Clinical signs and diagnosis of osteogenesis imperfecta in three dogs

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- Osteogenesis imperfecta is a heritable disease characterized by brittle bones.
- It is important to distinguish cases of osteogenesis imperfecta from trauma and metabolic disease when examining young dogs with multiple fractures.
- Osteogenesis imperfecta can be diagnosed by analyzing type-I collagen from cultured skin fibroblasts.

Three young dogs with multiple fractures and without a history of substantial trauma were examined at Cornell University's veterinary hospital during the last 2 years. Clinical signs and results of serum biochemical analyses, radiography, histologic examination, and type-I collagen analysis for each dog were consistent with **osteogenesis imperfecta (OI)**. The most common cause of OI is a mutation in 1 of the 2 genes coding for type-I collagen. Structural defects in type-I collagen result in brittle bones.

A 12-week-old sexually intact male Golden Retriever (dog 1) was examined because of muscle weakness. The puppy came from a litter of 13, 3 of which died shortly after birth. When purchased at 8 weeks old, the puppy was smaller than its littermates and had a hind limb lameness. Lameness resolved within 1 week, but was followed by recurrent episodes of a hobbled gait and 1 incident of the puppy being unable to walk. Trauma was not included in the history. The puppy was referred for evaluation of suspected neuromuscular disease.

On examination, the puppy was thin and small for its age (6 kg [13.2 lb] at 3 months). It had a crouched posture (Fig 1) and walked with a stilted gait, shifting its weight to the forelimbs. There was generalized muscle atrophy and weakness. The puppy's teeth were pink. Joint or skeletal abnormalities were not identified on palpation. Results of neurologic examination were consistent with neuromuscular or skeletal disease.

Results of a CBC, urinalysis, and most serum biochemical analyses were within reference ranges for a 12-week-old puppy. High activities of lactate dehydrogenase (1,389 U/L) and creatine kinase (920 U/L) indicated possible muscle injury or disease. Muscular dystrophy was considered unlikely, because activity of creatine kinase was considerably less than the 30,000 U/L often detected with this disease. Baseline concentration of triiodothyronine (T_3) was low (0.58 ng/ml; reference range, 1 to 2 ng/ml), but baseline concentration of thyroxine (T_4) was within the reference range (2.58 µg/dl; reference range, 1.5 to 3.0 µg/dl). The puppy was seronegative for *Neospora caninum* IgG.

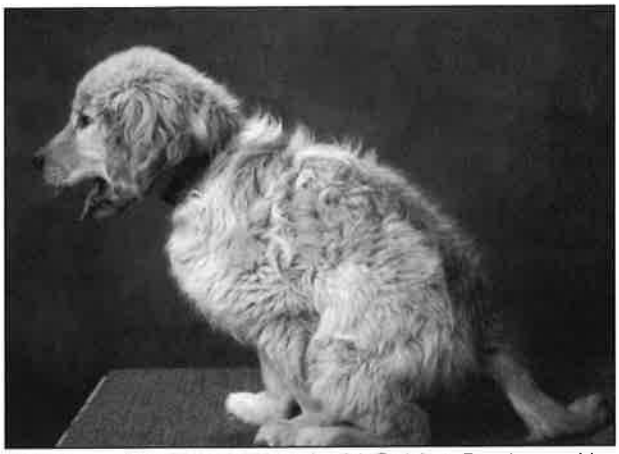

Figure 1—Dog 1, a 12-week-old Golden Retriever. Notice the crouched position and small size of the dog. Physical examination revealed pink teeth.

Radiography revealed long bone cortices that were less opaque than normal. There were multiple incomplete fractures involving both ulnae, femurs, and tibiae and the left ischium and right fifth rib. Callus formation and medullary sclerosis accompanied long bone fractures. Abnormalities were not detected on radiographs of the vertebral column. These findings were consistent with abnormal bone metabolism or formation.

Differential diagnoses included renal or nutritional secondary hyperparathyroidism and OI. Concentrations of BUN and serum creatinine and results of urinalysis were within reference ranges, so renal disease was unlikely. The puppy had been adequately nourished. Parathyroid hormone and vitamin D concentrations were not measured, but serum calcium (10.8 mg/dl) and phosphorus (6.6 mg/dl) concentrations were within reference ranges for the puppy's age.

