Clinical Vignette

Low Bone Mass and High Material Bone Density in Two Patients with Loeys-Dietz Syndrome Caused by Transforming Growth Factor Receptor 2 Mutations

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Running title: TGFBR2 Mutations and Material Bone Density

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Abstract

Loeys-Dietz syndrome (LDS) is a rare autosomal-dominant connective tissue disorder caused by heterozygous mutations in the genes encoding transforming growth factor beta receptor 1 or 2 (*TGFBR1* or *TGFBR2*). Although an association between LDS and osteoporosis has been reported, the skeletal phenotype regarding bone mass is not well characterized. Here we report on two LDS patients with mutations in *TGFBR2*. Patient 1 was a 24 year old man who had a total of three fractures involving the left radius, the left metacarpal and the right femur. At the age of 14 years, lumbar spine areal bone mineral density z-score was -4.0 and iliac bone histomorphometry showed elevated bone turnover (bone formation rate per bone surface: 91 $\mu m^3/\mu m^2$/year; age-matched control values, 37 [10], mean [SD]) and mildly low trabecular bone volume per tissue volume (17.2%; age-matched control values: 25.7 [5.3]). Bone mineralization density distribution (BMDD) in trabecular bone was increased (Ca_{Peak}: 22.70 wt% Ca; age-matched control values, 21.66 [0.52]). Patient 2, a 17 year old girl, suffered from diffuse bone pain but had not sustained fractures. At 14 years of age, her lumbar spine areal bone mineral density z-score was -3.4. Iliac bone histomorphometry at that age confirmed low bone mass (bone volume to tissue volume 10.1%, same control values as above) and high bone turnover (bone formation rate per bone surface: 70 $\mu m^3/\mu m^2$/year). BMDD in trabecular bone was significantly shifted towards increased mineralization (Ca_{Peak}: 22.36 wt% Ca). Thus, it appears that LDS can be associated with low bone mass and high bone turnover but increased matrix mineralization of trabecular bone.

Key words:

Backscattered electron imaging; bone mineral density; Loeys Dietz syndrome; transforming growth factor beta receptor 2; transforming growth factor beta

Introduction

Loeys–Dietz syndrome (LDS, OMIM ID #609192) is an autosomal dominant disorder of connective tissue that is typically characterized by the triad of (1) hypertelorism, (2) cleft palate or bifid uvula, (3) arterial/aortic aneurysms and/or arterial tortuosity. LDS is caused by heterozygous mutations in the genes encoding transforming growth factor beta receptor 1 or 2 (*TGFBR1* or *TGFBR2*). Mutations in *TGFBR2* are also associated with a large variety of skeletal manifestations, including craniofacial abnormalities and malformations of the feet, thorax and spine (1, 2).

TGFBR1 and *TGFBR2* are ubiquitously expressed and play a key role in transforming growth factor (TGF) beta signalling. TGF beta binds to cell surface TGFBR2, which forms a heterotetrameric complex with TGFBR1 (3). TGFBR2 contains a kinase domain that phosphorylates and activates TGFBR1. The intracellular downstream targets of TGFBR1 then regulate the transcription of a large number of target genes.

More than 70 different *TGFBR2* mutations have been found in humans (4). The large majority of these are missense mutations that affect the kinase domain of TGFBR2. Such mutations decrease the expression and function of TGFBR2 protein and thereby are thought to decrease the activity of TGF beta signaling (5). However, in some tissues such mutations seem to be associated with paradoxically increased levels of intracellular downstream mediators of TGF beta signaling (6). These apparently contradictory observations have yet to be reconciled on the mechanistic level.

Animal experiments suggest that TGF beta signaling is an important determinant of bone mass, bone metabolism and the material characteristics of bone (7, 8). However, there is little information regarding these skeletal characteristics in patients with *TGFBR2* mutations, apart from a case report on two LDS patients with low-impact fractures in childhood (9). Here we report clinical, bone histomorphometric and bone material observations in two patients with LDS and low bone mass.

Methods

The two patients were followed in tertiary hospitals in Montreal for bone fragility. Clinical data were extracted by retrospective chart review.

