND; MIM 156550) is attributed to exon skipping [Wilkin et al., 1999]. Several spondyloepiphyseal dysplasias (SED), including achondrogenesis type 2 (ACG2; MIM 200610), hypochondrogenesis (HCG; MIM 200610), SED congenita (SEDC; MIM 183900), and SED late-onset (SEDT; MIM 184100, 604864), are related to missense mutations or in-frame derangement in the triple helical region [Korkko et al., 2000]. Mutations in the carboxy-terminal propeptide (C-propeptide) region are responsible for rare phenotypes, such as platyspondylic lethal skeletal dysplasia Torrance type

(PLSD-T; MIM 151210) [Nishimura et al., 2004; Zankl et al., 2005] and spondyloperipheral dysplasia (SPPD; MIM 271700) [Zankl et al., 2004]. These entities correspond to the manifestations in most affected individuals; however, a few cases are intermediate between these entities or are otherwise unclassifiable, which demonstrates the continuum of the collagenopathy spectrum.

We report on 2 sibs with a severe form of type II collagenopathy born to healthy parents. The clinical phenotype was most consistent with PLSD-T, and the sibs had an identical heterozygous missense mutation in the triple helical region of the *COL2A1* gene.

PATIENT REPORT

Patients 1 and 2 were boys, the products of the second and fifth pregnancies, respectively, of a healthy non-consanguineous Japanese couple. The first and third pregnancies were uneventful, and the mother gave a birth to a healthy girl and boy, respectively. The fourth pregnancy resulted in an intrauterine fetal death at 25 weeks of gestation, yet the fetus was not reported to have any abnormalities on prenatal ultrasonography.

Patient 1

In this male fetus, ultrasonography in the second trimester showed thoracic hypoplasia with thin, short ribs and marked limb shortness, suggesting a lethal skeletal dysplasia, and the pregnancy was interrupted at 21 weeks of gestation. Autopsy findings were a prominent forehead, micrognathia, short neck, thoracic hypoplasia, protuberant abdomen, and severe rhizomelic shortness of the limbs (Fig. 1A).

Patient 2

In this male fetus, ultrasonography at 23 weeks of gestation showed thoracic hypoplasia and severe micromelia. Fetal computed tomography (CT) at 28 weeks of gestation showed a hypoplastic thorax, absence of vertebral and pubic ossifications, broad ilia with short greater sciatic notches and triradiate acetabula, and shortness of the long bones with cupped metaphyses (Fig. 2A). A suspicion of PLSD-T was raised with an alternative diagnostic possibility of an autosomal recessive disorder such as spondylometaphyseal dysplasia Sedaghatian type.

The boy was born by spontaneous vaginal delivery at 37 weeks of gestation. Birth weight was 2972 g (+0.1 SD), length 36.0 cm (-5.5 SD), and occipitofrontal

circumference was 36.4 cm (+2.1 SD). He had a low nasal bridge, micrognathia, cleft palate, rhizomelic shortness of limbs, short neck, thoracic hypoplasia, and protuberant abdomen (Fig. 1B). He presented with severe respiratory failure that required intratracheal intubation and mechanical ventilation. The skeletal alterations in the postnatal radiographs were almost identical with those seen on fetal CT scan, but the metaphyses of the long bones were ragged rather than simply cupped (Fig. 2B, C). Brachydactyly was not evident. Radiographs at 6 months showed persistent ossification failure of vertebral bodies, absence of pubic ossification, and shortness of long bones with mildly splayed ends and cupped metaphyses (Fig. 2D, E). The patient is currently 1 year old and still needs a mechanical ventilator.

MOLECULAR ANALYSIS

Genetic testing performed on a fibroblast culture obtained from patient 2 demonstrated heterozygosity for a c.3545G>A transition (p.Gly1182Asp) in exon 50 of *COL2A1*. An analysis performed on the dried umbilical cord from patient 1 documented the same mutation in patient 2. Despite given information of the high probability of parental mosaicism, the parents did not agree to genetic testing for themselves and their

healthy children; therefore, further assessment was not feasible.

DISCUSSION

These of 2 sibs have a severe form of type II collagenopathy, due to heterozygosity for a c.3545G>A mutation in *COL2A1*. Although the parents did not consent to sampling, the presence of the same mutation in the two sibs was proof of germinal mosaicism in one of the parents. Severe type II collagenopathies are usually sporadic and due to *de novo COL2A1* mutations. Intrafamilial recurrence is rare in severe type II collagenopathies. To date, there have been only 3 reports on the recurrence of ACG2 [Faivre et al., 2004; Forzano et al., 2007; Comstock et al., 2010]. The findings of a dominant mutation in two offspring of phenotypically normal parents suggested germinal mosaicism.

The skeletal manifestations of these sibs were consistent with a type II collagenopathy of an unclassifiable, intermediate form. Absence of vertebral and pubic ossification resembled that seen in ACG2. Brachydactyly, commonly seen in PLSD-T, was absent in our patients. In contrast, the severe shortness of the greater sciatic notches and the triradiate acetabula were similar to those of PLSD-T. The degree of long bone

shortness was milder than that seen in ACG2. Severely ragged metaphyses were more consistent with PLSD-T. Taken together, the skeletal alterations in these cases were most consistent with those of PLSD-T, which is specifically caused by dominant-negative mutations in the C-propeptide of *COL2A1*. However, the *COL2A1* mutation in these sibs resided in the triple helical region, not in the C-propeptide region. Our observation illustrates the phenotypic variability and diagnostic difficulties in type II collagenopathies. However, it should be emphasized that a *COL2A1* mutation in the triple helical domain can be responsible for a phenotype that is most consistent with PLSD-T.

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FIGURE LEGENDS

Fig. 1: Patients 1 (A) and 2 (B) with prominent forehead, micrognathia, short neck, thoracic hypoplasia, a protuberant abdomen, and rhizomelic shortness of limbs.

Fig. 2: Computed tomography at 28 weeks of gestation (A) and radiography at birth (B

and C) of patient 2. They had similar abnormalities including a hypoplastic thorax, hypoplasia of lower ilia, ossification failure of vertebral and pubic bones, and shortness of long bones with cupped metaphyses. Ragged metaphyses are seen only on the plain radiograph. The radiograph at age 6 months (D and E) shows that metaphyseal ossification defects become less conspicuous than previously seen, but vertebral ossification is still retarded.