Twenty-four hours prior to euthanasia, 10 mg/kg of body weight of oxytetracycline hydrochloride was slowly injected intravenously to fluorescently label newly formed bone. Immediately prior to euthanasia, the puppy was anesthetized, and skin specimens were obtained for fibroblast culture. Skin on the dorsolateral aspect of the thorax (T10 to T11) was prepared for biopsy by shaving and cleaning with povidone-iodine

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Supported by NIH grant No. AR20793 and by a grant from The Iams Co.

The authors thank Drs. Sharon Center, Barry Cooper, Alexander DeLahunta, Cindy Jackson, Paul McNamara, John Randolph, and Eric Trotter for evaluation of the dogs and Barbara Hover for assistance with cell culture assays.

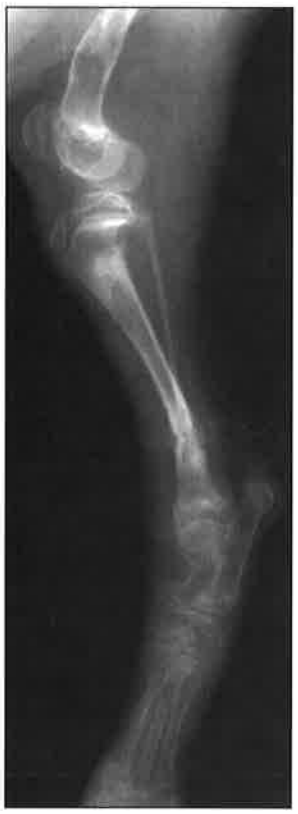

Figure 2—Radiographic view of the right hind limb of dog 3. There are healing fractures of the distal portions of the femur and tibia and the proximal portion of the tibia. Generalized osteopenia is evident.

and 70% ethanol. Full-thickness, 1.5 X 3-cm elliptical biopsy specimens were obtained using a No. 10 scalpel blade and were placed in Simm's balanced salt solution[1] containing 1% amphotericin B.[a] The skin was minced and transferred to tissue culture flasks containing Dulbecco's modified Eagle media with 20% fetal bovine serum, 1% amphotericin B, and a 1% penicillin-streptomycin solution.[b] Fibroblasts were allowed to grow out onto the flask wall from the tissue for 5 days. The minced skin then was removed, and fibroblasts were grown to confluence.[1]

Postmortem radiographs of the puppy were obtained. Generalized osteopenia was evident. Almost all long bones, both ilia, and multiple ribs had fractures in various stages of healing. The dentin of erupted and nonerupted teeth was thin (dentinogenesis imperfecta), and erupted teeth had multiple fractures. Bones were split longitudinally to allow examination of oxytetracycline-labeled regions with a UV lamp. Fluorescence was detected around fractures, beneath articular cartilage, and around growth plates, but was much less than expected in a 12-week-old puppy.

Microscopic examination of longitudinal and transverse sections of the bones revealed little, if any, primary spongiosa, and thin trabeculae of secondary spongiosa were lined by a few atrophic osteoblasts. Numerous cementing lines were evident. In the subperiosteal lamellae, there were many layers of osteoprogenitor cells, but few osteoblasts and little or no apposition of osteoid on subperiosteal lamellae. There were also few osteoclasts. In the inner cortex, Haversian and Volkmann's canals were wide and contained many bipolar osteoprogenitor cells, but osteoblasts were sparse and atrophic. In teeth, the dentin and predentin were thin and odontoblasts were sparse and atrophic. In contrast, ameloblasts were morphologically normal. Sections from the thyroid and parathyroid glands and kidneys were also histologically normal.

