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BRIEF COMMUNICATION

A novel frameshift deletion in *PLS3* causing severe primary osteoporosis

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Abstract

Mutations in the gene encoding plastin-3, *PLS3*, have recently been associated to severe primary osteoporosis. The molecular function of plastin-3 is not fully understood. Since *PLS3* is located on the X chromosome, males are usually more severely affected than females. *PLS3* mutations have thus far been reported in approximately 20 young patients with low bone mineral density (BMD). We describe an 8-year-old Greek boy with severe primary osteoporosis with multiple vertebral compression fractures and one low-energy long bone fracture. His clinical manifestations were consistent with osteogenesis imperfecta, including blue sclerae, joint hypermobility, low bone mineral density, kyphosis, bilateral conductive hearing loss, and mild dysmorphic features. The family history was negative for primary osteoporosis. *COL1A1* and *COL1A2* mutations were excluded by Sanger sequencing. However, Sanger sequencing of *PLS3* led to the identification of a de novo frameshift deletion, NM_005032: c.1096_1100delAACTT, p.(Asn366Serfs*5), in exon 10 confirming the diagnosis of PLS3 osteoporosis. In conclusion, we describe a novel frameshift deletion in *PLS3* causing severe primary osteoporosis in a boy. Our finding highlights the clinical overlap between type I collagen and PLS3-related skeletal fragility and underscores the importance of *PLS3* screening in patients with multiple fractures to enable proper genetic counseling.

Introduction

Primary osteoporosis is characterized by low bone mineral density (BMD) and recurrent low-energy peripheral and vertebral fractures. Primary osteoporosis is often classified as osteogenesis imperfecta (OI). Although brittle bones are the main hallmark, extraskeletal manifestations including blue sclerae, dentinogenesis imperfecta and hearing loss are also common in OI. The genetic and phenotypic variability is great and mutations in around 20 genes have been discovered to cause mild to progressively deforming OI so far. An X-linked form of primary osteoporosis with no extraskeletal manifestations has recently been assigned to mutations in *PLS3*, encoding plastin-3 [1–3]. To date, less than 20 point mutations and small to large deletions have been identified in *PLS3* in patients with early-onset primary osteoporosis but the full clinical and genetic spectrum remains inadequately described [1, 4–8]. Due to the X-chromosomal location of *PLS3*, males are usually more severely affected than females. The cellular functions of plastin-3 are not well known. Plastin-3, which contains 2 calcium-binding EF-hand motifs and 2 actin-binding domains, is an actin-binding and bundling protein that is important for the neuromuscular junction and acts as a protective modifier in spinal muscular atrophy (SMA) [9–11]. Furthermore, plastin-3 also plays a role in bone mineralization [2, 4, 5, 7], but the pathogenic mechanisms

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underlying PLS3 osteoporosis are not completely understood.

Here we describe a novel de novo frameshift deletion in *PLS3* in a Greek boy who presented with significant skeletal fragility but also with similar extra-skeletal manifestations to OI patients with *COL1A1* and *COL1A2* mutations.

Materials and methods

We recruited a Greek 8-year-old boy with primary osteoporosis. Before inclusion in our study, a written informed consent was signed by his parents. Blood sample collection from the proband and his parents as well as genetic investigations were performed according to our ethically approved protocol. Genomic DNA extraction and Sanger sequencing were performed according to standard procedures. Clinical data were collected retrospectively from hospital records.

Case report

In this study we report on an 8-year-old boy with primary osteoporosis. He had sustained a low-energy fracture of his left femur at the age of 2.5 years, after a fall from standing height. Kyphosis was noted clinically soon thereafter; subsequently, a lateral spine X-ray revealed fractures of thoracic vertebrae (T5, T6, and T8), confirmed with spine MRI (Fig. 1a–c). A full diagnostic work up for osteoporosis etiology was normal. Inborn errors of metabolism, hematological and endocrine disorders were excluded. Abdominal ultrasound, audiogram and cardiac assessments were all normal.

Antenatal and perinatal histories were unremarkable. He had surgery for pyloric stenosis and right-sided cryptorchidism in his infancy. There were no dental issues. His calcium intake was adequate and his physical activity was normal.

His parents were non-consanguineous and there was no family history for early-onset osteoporosis. Of note, his father had been operated for aortic aneurysm and his maternal aunt had a history of developmental dysplasia of the hips.

