Case report

Bulbous epiphysis and popcorn calcification as related to growth plate differentiation in osteogenesis imperfecta

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Summary

Background. Osteogenesis Imperfecta (OI) is an heritable systemic disorder of connective tissue due to different sequence variants in genes affecting both the synthesis of type I collagen and osteoblast function. Dominant and recessive inheritance is recognized. Approximately 90% of the OI cases are due to mutations in COL1A1/A2 genes. We clinically and radiologically describes an adult male with type III osteogenesis imperfecta who presents a rare bone dysplasia termed bulbous epiphyseal deformity in association with popcorn calcifications. Popcorn calcifications may occur with bulbous epiphyseal deformity or independently.

Methods. Molecular analysis was performed for COL1A1, COL1A2, LEPRE1 and WNT1 genes.

Results. An uncommon COL1A1 mutation was identified. Clinical and radiological exams confirmed a distinctive bulbous epiphyseal deformity with popcorn calcifications in distal femurs. We have identified four additional OI patients reported in current literature, whose X-rays show bulbous epiphyseal deformity related to mutations in CR-TAP, LEPRE1 and WNT1 genes.

Conclusion. The mutation identified here had been previously described twice in OI patients and no previous correlation with bulbous epiphyseal deformity was described. The occurrence of this bone dysplasia focuses attention on alterations in normal growth plate differentiation and the subsequent effect on endochondral bone formation in OI.

KEY WORDS: osteogenesis imperfecta; growth plate; chondrocyte; endochondral bone formation.

Background

Osteogenesis imperfecta (OI) is an heterogeneous group of disorders characterized by reduced bone mass, bone fragility and susceptibility to fracture (1). In the Online Mendelian Inheritance in Man database (OMIM), OI is classified as types I to XVI according clinical and histological features and related gene mutations (2). Approximately 90% of OI patients have structural or quantitative abnormalities of type I collagen caused by mutations in COL1A1 or COL1A2 genes associated with autosomal dominant inheritance (1). Recently, several genes related to collagen processing in the endoplasmic reticulum or to osteoblast function have been defined as causative of OI, frequently associated with recessive patterns of inheritance (1).

Histomorphometric studies have reported structural and material abnormalities in OI bone including alterations of the growth plate (3, 4). Disruption of the endochondral epiphysis with irregular and disorganized nests of chondrocytes causing incomplete replacement or fragmentation of growth plate cartilage are described as “popcorn calcification” (POC) (5-7). POC is not observed at birth and occurs predominantly in long bones of lower extremities, most frequently in the femur distal epiphysis (8). Bulbous epiphyseal deformity of the distal femur has been noted in a few OI patients but the significance of the lesion with regard to the pathophysiology of the growth plate in OI has not been addressed.

This report describes distinctive changes in the distal femur consisting of popcorn calcification associated with bulbous epiphyseal deformity in a patient with type III OI. This report addresses the putative relationship of these to altered growth plate function in individuals with mutations that alter the normal progression of growth plate differentiation to endochondral bone formation.

Methods

This report is in accordance with requirements of the Johns Hopkins Institutional Review Board. The clinical evaluation was performed at the Kennedy Krieger Institute; radiographic images and biochemical testing were obtained at Johns Hopkins Hospital (Baltimore, MD, USA). Data from existing medical records and radiographic images were assessed. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA; Horizon A, S/N 100164; Hologic Corp, Bedford, MA). The OI classification was based on the clinical criteria established by Silence (5).

Analysis of sequence variants was performed at Collagen Diagnosis Laboratory, University of Washington (Seattle, WA, USA). DNA was extracted by standard procedures. For the
sequence analysis of the type I collagen genes (COL1A1 and COL1A2), the coding exons and flanking intron sequences were amplified by PCR using 17 primer pairs for the COL1A1 gene and 23 primer pairs for the COL1A2 gene. The primer sequences were based on the 2006 freeze of the Human Genome sequence. The amplified fragments of the COL1A1 and COL1A2 genes were sequenced by automated sequencing in 26 and 35 reactions, respectively (total of 61 reactions). The mutations were confirmed by sequencing of a new amplification product in both directions. Sequence data were analyzed by the most recent version of the Mutation Surveyor Software (SoftGenetics, State College, PA, USA). DNA analysis was also performed for LEPRE1 and WNT1 genes with the finding of normal sequence.

