

81694_Auto_Edited.docx

Name of Journal: *World Journal of Clinical Cases*

Manuscript NO: 81694

Manuscript Type: CASE REPORT

Clinical and Genetic Diagnosis of Autosomal Dominant Osteopetrosis type II in a Chinese Family

Autosomal Dominant Osteopetrosis type II

Hongping Gong, Yan Ren, Panpan Zha, Wenyan Zhang, Jin Zhang, Zhiwen Zhang, Chun Wang

Abstract

BACKGROUND

Osteopetrosis is a rare genetic disorder characterized by increased bone density due to defective bone resorption of osteoclasts. Approximately, 80% of autosomal dominant osteopetrosis type II (ADO-II) patients were usually affected by heterozygous dominant mutations in the *CLCN7* gene and present early-onset osteoarthritis or recurrent fractures. In this study, we report a case of persistent joint pain without bone injury or underlying history.

CASE SUMMARY

We report a 53-year-old female with joint pain who was accidentally diagnosed with ADO-II. The clinical diagnosis was based on increased bone density and typical radiographic features. Two heterozygous mutations in the chloride voltage-gated channel 7 (*CLCN7*) and T-cell immune regulator 1 (*TCIRG1*) genes by whole exome sequencing were identified in the patient and her daughter. The missense mutation (c.857G>A) occurred in the *CLCN7* gene p. R286Q, which is highly conserved across species. The *TCIRG1* gene point mutation (c.714-20G>A) in intron 7 (near the splicing site of exon 7) had no effect on subsequent transcription.

CONCLUSION

This ADO-II case had a pathogenic *CLCN7* mutation and late onset without the usual clinical symptoms. For the diagnosis and assessment of the prognosis for osteopetrosis, genetic analysis is advised.

Key Words: Osteopetrosis; Autosomal dominant osteopetrosis type II; Diagnosis; Genetic Analysis; *CLCN7*; *TCIRG1*.

Gong H, Ren Y, Zha P, Zhang W, Zhang J, Zhang Z, Wang C. Clinical and Genetic Diagnosis of Autosomal Dominant Osteopetrosis type II in a Chinese Family. *World J Clin Cases* 2022; In press

Core Tip: ADO-II (autosomal dominant osteopetrosis) is an autosomal dominant form of osteopetrosis. In ADO-II patients, the clinical spectrum ranges from nonsymptomatic to recurrent fractures, anemia, and a favorable prognosis. We reported a 53-year-old female patient with persistent joint pain, who was accidentally diagnosed with ADO-II at a later age. Her asymptomatic daughter was also diagnosed with ADO-II, as confirmed by whole exome sequencing.

INTRODUCTION

Osteopetrosis, also known as "marble bone disease," is a rare genetic disease characterized by increased bone mass and density due to bone resorption failure^[1]. Dr. Albers-Schonberg, a radiologist in Germany, described it for the first time in 1904^[2]. It has a broad clinical spectrum, ranging from asymptomatic to life-threatening bone marrow failure and cranial nerve dysfunction. Based on clinical severity and inheritance patterns, osteopetrosis is classified into three types: a "malignant" autosomal recessive infantile form (ARO), a "benign" autosomal dominant form (ADO type II), and an intermediate recessive form (IRO)^[1, 3, 4]. To date, mutations in at least ten genes have been identified to cause failure of osteoclast differentiation or function in humans, including the T-cell immune regulator gene (*TCIRG1*), chloride voltage-gated channel 7 (*CLCN7*), TNF superfamily member 11 (*TNFSF11*), TNF receptor superfamily member 11a (*TNFRSF11A*), osteopetrosis-associated transmembrane protein (*OSTM1*), sorting nexin 10 (*SNX10*), pleckstrin homology and RUN domain containing M1 (*PLEKHM1*), and NF- κ B essential modulator (*NEMO*) genes^[3, 5].

The most prominent characteristic of ADO-II is its dense yet fragile bones. We present a rare case of limb joint pain that was accidentally diagnosed as ADO-II based on clinical findings and genetic analysis.

6

CASE PRESENTATION

Chief complaints

A 53-year-old woman was admitted to the hospital with a complaint of limb joint pain for 11 mo.