Further investigations were carried out at the age of 6 years. On examination, he had normal growth (height 25th centile, weight 50th centile), blue sclerae, joint hypermobility, and kyphosis. Also seen were subtle, but distinct, dysmorphic features featuring prominent epicanthic folds, narrow external ear canals, mild micrognathia, and a high-arched palate (Fig. 1).

His laboratory parameters for calcium homeostasis and metabolic bone markers showed mild hypercalciuria and slightly increased uDPD/uCreat (47 nmol/mmol; reference range = 10–35 nmol/mmol).

On DXA scan (GE Lunar Prodigy encore, Pediatric software) he had low lumbar spine (LS) BMD (L2–L4 *Z*-score −3.5) and low total body less head (TBLH) bone mineral content (*Z*-score −2.2), but normal height-adjusted lean body mass for age, compatible with a primary bone disorder.

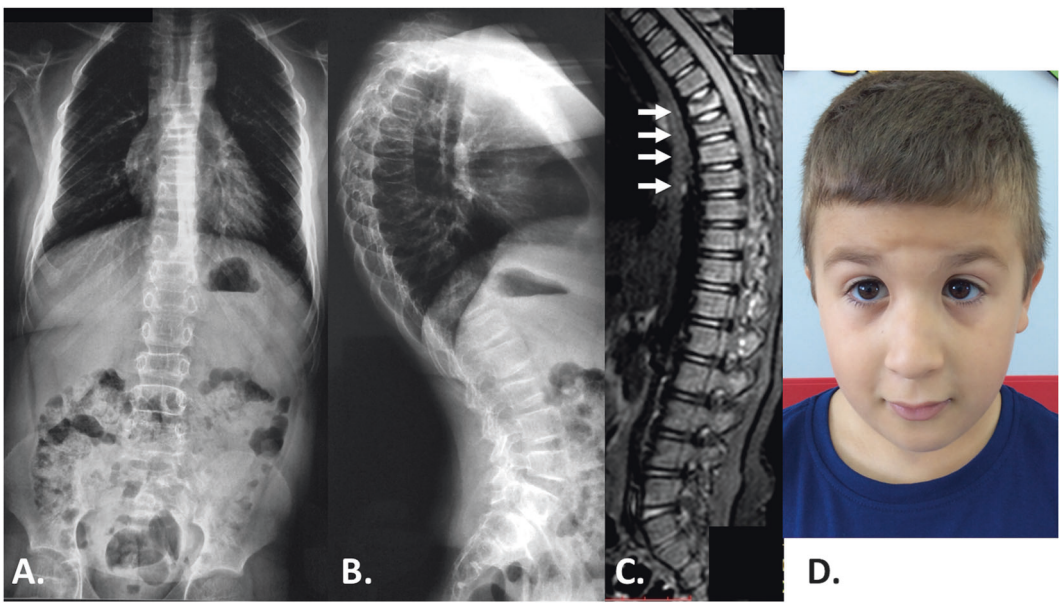

Fig. 1 Multiple fractures of the thoracic vertebrae and diffuse osteopenia on the plain X-rays of the spine (**a**, **b**), confirmed by MRI (white arrows) (**c**). Photo of the index patient: blue sclerae, hypertelorism and prominent epicanthic folds (**d**)