Results
A 29-year-old man of Asian ethnicity presented with osteogenesis imperfecta type III. He was non-ambulatory using a wheelchair however; he lives independently and is actively employed. Born with multiple fractures, he has experienced several fractures including in the last 5 years fractures of both arms, skull, right tibia, and right femur. The patient has never received bone active medications and had not had previous orthopedic surgery. His family included his parents and two healthy siblings currently living in California (USA) who have not been examined and have provided no specimens for testing. There is no familial history of OI. He displayed triangular facies with a narrow mandible, white sclerae, dentinogenesis imperfecta and short stature. His height was approximately 40 inches. There was a marked scoliosis with inequality of the two sides of the chest and extreme asymmetrical anterior protrusion of the sternum. Deformities due previous fractures were present in his upper and lower extremities. The lower extremities were more severely affected and were kept folded as he sat showing significant muscle wasting with very restricted motion except in both ankles, where motion was preserved. An additional feature included palpable enlargement of both distal femurs. The medical history included hypertension controlled by medications. Clinical laboratory results were: 25(OH) vitamin D 17 ng/mL (30-100), serum calcium 9.3 mg/dL (8.5-10.2), serum phosphorus 3.0 mg/dL (2.7-4.5), total alkaline phosphatase 68 U/L (38-126), bone-specific alkaline phosphatase 19.5 mcg/L (8.4-19.3), osteocalcin 28 ng/mL (9-38), and C-telopeptide 381 pg/mL (16-584). Bone densitometry for sites left distal femur, 1/3 right radius and whole body showed marked osteoporosis with right radius 1/3 BMD: 0.606 g/cm² Z-score -3.9 and whole body BMD: 0.640 g/cm² Z-score -7.3 (9). Radiographic study demonstrated severe skeletal deformities consistent with OI (Figure 1). Figures 2 A, B and C illustrated the unusually prominent bulbous circular enlargement of both distal femurs encircling multiple “popcorn calcifications”. Similar but less pronounced changes were seen in the proximal tibias. A chronic nonunion fracture was present in the proximal right femur diaphysis with a more acute fracture at the right mid diaphysis. Gene sequencing of COL1A1 and COL1A2 identified an heterozygous missense mutation (c.1111G>A; p.Gly371Ser, Gly1935Ser) of COL1A1 gene in exon 17 and an heterozygous variant of unknown significance (c.671G>A) in exon 14 of the COL1A2 gene.

Discussion
This patient with severe OI presents distinctive alterations in the distal femur and proximal tibia consisting of bulbous epiphyseal deformity with popcorn calcifications. In a recent review of the literature we recognized similar deformity in X-ray illustrations from four patients with severe and recessively inherited OI expressing different gene variants related to both type I collagen processing and osteoblast function (10-13). We suggest that the significance of this unusual lesion is that it focuses attention on the relationship of these gene variants to disruption of the epiphyseal growth plate and the subsequent longitudinal effect on diaphyseal bone formation.

To date, bulbous epiphysis is reported in a patient with type III OI and in few cases with recessive inheritance due to its limited recognition. POC is related to severe OI types, most often OI type III, but it is also described in patients with OI types IV, VII, VIII and XV (7, 8, 10, 11, 13). POC may occur with either autosomal dominant and recessive inheritance (8, 10). Bulbous epiphyseal deformity and POC may coexist at the distal epiphysis; however, independent occurrence of each lesion has also been observed suggesting that the mechanisms underlying the development or progression of each lesion may be different (7, 8).

Longitudinal bone growth occurs at the epiphyseal growth plate through the process of endochondral ossification (14, 15). Growth plate chondrocytes at different stages of differentiation secrete a cartilage matrix rich in types II, IX and XI collagens and type I collagen secretion is suppressed at this stage (14, 16). An orderly progression of chondro-osseous differentiation occurs from the epiphysis to the mid-shaft of the bone that includes stages of chondrocyte proliferation and hypertrophy with differentiation to osteoblasts leading to endochondral bone formation (14, 16).
The process of endochondral bone formation begins with the condensation of mesenchymal stem cells, which then differentiate into chondrocytes (17). In postnatal long bones, primary and secondary ossification centers form but remain separated by the growth plate cartilage. This structure is composed of three layers, the resting, proliferative and hypertrophic zones. The resting zone contains progenitor chondrocytes (18). The proliferative zone contains columnar clones of chondrocytes that undergo rapid proliferation. In the hypertrophic zone, the chondrocytes cease proliferating and undergo hypertrophy, increasing particularly in their height. These processes result in chondrogenesis. The resulting new cartilage is remodeled at its metaphyseal surface into bone (18).