Dual-energy X-ray absorptiometry was performed in the anterior–posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 2000W or 4500 device (Hologic Inc., Waltham, MA, USA). Lumbar spine areal bone mineral density (LS-aBMD) results were transformed to age-specific z-scores using reference data provided by the densitometer manufacturer. Metacarpal morphometry was performed on the second metacarpal and results were compared to reference data established by Garn et al (10).

Bone samples were obtained before the start of bisphosphonate treatment, at a site 2 cm posterior of the superior anterior iliac spine. Tetracycline double labeling was performed prior to biopsy. Sample preparation and histomorphometric analyses were performed using previously described procedures (11). Measurements were carried out using a digitizing table with Osteomeasure® software (Osteometrics Inc., Atlanta, GA, USA). Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research (12). Results were compared to the average value of the age-specific reference range using reference data established in our laboratory (11).

The bone mineralization density distribution (BMDD) in trabecular bone from these samples was analyzed by quantitative backscattered electron imaging (qBEI), as described elsewhere (13). Results were compared to the average values of a normative young reference data base established previously (14).

Clinical Vignette

Patient 1

Patient 1 is a 24-year-old man who was born at term with club feet and a left genu recurvatum. Physical examination at 8 years of age revealed hypertelorism, downslanting palpebral fissures with bilateral ptosis, retrognathia, a broad uvula and a high palate. There was pectus excavatum, hyperextensible joints (Beighton score 6/9) and a mild thoracic scoliosis at 16 degrees. At 11 years of age, frequent supraventricular premature beats and episodes of supraventricular tachycardia were noted on cardiac monitoring. Echocardiography showed an aortic root dilatation at 32 mm (z-score: +5.9) (15). No significant arterial tortuosity was found on chest computed tomography and magnetic resonance imaging at 24 years of age. Sequence analysis of *TGFBR2* revealed that the patient was heterozygous for a 1067G>C mutation in exon 4, resulting in a missense R356P change in the TGFBR2 kinase domain. This mutation had been previously reported in a Korean LDS patient (16).

Regarding skeletal findings, at 9 years of age LS-aBMD z-score was -2.1 (Figure 1) and bone age corresponded to chronological age. Three years later, the boy had a left distal radius fracture, followed by a left wrist fracture. At the age of 14 years, height was 168 cm (z-score: 0.3) and weight was 44 kg (z-score:-1.2, corresponding to 85% of the age-specific mean value in healthy boys). LS-aBMD z-score was low (-4.0), but lumbar bone size, as judged from the L1 to L4 bone surface area of the bone density scan, was normal (50.3 cm^2, corresponding to 87% of the age-specific mean value in healthy boys, thus corresponding to the analogously expressed result for body weight).

Histomorphometric analysis of a transiliac bone biopsy specimen revealed normal external bone size (i.e. normal core width), thin cortices, slightly low trabecular bone volume, elevated bone formation rate, and normal mineralization parameters (osteoid thickness and mineralization lag time) (Table 1; Figure 2). Bone resorption parameters (osteoclast surface per bone surface and

eroded surface per bone surface) were close to the mean value of the reference range. However, qBEI showed that the calcium content of trabecular bone matrix was elevated (Table 1, Figure 3). Following bone biopsy, cyclic intravenous pamidronate treatment was started at an annual dose of 9 mg per kg body weight (17). The only fracture during pamidronate therapy involved the 5th metatarsal of the left foot. Pamidronate was discontinued at 19 years of age, when LS-aBMD z-score was -1.1. At the age of 22 years a right femur fracture occurred. At the time of last follow up, the patient was 24 years old, with a height of 182 cm (z-score: 0.7), a weight of 65.9 kg (z-score:-0.3) and a LS-aBMD z-score of -1.0.

