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Case Report

Family with mild form of osteogenesis imperfecta with recurrent fractures due to novel mutation of *COL1A1*

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Abstract

Osteogenesis imperfecta is a congenital connective tissue disease characterized by bone vulnerability leading to fractures and deformation, as well as many other connective tissue symptoms. We here report a case of osteogenesis imperfecta diagnosed based on recurrent fractures and blue sclerae in a young boy. The mother also had blue sclerae and had experienced a compression fracture during pregnancy. They were both found to harbor a novel mutation of *COL1A1* (c.2559+1G>T). Genomic study is useful in identifying the mild phenotype of osteogenesis imperfecta.

Introduction

Osteogenesis imperfecta (OI) is a congenital connective tissue disease characterized by bone vulnerability leading to fractures and deformation, as well as many connective tissue symptoms, including dentinogenesis imperfecta, joint hypermobility, and mitral valve prolapse. We here report a case of osteogenesis imperfecta diagnosed based on recurrent fractures and blue sclerae in a young boy and his mother, both of whom were found to harbor a novel mutation of *COL1A1*.

Case Report

The patient was a Japanese boy with Kawasaki disease who had been undergoing follow-up at our hospital from the age of 10 months. When he was 13 years old, he visited our hospital due to recurrent bone fractures (5 times within 13 years). He was born at 38 weeks of gestation (height, 47 cm ; body weight, 2,574 g), and was admitted to our hospital due to Kawasaki disease at the age of 10 months without any sequelae, including regurgitation or aortic dilatation. His history of fracture was as follows : left tibia (18 months) ; left tibia after falling (2 years 5 months) ; right 1st toe after falling (6 years 7 months) ; right thumb (6 years 8 months) ; and right radius after falling (12 years). His height was 158.3 cm (−0.1 SD) ; weight, 34.7 kg (−1.38 SD) ; blood pressure, 98/39 mmHg ; and pulse, 72/min. He had blue sclerae but no visual disturbance. No abnormalities were observed on physical examination, with no scoliosis and normal balance of extremities. No hearing loss, amelogenesis imperfecta, or dentinogenesis imperfecta was observed.

The patient's serum alkaline phosphatase level was extremely high (1,207 U/L), whereas his serum calcium and 25-hydroxy vitamin D levels were normal. Osteocalcin and bone specific alkaline phosphatase (BAP) levels were high, at 328 ng/mL (normal range, 8.4-33.1 ng/mL) and 136 μg/L (normal range, 3.7-20.9 μg/L), respectively. The results of echocardiography was

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normal. The patient's bone density was low (0.52 g/cm^2), which was approximately 64% of that in healthy children of the same age. The mother also had blue sclerae and had experienced a compression fracture during pregnancy. Her height subsequently decreased by 5 cm. Her BAP and TRACP-5b levels were normal, at 13.4 ng/mL and 336 mU/dL (normal range, 120-420), respectively.

A whole exome sequencing (WES) study was suggested and approved by the Ethics Committee of Tokyo Medical University. Written informed consent was obtained from the patient and both of his parents prior to performance of the proband-WES.

Peripheral blood mononuclear cells were isolated from 5 mL whole blood with Ficoll-Paque PLUS (GE Healthcare) and genomic DNA extracted using the QIAamp DNA Blood Mini Kit (Qiagen). Exome enrichment and library preparation were performed with Ion Ampliseq Exome RDY Kit PI v3 (Thermo Fisher Scientific) and Ion Xpress Barcode Adapters (Thermo Fisher Scientific). Library concentration was measured with Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific). After template preparation using the Ion PI Hi-Q Chef kit (Thermo Fisher Scientific) on the Ion Chef system (Thermo Fisher Scientific), sequencing was performed with the Ion Proton sequencer (Thermo Fisher Scientific). The sequence data were analyzed using the Torrent Suite™ software on the Torrent Server. All parameters were used at the default settings. The result file was annotated using the ANNOVAR software[1].

The candidate variant was filtered using the settings detailed below. No variant on exon or splice-sites of OI-causing genes (*BMP1, COL1A1, COL1A2, CRTAP, FKBP10, IFITM5, OI16, P3H1, PPIB, SERPINF1, SERPINH1, SP7, SPARC, TMEM38B,* and *WNT1*), or an allele frequency of less than 1% in the Human Genomic Variation Database[2] or 1000 genome project[3], was observed in our in-house data. The only remaining candidate was a heterozygous splice-site variant, NM_000088.3 (*COL1A1*) : c.2559+1 G>T. It was validated by Sanger sequencing with the ABI PRISM 310 Genetic Analyzer. In addition, Sanger sequencing results for his parents revealed maternal inheritance.

The proband was diagnosed as OI type 1, which was subsequently treated with periodic intravenous bisphosphonate (pamidronate : 1 mg/kg ×3 days every 4 months). During the first round of treatment, the patient experienced temporary vomiting and discomfort owing to hypocalcemia, which improved with administration of a calcium agent. No hypocalcemia was observed after the second round of treatment. Bone density increased from 0.52 g/cm^2 to 0.68 g/cm^2. No fractures occurred during the 2 years after commencement of treatment.

