

## A Case of Autosomal Dominant Osteopetrosis Type II with a *CLCN7* Gene Mutation

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### What is already known on this topic?

Autosomal dominant osteopetrosis type II (ADO-II) is the benign form of osteopetrosis, which is characterized primarily by vertebral endplate thickening and includes increased cortical, and fragile bones with multiple fractures later in life. In families with ADO-II, the penetrance ranges from 60 to 90%.

### What this study adds?

This is the first case of autosomal dominant ADO-II with a confirmed mutation in the *CLCN7* gene in Korea. The patients showed typical radiologic findings of ADO-II with hearing loss. However, father with same mutation was asymptomatic, with no clinical or radiologic signs. Thus, we conclude that the exact prevalence is not known.

### Abstract

Osteopetrosis is a rare genetic disease characterized by increased bone density and bone breakage due to defective osteoclast function. Autosomal dominant osteopetrosis type II, Albers-Schonberg disease, is characterized by the sclerosis of bones, predominantly involving the spine, pelvis, and the base of the skull. Here, we report a typical case of osteopetrosis in a 17.7-year-old male who carries a heterozygous c.746C>T mutation in exon 9 in the *chloride voltage-gated channel 7* gene. The patient spine showed multiple sclerotic changes including sandwich vertebra. However, his father with same mutation showed normal skeleton radiographs.

**Keywords:** Osteopetrosis, bone density, osteoclast, sclerosis, mutation

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05.10.2018

10.02.2019

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### Introduction

Osteopetrosis, also known as marble bone disease, is an extremely rare bone disease characterized by increased bone mineral density, where bones are prone to breakage due to defective osteoclast function despite the increased bone mineral density<sup>1-3</sup>. According to The Nosology Group of the International Skeletal Dysplasia Society, osteopetrosis is classified based on clinical features, mode of inheritance and molecular mechanism<sup>2</sup>. There are various clinical features and genes involved in different types of osteopetrosis. Among all, inheritance types are autosomal recessive, autosomal dominant and X-linked. Autosomal recessive osteopetrosis (ARO), which is a malignant form of osteopetrosis that can be seen in infancy, can result in growth failure and increased frequency of fractures. Patients with ARO suffer from anemia and recurrent infections. It is considered that this is due to the bone expansion which leads to bone marrow space narrowing and results in extramedullary hematopoiesis. Some patients with ARO also suffer from blindness, facial paralysis, and deafness, due to pressure on the cranial nerves by the narrowing of spaces due to bone expansion.<sup>1-3</sup> However, autosomal dominant osteopetrosis (ADO), which is the benign form of osteopetrosis, may present no symptoms and most of them are found incidentally. ADO type II (ADO-II), also known as Albers-Schonberg disease, is characterized primarily by vertebral endplate thickening ("sandwich vertebrae") and includes increased cortical but normal cancellous bone volume, and fragile bones with multiple fractures later in life<sup>4-7</sup>. Most common cause of ADO-II is inactivating mutations in the chloride channel 7 (*CICN7*) gene, which results in ineffective osteoclast-mediated bone resorption through disrupt acidification of the osteoclast resorption lacunae that in turn prevents degradation of the mineral component of bone<sup>3</sup>. Here, we present a case of ADO-II in a 17.7-year-old male, who carries a heterozygous gene mutation in *CLCN7*.

### Case Report

A 17.7-year-old male was referred to our hospital due to sclerotic changes in bony structures. Approximately one month ago, he started complaining of pain in the right shin. X-rays in a local clinic revealed a generalized increase in bone density. He weighed 3.8 kg (75th percentile) at birth. He had no history of chronic diseases such as hypertension, diabetes, or hepatitis. The patient and his family members including his parents and younger sister had no history of bone fractures. His aunt was suspicious of having bone-related disease, but she did not get screened. He suffered from chronic otitis media and was diagnosed with partial hearing loss when he was 16 years old. On physical and neurological examination, no specific findings were noted. His current height and weight were 170.6 cm (50th percentile) and 69.0 kg (75th percentile), respectively.