Patient 2

Patient 2 is a 17 year-old girl who was born with submucous cleft palate with bifid uvula, mitral valve prolapse, ventricular septal defect and camptodactyly. Her parents and her two sisters were healthy, and there was no known consanguinity. At the age of 11 years, aortic root dilatation of 40.4 mm (z-score: +10.4) was noted. Treatment with Losartan was started at the age of 12 years. Ophthalmic examination was normal and arterial magnetic resonance imaging did not find any arterial tortuosity. DNA sequence analysis of *TGFBR2* revealed a heterozygous missense mutation in exon 7, 1336G>A (D446N, kinase domain of TGFBR2), which had previously been reported in Loeys-Dietz syndrome (18).

At the age of 12 years, Patient 2 was first evaluated for bone pain in the absence of a history of fractures. Height was 134 cm (z-score: -2.3) and weight was 27 kg (z-score: -2.6). Physical examination revealed mild hypertelorism and evidence of repaired submucous cleft palate. LS-aBMD was 0.590 g/cm^2, corresponding to an age-matched z-score of -3.4 (Figure 1). Skeletal abnormalities included dolichostenomelia, arachnodactyly, bilateral camptodactyly of the fifth fingers, joint laxity, scoliosis and spondylolisthesis that was eventually surgically repaired two years later. At 14 years of age, LS-aBMD had decreased to 0.530 g/cm^2, corresponding to an age-matched z-score of -4.4. Lumbar bone area was not evaluated due to the presence of scoliosis

(Figure 4). In addition, acetabular protrusion was found. Bone age was delayed by 3 years compared to chronological age (Figure 4). Second metacarpal length z-score (-1.9) was similar to height z-score. Metacarpal outer diameter was normal (z-score of -0.4), whereas metacarpal cortical width was very low (z-score of -4.4).

A transiliac bone biopsy was performed at the age of 14 years. Histomorphometric analysis showed normal outer bone size, thin cortices, low trabecular bone volume, elevated bone formation rate and normal mineralization parameters (Table 1; Figure 2). Bone resorption parameters were below the mean of the reference range. However, calcium content of trabecular bone matrix was elevated (Table 1, Figure 3).

Because of bone pain and low LS-aBMD, treatment with cyclical intravenous infusions of zoledronic acid was started at a dose of 0.05 mg per kg body weight. Infusions were repeated every 6 months. LS-aBMD initially did not increase (Figure 1). After estrogen was given to treat central pubertal delay at 16 years of age, LS-aBMD started to increase (Figure 1).

Discussion

Here we report histomorphometric and qBEI observations in two patients with LDS caused by heterozygous missense mutations in *TGFBR2*. Both patients had low bone mass in the presence of normal outer bone size. Histomorphometric findings were similar between the two patients, with thin cortices, high bone turnover and absence of a mineralization defect. Quantitative BEI demonstrated that both patients had elevated bone matrix mineralization on the level of individual trabecula.

Skeletal fragility in LDS has not been characterized in any detail. One of the early reports on the disorder mentioned that 4 out of 40 LDS patients had 'osteoporosis and fractures at a young age', but gave no further details (1). A recent report described two LDS patients with *TGFBR2* missense mutations who had low aBMD and fractures (9). Bone histological features were

mentioned in one of these patients, but as the bone biopsy had been performed after several years of intravenous pamidronate treatment (which significantly changes the appearance of bone tissue), such results are difficult to interpret.

Our observation that bone turnover was elevated and bone mass was low in both patients seems to mirror findings in mouse models of increased TGF beta signaling. For example, overexpression of TGF beta in osteoblasts results in high bone turnover and low bone mass (19). Thus, the bone turnover and bone mass results are compatible with the hypothesis that the *TGFBR2* mutations in these patients were associated with increased TGF beta signaling.

However, this interpretation is in contrast to the findings of the qBEI analyses. Both our patients had elevated calcium content in trabecular bone matrix, while in mouse models with increased TGF beta signaling it is decreased (8). Thus, the results of the histomorphometric analyses and of the qBEI measurements in our patients point in opposite directions with regard to the underlying TGF beta signaling activity. Interestingly, a situation of high bone turnover and high bone matrix mineralization has been previously described in osteogenesis imperfecta (20). It is difficult to reconcile these contrasting results on the basis of the present observations. Detailed analyses in larger patient groups are required.