A female spayed Standard Poodle (dog 2) was examined by a referring veterinarian for multiple episodes of lameness. Physical examination and radiographic findings obtained at 11 months of age were suggestive of panosteitis, and the dog was treated with rest and analgesics. At 1 year of age, the dog suffered a nondisplaced compression fracture of the proximal portion of the left humerus. Trauma had not been observed. At 16 months of age, the dog's right metacarpal bones II, III, and IV were fractured after blunt trauma. At this time, the referring veterinarian considered the possibility of metabolic bone disease. The dog had been on a well-balanced commercial dog food diet. Primary and secondary hyperparathyroidism were ruled out by determining that serum concentrations of $1,25(OH)_2$ vitamin D, 25-hydroxy vitamin D, intact parathyroid hormone, and ionized calcium were within reference ranges. The dog was referred for further assessment.

On physical examination, the dog had reduced muscle mass and was lame on the right forelimb. Its teeth were white. Results of CBC, serum biochemical analyses, and urinalysis were within reference ranges. Radiography of the left brachium, right antebrachium, and carpus revealed generalized decreased bone opacity and thinning of the cortices. Previous fractures had healed. Osteogenesis imperfecta was the presumptive diagnosis. The dog was sedated with acepromazine maleate and oxymorphone hydrochloride, a biopsy site on the dorsolateral aspect of the back was prepared, and a full-thickness skin biopsy specimen was obtained for type-I collagen analysis as described for dog 1.

This dog was examined again at 2 years of age after another episode of blunt trauma. The dog sustained transverse, slightly comminuted fractures of the distal portions of the right radius and ulna. Generalized osteopenia was evident on radiographs. Whereas in most instances a single plate on the radius would be sufficient internal fixation, the radius and ulna were plated because of the inherent fragility of bone in dogs with OI.

A 3-month-old female Beagle (dog 3) was examined because it was unable to stand and was small for its age. Its teeth were white. The puppy had fractured bones at 2 (right femur), 5 (both forelimbs), 11 (right tibia), and 12 weeks of age (left femur). Most fractures occurred after jumping down from a bed or the owner's arms. The puppy had been on a commercial dry dog food diet, and littermates were not known to be affected.

Results of a CBC and serum biochemical analyses, including concentrations of parathyroid hormone, ionized calcium, and vitamin D, were within reference ranges. Radiography revealed generalized osteopenia with fractures of the distal portions of the humeri, right femur, and right tibia and proximal portions of the right tibia and left femur with varying degrees of healing and callus formation (Fig 2). Laminae durae vis-