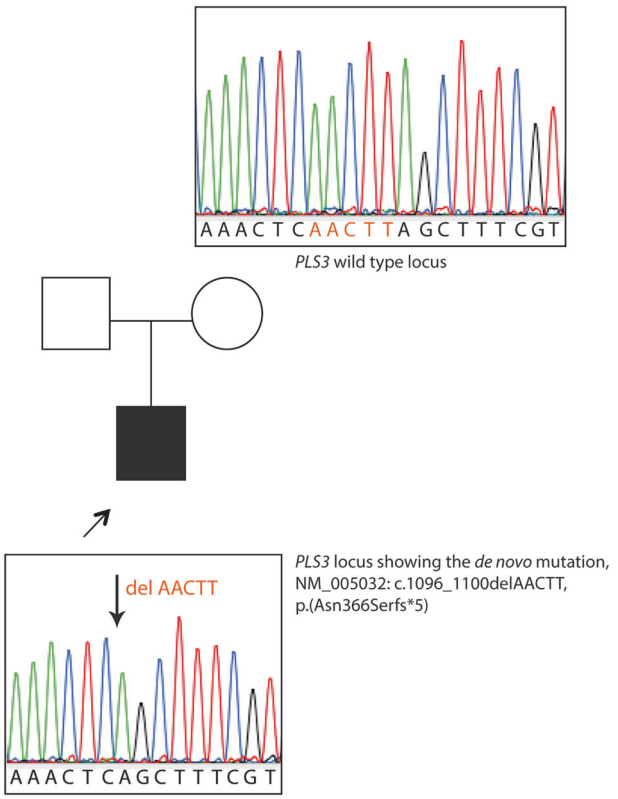

Fig. 2 Pedigree of the family and Sanger electropherograms showing the de novo frameshift *PLS3* deletion, c.1096_1100delAACTT, in the index patient. The deleted DNA sequence is labeled with orange color. His mother has instead a wild type sequence

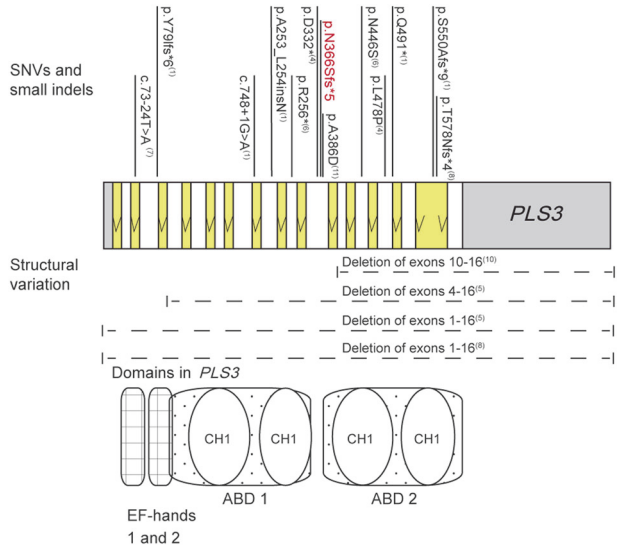

Fig. 3 Schematic representation of both the PLS3 gene and protein showing all the mutations causing osteoporosis that have been identified so far. The mutation here reported is marked in red color

A clinical diagnosis of OI was suspected and sequencing of *COL1A1* and *COL1A2* was undertaken. No mutation was found. He was started on oral alendronate, at 35 mg/week and 400 IU/d cholecalciferol; IV zoledronic acid was considered but could not be initiated because of a long distance to the hospital.

Since the phenotype of our patient resembled that of children with *PLS3* osteoporosis, we proceeded by *PLS3* sequencing. A novel frameshift deletion, NM_005032: c.1096_1100delAACTT, in exon 10 was subsequently identified (Fig. 2). The mutation leads to frameshift in the coding sequence and early termination of amino acid sequence p.(Asn366Serfs*5) (Fig. 3). It was absent in the mother and thus determined as a de novo change (Fig. 2). This variant is classified as pathogenic according to the ACMG guidelines [12].

After one year of alendronate treatment, he was reviewed at age 7.5 years. His growth was normal (height 25th centile, weight 50th–75th centile) and no new fractures occurred, bone markers were appropriately suppressed and BMD was improved: LS BMD *Z*-score −2.3 and TBLH Z-score −0.8. Kyphosis improved both clinically and radiologically (Fig. 1). A significant concern presently is his bilateral conductive hearing loss and very narrow ear canals.

Discussion

Here we describe a young boy with early-onset primary osteoporosis caused by a novel hemizygous *PLS3* deletion c.1096_1100delAACTT, leading to a frameshift and an early stop codon in the mRNA (p.Asn366Serfs*5). The aberrant transcript is likely to be degraded by nonsense-mediated mRNA decay. Since the patient is male and lacks a normal *PLS3* allele, plastin-3 would be completely absent and this would explain his severe phenotype. Alternatively, the deletion may lead to a truncated and most likely non-functional protein. Unfortunately, no additional patient samples were available for further studies of the mutation. Our finding expands the genetic spectrum of PLS3 osteoporosis (Fig. 3).