In our patients, bisphosphonate treatment seemed to be beneficial in one case but elicited a less obvious response in the second case, which was complicated by delayed central puberty. It is worth noting that Losartan treatment did not have a clear effect on LS-aBMD in Patient 2. In future, pharmacologic inhibition of TGFBR1 may become a possibility which in animal models had a combined anabolic and anticatabolic effect on bone (7). Such therapy might become a logical treatment approach in patients with LDS.

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Roles of the authors: IMBA drafted the original version of the manuscript; TE and GC contributed the history of Patient 2; FHG and MT contributed the history of Patient 1; PR and KK performed and interpreted qBEI analyses; FR conceptualized the project and supervised the writing of the report. All authors have read and approved of the final version of the manuscript.

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Figure Legend

Figure 1: Time course of LS-aBMD results in Patient 1 (A) and Patient 2 (B). The arrow in Patient 1 indicates the start of pamidronate treatment. The arrows in Patient 2 indicate the start of treatment with Losartan, followed by therapy with zoledronic acid and estrogen.

Figure 2: Transiliac bone biopsy specimen of a control subject (A, B, C), Patient 1 (D, E, F) and Patient 2 (G, H, I). All samples were obtained at 14 years of age. A, D, G: View of entire samples. Both patients had thinner cortices and lower trabecular bone volume than the control but outer bone size was similar. B, E, H: Goldner stained sections, showing bone structure of normal appearance and a normal amount of osteoid (magnification: 50x). C, F, I: Unstained sections seen under fluorescent light, demonstrating normal tetracycline label uptake (magnification 100x).

Figure 3: Bone mineralization density distribution (BMDD) in cancellous and cortical bone in Patient 1 (A) and Patient 2 (B) compared to age matched reference data in cancellous bone (Cn-Young) (14). In both patients the distribution curve is shifted to the right compared to control values.

Figure 4: Radiographs in Patient 2 at the age of 14 years: (A) Presence of scoliosis. (B) Anteroposterior view of the pelvis, showing acetabular protrusion and presence of internal fixation screws for spondylolisthesis repair. (C) Gand and wrist x-ray, showing delayed bone maturation.

Table 1. Histomorphometric and qBEI results in Patient 1 and 2 compared to age-matched control values (11, 20).

	Patient 1	**Patient 2**	**Controls**
Core width (mm)	5.4	6.2	8.6 (2.4)
Cortical width (µm)	617	296	1178 (349)
Bone volume per tissue volume (%)	17.2	10.1	25.7 (5.3)
Bone formation rate per bone surface ($\mu m^3 * \mu m^{-2} * y^{-1}$)	91.4	69.6	37 (10)
Mineralization lag time (d)	11.6	10	15.3 (3.6)
Osteoid thickness (µm)	7.8	5.7	6.3 (10)
Osteoclast surface per bone surface (%)	1.1	0.9	1.1 (0.7)
Eroded surface per bone surface (%)	15	13	18 (6)
Quantitative Backscattered Electron Imaging			
Ca_{Mean} (wt% Ca)	21.38	21.67	20.95 (0.57)
Ca_{Peak} (wt% Ca)	22.36	22.70	21.66 (0.52)
Ca_{Width} (Δwt% Ca)	3.64	3.12	3.47 (3.12; 3.64)
Ca_{Low} (%)	7.49	6.98	6.14 (4.90; 7.99)
Ca_{High} (%)	3.13	2.59	0.89 (0.43; 1.47)

Ca_{Mean}: weighted mean Ca content, Ca_{Peak}: most frequently measured Ca content, Ca_{Width}: full width at half maximum of BMDD peak (heterogeneity of mineralization), Ca_{Low}: fraction of low mineralized bone (<17.68 wt %), Ca_{High}: fraction of high mineralized bone (>25.30 wt %).