Plain radiographs showed a generalized increase in bone density involving the skull, vertebrae, and pelvis. X-rays of the skull showed thickening and increased skull-base density (Fig. 1A). X-rays of the spine showed typical end-plate thickening and sclerosis producing the classic "sandwich vertebrae" appearance (Fig. 1B). Sandwich vertebra is a radiologic finding in which the endplates are densely sclerotic, presenting the appearance of a sandwich. X-rays of the pelvis showed the "bone-within-bone" appearance, primarily in the iliac wings (Fig. 1C). Bone-within-bone is a term applied to bones that appear to have another bone within them. The other family members, including his younger sister, mother and father, showed normal bone density. Fig. 1D shows normal bone appearance of the patient's father. Bone mineral densitometry (BMD) of the antero-posterior lumbar spine vertebrae one through four was measured as 2.466 g/cm<sup>2</sup> (Z-score = 10.7) by dual-energy X-ray absorptiometry (DXA) on a Lunar Prodigy (Lunar, Madison, WI, USA). The BMD of the left femoral neck, trochanter, and Ward's triangle were measured as 1.966 g/cm<sup>2</sup> (Z-score = 7.0)<sup>8</sup>, 1.825 g/cm<sup>2</sup>, and 1.943 g/cm<sup>2</sup>, respectively. Blood chemistry showed serum albumin at 4.4 g/dL (reference range 3.5-5.2 g/dL), total calcium at 9.5 mg/dL (8.6-10.2 mg/dL), phosphorus at 5.0 mg/dL (2.7-4.5 mg/dL), ionized calcium at 4.81 mg/dL (4.48-4.92 mg/dL), alkaline phosphatase at 108 U/L (40-129 U/L), sodium at 145 mmol/L, potassium at 4.4 mmol/L, chloride at 105 mmol/L, and bicarbonate at 28.4 mmol/L. The intact parathyroid hormone (PTH) level was slightly elevated at 79.5 pg/mL (reference range 14-72 pg/mL), the 25-hydroxy-vitamin D<sub>3</sub> was 25.7 ng/mL (insufficiency range 10-30 ng/mL), and thyroid-stimulating hormone (TSH) was 5.38 uIU/mL (reference range 0.27-4.20 uIU/mL).

For evaluation of osteopetrosis, targeted gene panel sequencing was performed to check for the presence of pathogenic variants of multiple associated genes responsible for osteopetrosis. After informed consent, 3 cc of blood was obtained from the patient, sister, and both parents. Library preparation was done using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA), which enriches a 12-Mb region spanning 62,000 target exons of a total of 4,813 clinically relevant genes. Massively parallel sequencing was performed on the Illumina NextSeq platform. Sequence reads were mapped to UCSC hg19 standard base for comparative analysis. The results of targeted gene panel sequencing revealed heterozygous missense mutation c.746C>T (p.Pro249Leu) in exon 9 of *CLCN7* gene, which was previously reported in patient with ADO type II<sup>9</sup> and there was no pathogenic variant in other genes. Sanger sequencing confirmed the presence of this variant, and the same heterozygous variant was only found in his father (Fig. 2). However, his father denied all symptoms including history of fracture, osteomyelitis, visual impairment, and hearing problem. His skeleton radiographs also showed normal (Fig. 1D). We did not evaluate bone mineral density of the patient's father, as his X-ray showed normal appearance.

## Discussion

This is the first case of autosomal dominant ADO-II in Korea, with a confirmed mutation in the *CLCN7* gene.

Previously, a case of infantile malignant *CLCN7*-related ARO, with neonatal thrombocytopenia was reported in Korea. There are two mutations in that case: (1) a deletion of an A at nucleotide 17631, in the paternally derived allele causing a frame shift and a premature stop codon at codon 395, and (2) an intronic point mutation G23742A in the maternal allele<sup>9</sup>.