Interestingly, our patient presented with skeletal and extra-skeletal features that are typical for *COL1A1* and *COL1A2* related OI, including low BMD, recurrent fractures, blue sclerae as well as hearing loss. Blue sclerae and hearing loss are not common features in PLS3 osteoporosis [1, 4–7, 13–15]. Although OI-related hearing loss usually appears in adulthood due to otosclerosis [16], hearing deficit in our patient is likely due to his abnormal ear anatomy. Furthermore, our patient also presents with dysmorphic facial features, which have been reported in PLS3 osteoporosis once before [5], suggesting that this may be a part of the disease spectrum. These findings support previous speculations that *PLS3* is widely expressed in many tissues

and its total depletion would therefore not affect only bone [1, 4, 6, 13]. Similar to other patients with *PLS3* mutations [1, 6, 13], bisphosphonate treatment in our patient has increased BMD, improved spinal changes and prevented new fractures.

In summary, this is a case of PLS3 primary osteoporosis, which comprises some OI features, comorbidities and dysmorphic features not previously described in this context. Lateral thinking in atypical cases like this is required to detect rarities like PLS3 osteoporosis and to enable appropriate genetic counseling. Detailed, regular follow-up is imperative, in an effort to record special features of this particular group of patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Van Dijk FS, Zillikens MC, Micha D, Riessland M, Marcelis CL, de Die-Smulders CE, et al. PLS3 mutations in X-linked osteoporosis with fractures. N Engl J Med. 2013;369:1529–36.
2. Kampe AJ, Makitie RE, Makitie O. New genetic forms of childhood-onset primary osteoporosis. Horm Res Paediatr. 2015;84:361–9.
3. Makitie RE, Kampe AJ, Taylan F, Makitie O. Recent discoveries in monogenic disorders of childhood bone fragility. Curr Osteoporos Rep. 2017;15:303–10.
4. Fahiminiya S, Majewski J, Al-Jallad H, Moffatt P, Mort J, Glorieux FH, et al. Osteoporosis caused by mutations in PLS3: clinical and bone tissue characteristics. J Bone Mineral Res. 2014;29:1805–14.
5. Kampe AJ, Costantini A, Levy-Shraga Y, Zeitlin L, Roschger P, Taylan F et al. PLS3 deletions lead to severe spinal osteoporosis and disturbed bone matrix mineralization. J Bone Miner Res. 2017;32:2394–2404.
6. Kampe, AJ, Costantini, A, Makitie, RE, Jantti, N, Valta, H, Mayranpaa, M et al. PLS3 sequencing in childhood-onset primary osteoporosis identifies two novel disease-causing variants. Osteoporosis Int. 2017;28:3023–3032.
7. Laine CM, Wessman M, Toiviainen-Salo S, Kaunisto MA, Mayranpaa MK, Laine T, et al. A novel splice mutation in PLS3 causes X-linked early onset low-turnover osteoporosis. J Bone Mineral Res. 2015;30:510–8.
8. Kannu P, Mahjoub A, Babul-Hirji R, Carter MT, Harrington J. PLS3 mutations in X-linked osteoporosis: clinical and bone characteristics of two novel mutations. Horm Res Paediatr. 2017;88:298–304.
9. Oprea GE, Kroeber S, McWhorter ML, Rossoll W, Mueller S, Krawczak M, et al. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. Science. 2008;320:524–7.
10. Heesen L, Peitz M, Torres-Benito L, Holker I, Hupperich K, Dobrindt K, et al. Plastin 3 is upregulated in iPSC-derived motoneurons from asymptomatic SMN1-deleted individuals. Cell Mol Life Sci. 2016;73:2089–104.
11. Lyon AN, Pineda RH, Hao LT, Kudryashova E, Kudryashov DS, Beattie CE. Calcium binding is essential for plastin 3 function in Smn-deficient motoneurons. Hum Mol Genet. 2014;23:1990–2004.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
13. Lv F, Ma M, Liu W, Xu X, Song Y, Li L, et al. A novel large fragment deletion in PLS3 causes rare X-linked early-onset osteoporosis and response to zoledronic acid. Osteoporos Int. 2017;28:2691–2700.
14. Nishi E, Masuda K, Arakawa M, Kawame H, Kosho T, Kitahara M, et al. Exome sequencing-based identification of mutations in non-syndromic genes among individuals with apparently syndromic features. Am J Med Genet A. 2016;170:2889–94.
15. van de Laarschot DM, Zillikens MC. Atypical femur fracture in an adolescent boy treated with bisphosphonates for X-linked osteoporosis based on PLS3 mutation. Bone. 2016;91:148–51.
16. Nager GT. Osteogenesis imperfecta of the temporal bone and its relation to otosclerosis. Ann Otol Rhinol Laryngol. 1988;97:585–93.