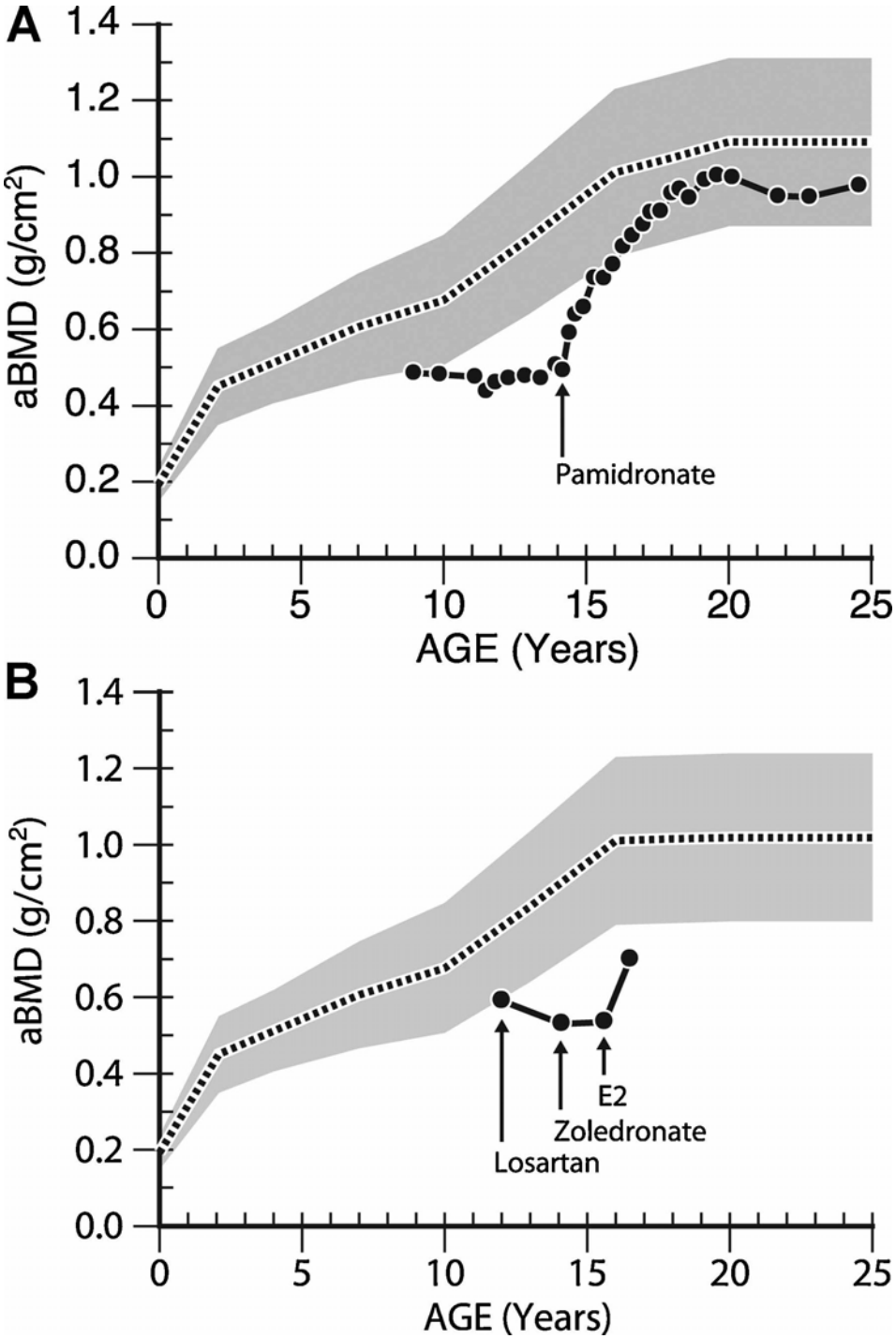

Figure 1