ADO-II is the most common form of osteopetrosis and is characterized by sclerosis, predominantly involving the spine, pelvis, and base of the skull<sup>4-7</sup>. The fragility of bones and dental abscess are common complications. The gene that is mutated in ADO-II was reported to be localized on chromosome 16p13.3 and was later identified to be *CLCN7*<sup>5,6</sup>. The *CLCN7* gene encodes the 803 amino acids, chloride channel 7 protein subunit (ClC-7), which plays a role in efficient proton pumping in the osteoclast ruffled membrane<sup>3</sup>. Thus, patients with the *CLCN7* mutation have reduced bone resorption, which leads to osteopetrosis<sup>10</sup>. Previously, over 70 different mutations in *CLCN7* have been identified in ADO-II families, and almost all cases have been associated with heterozygous mutations in the *CLCN7* gene<sup>5, 7, 11, 12</sup>.

The spectrum of *CLCN7*-related osteopetrosis includes infantile malignant ARO, intermediate autosomal osteopetrosis (IAO), and ADO-II<sup>1</sup>. ADO-II is a benign condition and the disease onset is usually in late childhood or adolescence. The diagnostic criterion for ADO-II is osteosclerosis of the spine with a "sandwich vertebra or rugger-jersey" appearance. Most affected subjects have a "bone-within-bone" appearance, primarily in the iliac wings, but also in other long bones. Erlenmeyer-shaped femoral metaphyses, transverse bands of sclerosis, and mild osteosclerosis in the base of the skull are often observed<sup>1-3, 4, 7</sup>.

The complications of infantile malignant ARO include poor growth and fractures, with a life expectancy of fewer than 10 years. However, most ADO-II cases are benign, with normal life expectancy. Long-term complications of ADO-II

include fractures in long bones or vertebrae, scoliosis, hip osteoarthritis, and osteomyelitis<sup>4</sup>). One study, including longitudinal data, suggest that the ADO clinical phenotype especially fracture worsens over time<sup>13</sup>). Thus, vigorous physical activities should be avoided to prevent fractures, and routine dental examination and oral hygiene are important to prevent osteomyelitis of the mandible. In treating fractures, orthopedic surgeons should pay special attention to delayed union or non-union fractures. Cranial nerve compression is a rare occurrence. Hearing loss and vision loss occur in fewer than 5% of affected subjects. In our case, the patient had hearing loss due to osteopetrosis, but we did not perform further evaluation in order to identify the cause of right ear hearing loss.

The prevalence of ADO-II is estimated to be as low at 0.2 in 100,000 to 5.5 in 100,000 cases<sup>14-16</sup>). However, asymptomatic carriers were found with some mutations, and non-penetrance rates were 24 to 41% depending on mutations in families with ADO-II and most of them are asymptomatic in younger age<sup>4, 15, 17</sup>). Furthermore, no *CLCN7* mutation could be found in up to 30% of patients presenting with a clinical phenotype of ADO<sup>10, 18</sup>). Given the reduced penetrance of the ADO phenotype, the spectrum of disease expression can range from radiographically unaffected gene carriers to skeletally affected yet asymptomatic subjects to severely affected patient with fractures and increasing severity over time, the prevalence of ADO-II is even higher<sup>13</sup>). In our case, the father had the same mutation but was asymptomatic, with no clinical or radiologic signs. We believe it was due to reduced penetrance of phenotype. Therefore, it is important that *CLCN7* gene mutations be considered when patients have increased bone density, with radiologic findings such as “bone-within-bone” appearance. In future studies, we hope to perform genetic testing to confirm additional cases of asymptomatic Korean ADO-II cases.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

This study was approved by Korea Cancer Center IRB (No: K-1711-002-026)

#### **Acknowledgments**

The authors thank the patients and his family members for their participation. We also thank Professor Jin, Dong-Kyu and Cho, Sung Yoon of Samsung Medical Center for valuable comments.

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