Key factors in chondrocyte differentiation are Indian hedgehog (IHH), parathyroid hormone-related peptide (PTHrP), bone morphogenetic proteins (BMPs) and components of the Wnt signaling pathway (15, 17, 19). IHH, which expressed in pre-hypertrophic chondrocytes, regulates the onset and progression of hypertrophic differentiation by forming a negative feedback loop with PTHrP. In embryonic long bones, PTHrP is synthesized in the perichondrial chondrocytes whereas in the postnatal growth plate the source of PTHrP shifts to the resting zone of the growth plate (20). PTHrP acts through PTH1R to inhibit chondrocyte hypertrophy (17, 19). Thus, IHH-dependent activation of PTHrP expression maintains a population of immature chondrocytes in the resting and columnar zones of the growth plate (21). Wnt/beta-catenin signaling regulates initiation of chondrocyte hypertrophy by inhibiting PTHrP signaling activity. In addition, Wnt/beta-catenin signaling regulates chondrocyte hypertrophy in a non-cell autonomous manner. Wnt/beta-catenin signaling also controls final maturation of hypertrophic chondrocytes independent of PTHrP signaling (17, 21).

Also interacting in this process are growth hormone, thyroid hormone, sex steroids, growth factors, fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), the transforming growth factor-β (TGF β) superfamily and, transcription factors including Sox9, Runx2 and FoxA2 (15, 19, 22). Specific MicroRNAs (miRNAs) have also been described as mediators in the regulation of chondrocyte differentiation (23). Deficiency of vitamin D and mechanical trauma are also associated with dis-
rupture of the growth plate (24, 25). In addition, chronic malnutrition directly affects growth plate chondrocytes by decreasing sensitivity to GH (22).

To our knowledge, chondrocyte differentiation is not described in bone biopsies reported from patients with OI. The oim/oim mouse model of OI simulates moderate to severe OI due to a mutation in COL1A2 resulting in the synthesis of collagen type I pro-c1(I) homotrimer (26). Evans et al. observed that growth plate areas were approximately 30% greater in the oim/oim mouse compared to the oim/wt animals (27). Chondrocyte development in the larger growth plates differed in the oim/oim mouse compared to oim/wt mice or wild type mice, the oim/oim animals showing a marked increase in the apparent number of hypertrophic cells within the growth plate (27). Additionally, oim/oim hypertrophic cell nuclei remained intact at the chondro-osseous junction while wt/wt and oim/wt animals displayed homogeneous vacant hypertrophic cell lacunae at the junction (27). A case histologically analyzed by Bullough showed “polared maturation of the chondrocytes with columnation and irregular direction of maturation relative to the long axis of the bone” (7). These results suggest that chondrocyte differentiation may be altered at an early stage in the presence of a defect in COL1A1 synthesis as in this patient, although type I collagen synthesis occurs at a later stage in growth plate differentiation.

We have identified four reports with illustrations showing characteristic bulbous epiphysis associated with POC, one case caused by CRTAP mutation, one by an LEPR1 mutation and two related to two distinct WNT1 mutations (10-13). The COL1A1 c.11111G>A mutation identified here was previously described in two cases which also were compatible with type III phenotype (28, 29). In the Zhang study, the individual with this same mutation did not have POC or bulbous epiphysis and in Marini study bulbous deformity was not reported (28, 29). In addition, the variant of unknown significance here in the COL1A2 gene results in substitution of arginine by histidine at position 134 of the pro-2(I) triple helical domain; the arginine at this position is conserved in all 100 vertebrate species surveyed so that its role in this patient is not defined.

Morello et al. described two patients with POC, one having a homozygous missense mutation in CRTAP and the second with an homozygous null mutation in the prolyl 3-hydroxylation complex (P3H1) (30). This abnormality may represent a cartilaginous dysplasia at the developing growth plate associated to the primary bone defects, since was described a lack of prolyl 3-hydroxylation at the developing growth plate associated to complex (P3H1) (30). This abnormality may represent a cartilage with an homozygous null mutation in the prolyl 3-hydroxylation

Conclusion

In conclusion, this is the first case describing an individual with severe OI and a COL1A1 mutation associated with the formation of bulbous epiphysis deformity. This report and the other studies involving this lesion in CRTAP, LEPR1 and WNT1 genes indicate the need for further investigation into the role each of them plays in growth plate maturation and endochondral bone formation.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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