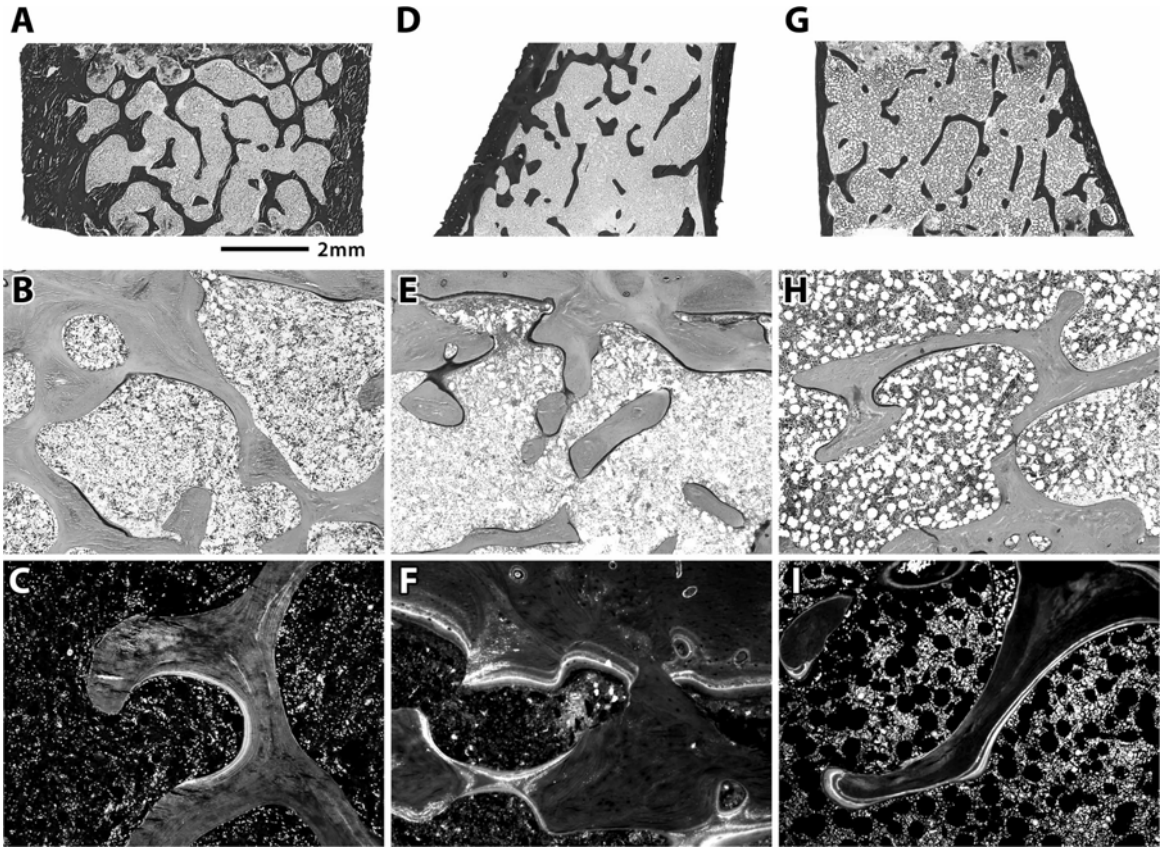

Figure 2

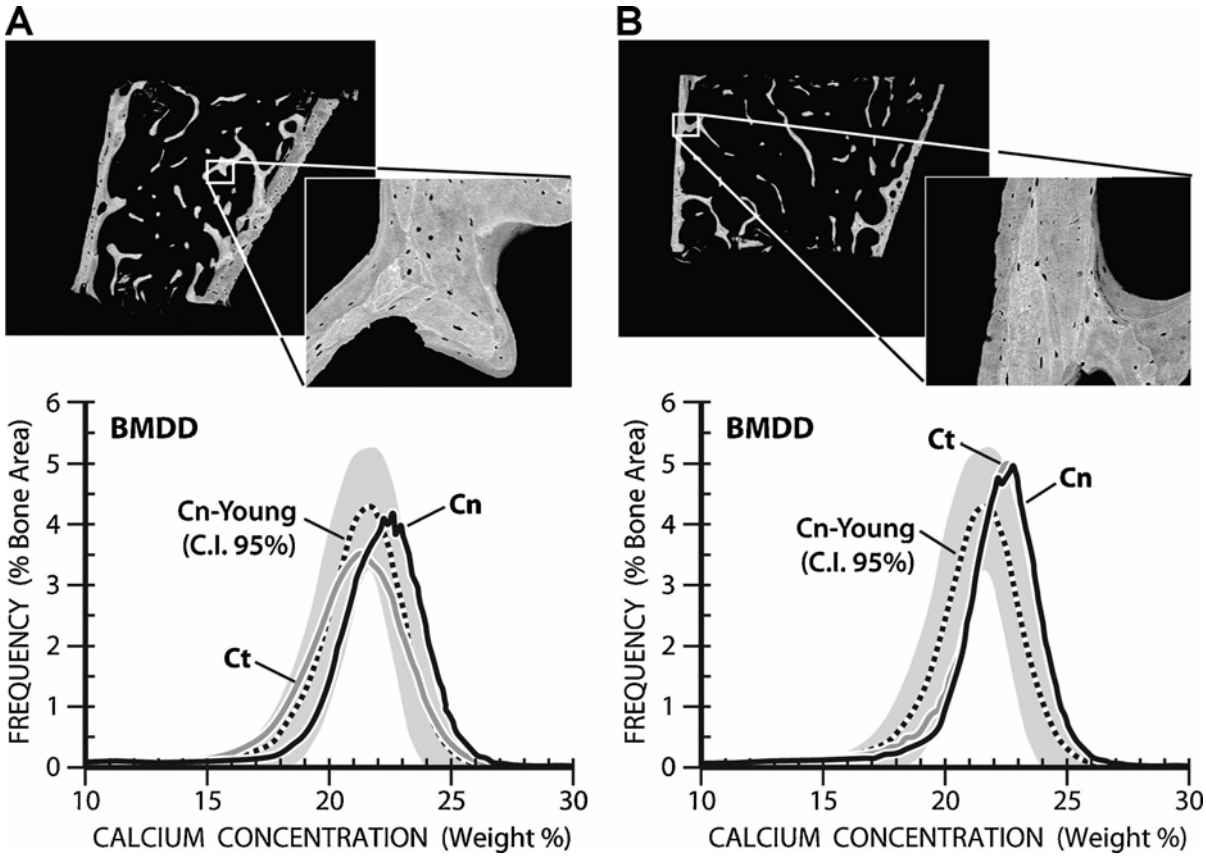