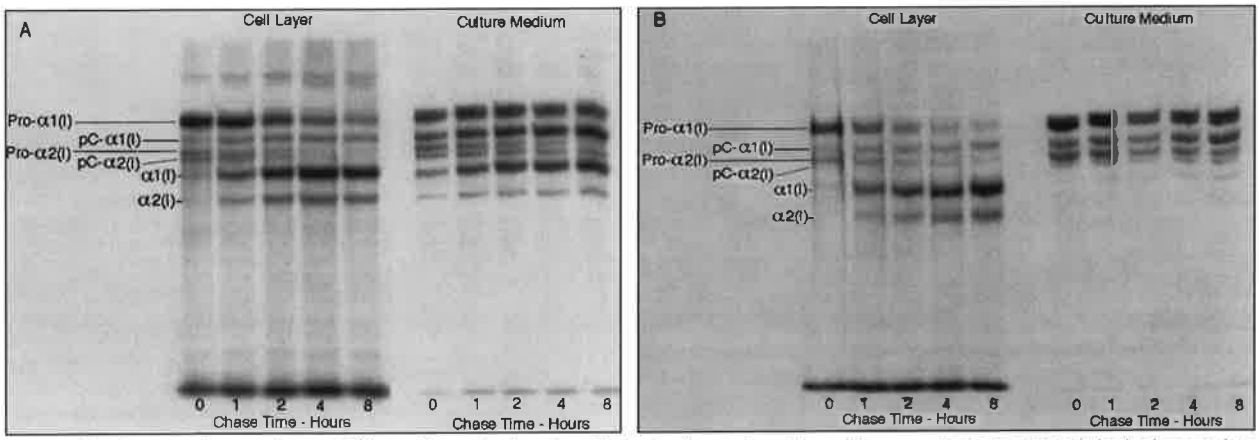

Figure 3—Autoradiographs of 5% sodium dodecyl sulfate polyacrylamide gels used to separate labeled proteins in the cell layer and culture media from dogs with osteogenesis imperfecta (OI). Each lane contains ^{3}H-proline-labeled proteins from the cell layer or culture medium collected from 1 dish of dog fibroblasts at the postlabeling incubation time indicated. (A) Dog 3: 50 to 90% of pro-α chains were processed to α chains in the cell layer and culture medium. In the cell layer, the electrophoretic mobility of pro-α and α chains was identical at all time points. In contrast, in culture medium, the electrophoretic mobility of the chains was noticeably slower at all time points after the 0-hour postlabeling incubation time. (B) Dog 2: 50 to 90% of pro-α chains were processed to α chains in the cell layer, but in the culture medium, there was almost no processing to mature α chains during the 8-hour postlabeling incubation period. In culture medium, the electrophoretic mobility of these chains was also slower at all time points after the 0-hour postlabeling incubation period. Abnormalities identified in specimens from dog 1 were similar to those shown in (B).

ible on radiographs of the dentition appeared normal. Generalized osteopenia indicated a metabolic cause for the multiple fractures, but disuse could not be ruled out because the puppy had spent most of its life with splints on various limbs.

Internal fracture fixation was not considered a viable option because of the extensive osteopenia. The puppy was discharged from the hospital with instructions to the owners for strict rest; external coaptation was not applied. Prognosis for recovery was guarded to grave. One month later, the puppy was examined, found to have additional long bone fractures, and euthanatized. For fibroblast culture, a skin biopsy specimen was obtained from the dorsolateral aspect of the thorax, using the procedures described previously.

On necropsy, all bones, including the vertebrae, were brittle and snapped easily. Dentinogenesis imperfecta was not evident. Histologic examination revealed severe osteopenia in the trabecular and cortical bones of the calvarium, ribs, proximal portion of the humerus, vertebra, distal portion of the femur, and tibia. Failure of osteoprogenitor cells to mature into osteoblasts was supported by the existence of multiple layers of bipolar osteoprogenitor cells between the periosteum and trabeculae and by a generalized paucity of osteoblasts on trabecular surfaces. The density of collagen in skin was histologically normal.

Because type-I collagen constitutes > 85% of the organic matrix of bone and provides the structural framework for mineralization, COL1 gene mutations often manifest clinically as brittle bone. In clinically normal animals, type-I procollagen is composed of 2 pro-α 1(I) chains (coded for by the COL1A1 gene) and 1 pro-α 2(I) chain (coded for by the COL1A2 gene). After half of the proline and 25 to 35% of the lysine residues in each chain are hydroxylated, the 3 pro-α chains spontaneously wind up to form a triple helical procollagen molecule. Procollagen is secreted into the extracellular matrix, where the C- and N-terminal propeptides are enzymatically cleaved to produce collagen. The collagen monomers spontaneously polymerize into collagen fibrils, and cross-links form between monomers within fibrils. In > 90% of cases of OI in human beings, a mutation in COL1A1 or COL1A2 produces an altered pro-α chain that disrupts formation of the triple helix.[2] Structural defects in pro-α chains include insertions or deletions of a few amino acids and substitution of 1 amino acid for another. These primary structural defects can lead to excessive hydroxylation of lysine, premature degradation of structurally abnormal collagen monomers, inability to process procollagen to collagen, and kinks in the triple helix.[3] The resulting collagen fibrils are thin and irregular, and because type-I collagen is the scaffold for bone mineralization, COL1 mutations result in brittle bones.

For the dogs of this report, fibroblast cultures were produced from skin biopsy specimens. Although clinical signs of OI usually are not apparent in the skin, mutations in the COL1 genes are reflected in the structure of type-I collagen synthesized by skin fibroblasts. Because the proline content of collagen is much higher than that of other proteins, collagens produced by cultured fibroblasts can be preferentially labeled with ^{3}H-proline. The effects of a COL1 mutation are seen as a change in procollagen processing, a change in the electrophoretic mobility of type-I procollagen or collagen, or both.