Discussions & Conclusion

Osteogenesis imperfecta is a congenital connective tissue disease with a prevalence of approximately 1 : 20,000-30,000. Approximately 90% of such patients carry the mutation in the genes encoding type I collagen (*COL1A1* or *COL1A2*), which is a major component of connective tissue. Recently, other gene mutations (*FKBP10*, *LEPRE1*, *CRTAP*, *PPIB*, *SERPINH1*, *SERPINF1*, and *BMP1*) have also been reported[4]. Osteogenesis deficiency is currently classified into 8 types, and the present case was considered to be type I. Two cases with a similar mutation (c.2559+1G>A) have been reported in the Osteogenesis Imperfecta Variant Database (https://oi.gene.le.ac.uk/variants.php?select_db=COL1A1&action=search_all&search_Variant%2FDNA=c.2559%2B1G%3EA), both of which were concluded to be pathogenic. To our knowledge, however, this is the first case of a patient with the mutation c.2559+1G>T. According to research on phenotype and genotype, 56 causative *COL1A1* and *COL1A2* mutations, and 23 haploinsufficiency mutations in *COL1A1* (8 splicing, including c.2559+1G>A, 7 nonsense, and 8 frameshift) have been reported. In one study, <18-year-old patients with *COL1A1* haploinsufficiency were no taller than patients with helical mutations ; in the >18-year-old group, however, the average height of such patients was significantly greater than that in those with helical mutations. Moreover, patients with haploinsufficiency had higher femoral neck bone mineral density than patients with helical mutations[5]. The symptoms in the mother and child observed in the present study suggest that this mutation causes mild phenotypes. However, pregnant women with type I OI have been reported to be at increased risk for osteoporosis, restrictive pulmonary disease, cephalopelvic disproportion, and other problems associated with connective tissue disorders, including capillary fragility[6]. To avoid these conditions, early diagnosis is favorable before pregnancy in women with blue sclerae.

Markers for osteogenesis, including osteocalcin, BAP, and type I collagen C-terminal propeptide, are indicative of increased osteoblastic function. Osteoblasts differentiate from preosteoblasts and mature into cells with mineralization ability. Osteocalcin, however, is considered a late marker of osteoblastic differentiation. Osteogenic markers showed an increase in the present patient. Unfortunately, levels of type I collagen C-terminal propeptide were not determined before commencement of treatment. Recently, type I collagen C-terminal propeptide was shown to be a good marker of Hip Dysplasia in Children with OI[7]. These markers may hence be useful as diagnostic screening markers for OI.

Bone fractures in OI patients have been reported to occur frequently in infancy and at the ages of 2 to 3 years

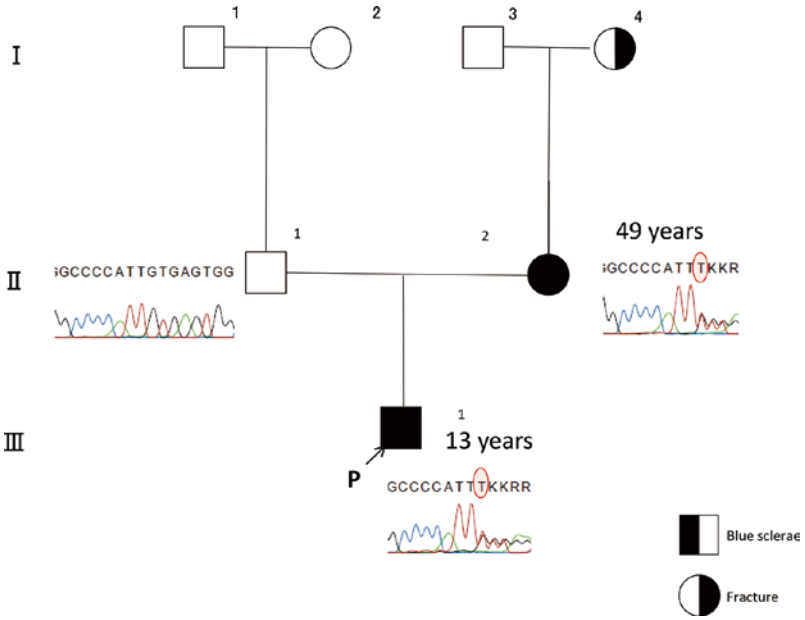

Fig. 1 Family pedigree
Red oval indicates mutation.

due to instability in walking. Some patients have been reported to have a history of more than 5 fractures a year[8]. In addition, bilateral fractures and recurrent bone fractures often occur in OI, and often require invasive treatment due to fragment dislocation[9]. These fractures are sometimes mistaken for abuse. The frequency of fractures in abused children in Japan is approximately 43%. The majority of such fractures are not attributable to abuse, however, with 85% occurring in children over 5 years old, whereas 80% of fractures resulting from abuse occurred in children younger than 18 months. One study demonstrated that among 504 cases of suspected abuse, 18 cases were actually due to OI[10]. Since OI is a treatable disease, it is important to distinguish it from abuse.

Acknowledgment

Informed consent was obtained from the family prior to commencement of this study.

The authors declare no conflict of interest.

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新規変異を伴った頻回骨折を示した軽症骨形成不全症の一家系

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【要旨】 骨形成不全症は骨折と変形を伴う骨の脆弱性と他の結合組織の症状を特徴とする先天性の結合織疾患である。今回、頻回に骨折歴と青色強膜から診断に至った小児例を経験した。母も同様に青色強膜を持ち、出産時に圧迫骨折の既往があった。母児は *COL1A1* に新規変異（c.2559+1G>T）を伴っていた。遺伝学的検査が骨形成不全症の軽症な表現型の診断に有用である。

〈キーワード〉 *COL1A1*、青色強膜、妊娠、オステオカルシン、骨型アルカリホスファターゼ