History of present illness

The patient presented with pain in her shoulder, elbow, wrist, and metacarpophalangeal joints with swelling, tenderness and numbness for 1 mo before pain began in her bilateral knee, ankle, toe and finger joints for approximately 10 mo.

4

History of past illness

The patient had no history of fractures and bone injury.

Personal and family history

Her parents were not consanguineous. Her father and mother died of bone cancer and esophageal cancer, respectively. She has a daughter, a brother and two sisters who are asymptomatic. The pedigree of her family is shown in **Figure 1**.

Physical examination

1

On admission, her blood pressure was 120/70 mmHg, pulse rate 98/min, and respiratory rate 20/min. Her height and body weight were 155 cm and 63 kg, respectively. Physical examinations showed slight swelling of her wrists, hands, knees and ankles, accompanied by tenderness, limited retral swing of the upper limbs and slightly restricted movement of the lower limbs.

Laboratory examinations

The patient had mild anemia; decreased levels of serum bone alkaline phosphatase (BALP), 25-dihydroxy vitamin D3, urinary calcium and phosphorus; and an elevated

level of serum phosphorus. The levels of serum calcium, urinary fluoride, lactate dehydrogenase, creatine kinase, C-telopeptide of type I collagen (CTX), N-terminal mid-fragment of osteocalcin (N-MID OC), and parathyroid hormone (PTH) were within the normal reference ranges. Her spectrum of antinuclear antibodies (ANAs), anti-cyclic citrullinated peptide (anti-CCP), anti-streptolysin O (ASO) and rheumatoid factor were negative.

The biochemical measurements of her daughter, brother and sister were normal except that her daughter had mild anemia (partial data not shown). All laboratory findings are summarized in **Table 1**.

Imaging examinations

The bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (iDXA, GE Lunar, USA). The results showed that the hips and lumbar spines of the patient and her daughter significantly increased. The T scores of the patient were higher than those of Chinese female youth, and the Z scores of her daughter were also higher than those of the age-matched Chinese women (**Table 1**). The results of the BMD tests for her brother and sister revealed low bone mass (osteopenia) compared with the Chinese sex-matched adolescents (data not shown).

X-ray images of the limbs, including the wrist and ankle joints of the patient, showed increased bone density in the pelvis, femurs, humerus, knees and shoulder joints; mild degeneration in the hips, knees, ankles, shoulders, elbows and wrist joints; and slight soft tissue swelling around the wrist joints (**Figure 2 A B C**). The chest computerized tomography (CT) scan of the patient showed that bone mineral density increased in the bilateral humeral head, sternum, scapula, ribs and multiple thoracic vertebrae (**Figure 2 D**). Whole-body bone single-photon emission computed tomography (SPECT) of the patient revealed extremely high uptake in the long bone, ribs, and spine with no renal or bladder radioactivity visualization. The features from imaging showed a “bone super scan” (**Figure 2 E**). The results of the ultrasound examination of the patient showed synovitis in both the first metatarsophalangeal joints and the wrist joint, tenosynovitis

of the fourth compartment of the left wrist, and joint effusion on the right ankle (data not shown).

X-ray images of the limbs of the patient's daughter showed that the density of the bone tip and flat bone increased, and the marrow cavity became narrow (**Figure 2 F G H**). The CT scan of the lumbar spine of the patient's daughter showed that bone mineral density increased at the upper and lower edges from the twelfth thoracic vertebra (T12) to the first sacral vertebra (S1) and slightly decreased in their center (**Figure 2 I**).

FURTHER DIAGNOSTIC WORK-UP

Pathological study

A bone biopsy of the patient on the right posterior iliac crest showed cortical bone sclerosis, some thickened bone trabeculae, and active proliferation of bone marrow hematopoietic cells (**Supplementary Figure 1**). Immunohistochemical staining of the bone marrow biopsy showed a few CD20(+) or CD3(+) lymphocytes and scattered CD138(+), IGκ (+) or IGλ (+) plasma cells (data not shown).