Figure 3

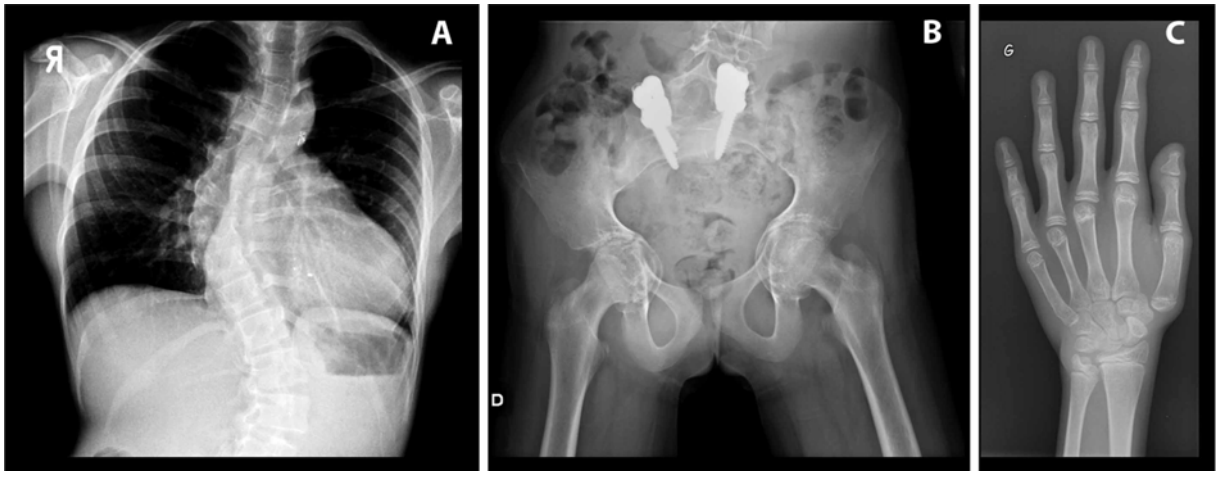

Figure 4