A cell culture assay is used to detect abnormalities of procollagen processing. Five dishes of postconfluent skin fibroblasts from clinically normal dogs and dogs with brittle bones are pulse labeled for 1 hour with ^{3}H-proline, then incubated with unlabeled media for 0, 1, 2, 4, or 8 hours.[1] At each time point, the culture medium and cell layer are collected from 1 dish. A few

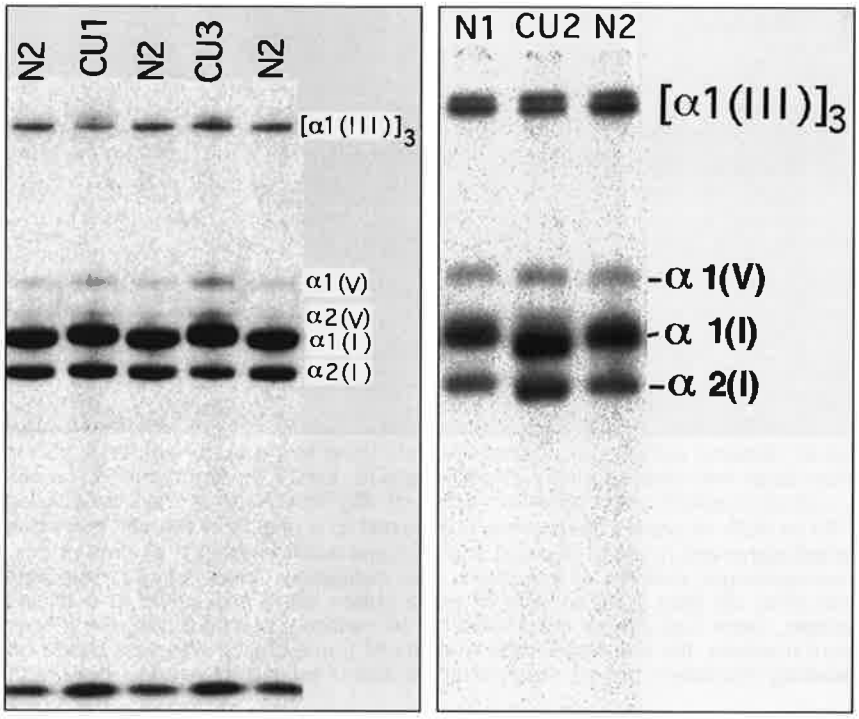

Figure 4—Autoradiographs of 5% sodium dodecyl sulfate polyacrylamide gels used to separate hydroxylated, pepsin-digested type-I collagen from skin fibroblasts of dogs with OI. (Left) The hydroxylated 1(I) and 2(I) chains from dogs 1 (CU 1) and 3 (CU 3) migrate more slowly than the adjacent controls from a clinically normal dog (N2). This abnormality is consistent with overhydroxylation caused by a delay in triple helix formation in type-I collagen of dogs with OI. (Right) The hydroxylated 1(I) and 2(I) chains of dog 2 (CU 2) migrate farther than the controls from clinically normal dogs (N1 and N2). This abnormality is consistent with a structural change in the type-I collagen of dog 2 that causes the collagenous triple helix to be susceptible to pepsin digestion.

mutations affect intracellular procollagens, whereas others affect soluble procollagens in the culture medium. Labeled proteins are separated by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis. If procollagen processing is normal, 50 to 90% of labeled protein will be processed to collagen by the end of the 8-hour incubation.[1,4] In cultured fibroblasts from dog 3, 50 to 90% of the procollagen was processed to α chains in the cell layer and culture medium (Fig 3A). In contrast, procollagen processing was delayed in the culture media of cells from dogs 1 and 2 (Fig 3B).

Structural defects of type-I collagen also may be detected with sodium dodecyl sulfate polyacrylamide gel electrophoresis of ^{3}H-proline labeled protein. Compared with clinically normal patients, the electrophoretic migration of hydroxylated collagen from patients with OI is often slowed.[3] This is because a COL1 mutation that delays helix formation allows additional lysine residues to be hydroxylated, increasing the bulk of the α chains. The electrophoretic mobility of procollagen was delayed in culture media of cells from all 3 dogs (Fig 3A and 3B).