Genetic analysis

Whole exome sequencing (¹whole-exome library construction by xGen Exome Research Panel v2.0 (IDT, Iowa, USA), high-throughput sequencing by a DNBSEQ-T7 sequencer (MGI, Beijing, CHN), and not less than 99% of target sequence were sequenced) identified two heterozygous mutations, including c.857G>A (p. Arg286Gln, rs760956030) in exon 10 of the *CLCN7* gene (NCBI reference sequence: NM_001287) and c.714-20G>A (-, rs200087340) in intron 7 of the *TCIRG1* gene (NCBI reference sequence: NM_006019.4). Her daughter carried the same heterozygous mutation in the *CLCN7* and *TCIRG1* genes by genetic analysis (**Figure 3**).

Prediction of functional effects of the CLCN7 and TCIRG1 gene mutations

The R286 position was highly conserved among various species in the *CLCN7* gene (**Supplementary Figure 2**). Moreover, Polymorphism Phenotyping v2 (PolyPhen-2) (<http://genetics.bwh.harvard.edu/pph>) prediction results of p. R286Q in the *CLCN7* gene was probably damaging, with a score of 1.000 (**Supplementary Figure 3**), and

Mutation Taster (<https://www.mutationtaster.org/>) predicted it to be a disease-causing variant. Mutation Taster predicted c.714-20(IVS7) G>A in the *TCIRG1* gene as no change in potential splicing sites.

FINAL DIAGNOSIS

Combined with the patient's medical history and radiological examination results, the final diagnosis was osteopetrosis. In light of genetic typing, the case belonged to ADO II.

TREATMENT

During hospitalization, celecoxib capsules, wet packing of Liuhedan (a traditional Chinese medicine recipe), ketoprofen gel, and flurbiprofen paste were all given. Her hemoglobin level increased from 99 g/L to 109 g/L with normal serum phosphorus after 16 days of treatment. Her regular activities were unaffected, and the discomfort and swelling in her joints subsided after she was discharged.

OUTCOME AND FOLLOW-UP

After 2 years of follow-up, her joints were no longer painful, and no complications developed. However, her daughter experienced mild lumbar spine discomfort and received analgesic treatment.

DISCUSSION

Osteopetrosis is a rare inherited metabolic bone disorder characterized by a generalized increase in bone density due to osteoclastic insufficiency, impaired bone absorption and poor bone remodeling^[4]. Once osteoclasts have defective proton pumps, chloride channels, or carbonic anhydrase II proteins, the mineral matrix is unable to be resorbed effectively^[6]. Published studies have shown that most defects in genes result in impaired acidification of bone^[7].

With a prevalence of approximately 1:20,000 Live births, ADO is far more common and less severe than ARO^[8, 9]. The clinical phenotype of osteopetrosis is highly variable, making diagnosis difficult for clinicians. Patients with osteopetrosis may present with no symptoms, fractures following minor trauma, osteomyelitis, early arthritis, anemia, hearing and vision problems due to cranial nerve compression, or all of the above^[4]. Some differential diagnoses should be ruled out, such as congenital diseases (e.g., hypoparathyroidism, pseudohypoparathyroidism), chemical poisoning (e.g., with fluoride, lead, or beryllium), and malignancies (leukemias and myeloproliferative diseases). This patient had normal urinary fluoride and PTH serum levels without arthritis, and she was eventually diagnosed with ADO-II. Her daughter was asymptomatic, and ADO-II was confirmed by increased BMD, radiological examination, and the p. R286Q mutation. Their anemia was mild due to relatively sufficient marrow cavity retention for normal hematopoiesis^[10]. It is worth noting that, in contrast to ARO, this patient had a mildly decreased BALP concentration. 5 The majority of ADO-II patients have an imbalance in osteoblast serum markers, with low BALP and high osteocalcin levels^[11]. As a result, bone biomarkers may be useful in disease classification.

Increased BMD and radiographic findings are most commonly used to diagnose osteopetrosis. The classic radiographic features of osteopetrosis are the bare minimum for diagnosis^[7]. Radiographic features of osteopetrosis include diffuse bone sclerosis with "bone-within-bone" in the pelvis, long bones, phalanges, and vertebrae. A bone marrow biopsy may be required to confirm the diagnosis and differentiate between osteoclast-poor and osteoclast-rich subtypes of osteopetrosis. Bone marrow biopsy can also distinguish hematological disorders such as myelofibrosis, sickle cell disease, leukemia, and osteoblastic bony metastases. It is, however, more invasive to the patient and carries some risks. Whole exome sequencing, on the other hand, is becoming less expensive and faster to obtain a diagnosis and is not invasive. Therefore, therapeutic approaches must be tailored to each patient.