Digestion of the nonhelical C- and N-terminal propeptides of procollagen with pepsin allows analysis of the mobility of the triple helical region of the molecule.[4] The mobility of pepsin-resistant α chains from dogs 1 and 3 was slowed, indicating overhydroxylation (Fig 4, left). In contrast, the migration of pepsin-resistant α chains from dog 2 was faster than usual (Fig 4, right). This finding is consistent with a structural abnormality in type-I collagen that allows pepsin to cleave the molecule within the normally resistant triple helix. Similar changes were observed in processed specimens from a human being with OI. In that instance, a COL1A1 mutation in a splice site introduced a noncollagenous insertion in the triple-helical region.[5]

The incidence of OI in human beings is approximately 1 case/25,000 births.[6] Osteogenesis imperfecta in cattle has also been reported. In the latter species, it is an autosomal dominant condition characterized by multiple spontaneous fractures, generalized joint laxity, and dentinogenesis imperfecta.[7-10] Excessive modification of collagen has been described for OI in cattle.[1] Osteogenesis imperfecta has been suspected as a cause of bone fragility in dogs,[11,12] cats,[13,14] a tiger,[15] and sheep.[16] However, other causes of pathologic fractures were not thoroughly ruled out for these animals, and biochemical studies of collagen were not performed.

Differential diagnoses for multiple fractures in young dogs include trauma, nutritional or renal secondary hyperparathyroidism, and OI. Neoplasia and primary hyperparathyroidism are less likely in young dogs, although congenital primary hyperparathyroidism in 2 German Shepherd Dogs has been repored.[17] A presumptive diagnosis of OI was made for each dog described in this report after serologic evaluation ruled out parathyroid-related disease. However, physical abuse remained a consideration.

The aforementioned cell culture assay provided the first evidence of a structural abnormality in type-I collagen in these dogs. Specifically, failure of procollagen processing in dogs 1 and 2, and abnormal electrophoretic mobility of pepsin-resistant α chains in dogs 1, 2, and 3 are consistent with a mutation in 1 of the COL1 genes.

More than 90% of human beings with OI have an autosomal dominant mutation in COL1A1 or COL1A2.[2] Four types of OI have been identified in human beings on the basis of the severity of disease.[18] The clinical expression of OI ranges from type I (a mild form with few fractures) to type IV and type III (in which there is increasing bone fragility and deformity) to type II (which

is lethal in the perinatal period because of multiple fractures and severe deformity). Dentinogenesis imperfecta and hearing loss are reported in types I, III, and IV, whereas blue sclerae are sometimes seen in types I and III. Children with multiple fractures caused by OI are often initially mistaken as victims of physical abuse.

Consistent with OI types III and IV, all 3 dogs in this study survived the perinatal period but suffered multiple fractures at a young age, with minimal to no associated trauma. Dogs 1 and 3 could be classified as having OI type III on the basis of their small stature, the existence of fractures in nearly every long bone, and evidence of disease at a young age (dog 1, 8 weeks; dog 3, 2 weeks). Dog 1 also had dentinogenesis imperfecta. In contrast, dog 2, which achieved a normal body size, had fewer fractures and did not have signs referable to OI until 11 or 12 months of age, could be classified as having OI type IV.

After ruling out other causes of metabolic fractures, OI should be considered in young dogs with multiple fractures and without a history of substantial trauma. It is possible that many cases of OI in dogs are misdiagnosed as physical abuse or just "bad luck" when a dog suffers from multiple fractures and serologic tests do not reveal a metabolic cause. It is also likely that perinatal lethal OI (type II) in dogs is missed, as necropsy of neonates is uncommon. The hallmark sign of OI is brittle bone. Findings of osteopenia and dentinogenesis imperfecta (seen as pink teeth) in a young dog are consistent with OI, but pink teeth are not found in every case of OI and there are many causes of osteopenia. Definitive diagnosis of OI is currently made by analyzing type-I collagen from cultured skin fibroblasts.

[a]GIBCO No. 6005295, GIBCO Laboratories, Grand Island, NY.
[b]GIBCO No. 6105300, GIBCO Laboratories, Grand Island, NY.

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