Approximately 80% of ADO-II patients are affected by heterozygous dominant negative mutations of the *CLCN7* gene, while 17% of ARO patients have recessive mutations in the *CLCN7* gene^[5, 12]. *CLCN7* encodes the chloride channel involved in osteoclast HCl secretion, which is critical in osteoclast dissolution of bone mineral and organic bone matrix^[13, 14]. A single nucleotide change (c.857G>A, p. R286Q) in exon 10 of *CLCN7* results in a protein with the amino acid glutamine instead of arginine. This variant appears to be located at one of the “hot spots” as the most common *CLCN7* mutations causing ADO and three known disease-related *CLCN7* mutations at the R286 position (p. R286P, p. R286W and p. R286Q) have previously been reported among Caucasians and Asians^[15-17]. The mutations (p. R286Q) in the *CLCN7* gene are located in the intramembrane α -helices, creating a positive electrical potential to prevent the fast flux of chloride at the binding site^[18]. Approximately 80% of *CLCN7*-dependent ADO-II patients discovered the disease after fractures, implying that osteoblast malfunction likely results in low-quality bone tissue^[5, 11]. However, no fractures have occurred in this patient thus far. As a result, even if the mutations are identical, the clinical phenotypes may differ.

Osteopetrosis may also be caused by an intronic nucleotide change in the *TCIRG1* gene^[19]. *TCIRG1* encodes the $\alpha 3$ subunit of H⁺ ATPase, and V-ATPase with $\alpha 2/\alpha 3$ is a major proton pump of osteoclasts^[20]. Mutations in *TCIRG1* account for approximately 50% of ARO cases^[5]. The *TCIRG1* variant (c.714-20 G>A) is a point mutation, but there is insufficient evidence to conclude that it is pathogenic. Furthermore, published reports of digenic inheritance suggested that *TCIRG1* and *CLCN7* interact in the two mutations^[15, 21].

The majority of benign ADO treatments are symptomatic and supportive. Good nutrition is critical for patients with osteopetrosis, especially for those who have hypocalcemia and require calcium and vitamin D supplements^[7]. Hematopoietic stem cell transplantation is reserved for osteopetrosis that is malignant^[4]. The majority of ADO patients have a better prognosis. Nonetheless, for mild osteopetrosis, it is critical to monitor the disease status and progression by the affected organs.

CONCLUSION

A case of rare ADO-II was accidentally diagnosed in a late-onset patient and her daughter based on increased BMD, classic radiographic features, and a *CLCN7* gene mutation. It was suggested that genetic testing be used to identify precision classifications of osteopetrosis and to provide useful information for therapeutic decisions and prognosis.

ACKNOWLEDGEMENTS

We thank the patient and her relatives for kindly contributing to this study.

8%

SIMILARITY INDEX

PRIMARY SOURCES

- | | | |
|---|---|---------------|
| 1 | www.ncbi.nlm.nih.gov
Internet | 47 words — 2% |
| 2 | www.spandidos-publications.com
Internet | 33 words — 1% |
| 3 | e-sciencecentral.org
Internet | 31 words — 1% |
| 4 | f6publishing.blob.core.windows.net
Internet | 28 words — 1% |
| 5 | A Del Fattore. "Clinical, genetic, and cellular analysis of 49 osteopetrotic patients: implications for diagnosis and treatment", Journal of Medical Genetics, 9/9/2005
Crossref | 17 words — 1% |
| 6 | Ling-Ling Yan, Xiu-Xiu Jin, Xiao-Dan Yan, Jin-Bang Peng, Zhuo-Ya Li, Bi-Li He. "Combined use of extracorporeal membrane oxygenation with interventional surgery for acute pancreatitis with pulmonary embolism: A case report", World Journal of Clinical Cases, 2022
Crossref | 16 words — 1% |
| 7 | ejcrim.com
Internet | 15 words — 1% |
| 8 | link.springer.com
Internet | |

12 words — < 1%

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY OFF

EXCLUDE SOURCES < 12 WORDS
EXCLUDE MATCHES < 12 WORDS