Histopathologic study of long-bone growth plates confirms the basset hound as an osteochondrodysplastic breed

Simón Martínez, Raúl Fajardo, Jesús Valdés, Raúl Ulloa-Arvizu, Rogelio Alonso

Abstract

Osteochondrodysplasias are caused by abnormal development and growth of cartilage and bone. These abnormalities have been reported in both humans and animals with dwarfism. The basset hound is considered a breed with a disproportionate prevalence of dwarfism, the cause of which is unknown. To determine the type of osteochondrodysplasia in this breed, we analyzed histologically the growth plates from the long bones of a basset hound and a Doberman pinscher, both 2 mo old. Tissue was fixed in 4% paraformaldehyde, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and analyzed by light microscopy. Our results suggest that by this method the basset hound can be defined only as a breed having osteochondrodysplasia due to a primary cartilage problem in the growth plate.

Résumé

Les ostéochondrodysplasies sont causées par un développement anormal et une croissance anormale du cartilage et des os. Ces anomalies ont été rapportées chez l’humain et les animaux atteints de nanisme. Le basset est considéré comme appartenant à une race avec une prévalence disproportionnée de nanisme, mais dont la cause est inconnue. Afin de déterminer le type d’ostéochondrodysplasie chez cette race, une analyse histologique des plaques de croissance des os longs d’un basset et d’un doberman miniature, tous deux âgés de 2 mois, a été effectuée. Les tissus ont été fixés dans de la paraformaldéhyde à 4 %, enrobés de paraffine, tranchés en section de 5 μm, colorés avec de l’hématoxyline et de l’éosine, et examinés par microscopie photonique. Nos résultats suggèrent qu’avec la méthode employée, le basset peut être défini uniquement comme étant une race souffrant d’ostéochondrodysplasie associée à un problème primaire du cartilage dans la plaque de croissance.
In animals, osteochondrodysplasias have been reported mainly in dogs, including the Alaskan Malamute, Scottish deer hound, Norwegian elk hound, Maremmano–Abruzzese shepherd, Great Pyrenees, German shepherd, miniature poodle, Labrador retriever, beagle, and cocker spaniel. Most of the alterations in these dogs result from a de novo mutation, but in some cases they appear to be due to autosomal recessive inheritance (9,10).

Other breeds, such as the bulldog, basset hound, dachshund, Pekingese, and shi tzu, are referred as achondroplastic, since they have a disproportionate rate of dwarfism, and some of them also have a nasal bridge depression and prognathism (11–13). Although the most frequent mutation in achondroplastic humans originates from a G/A transition in nucleotide 1138 of the transmembrane domain of gene \textit{FGFR3} (14), a similar mutation seems not to be involved in the bulldog, basset hound, and dachshund with the osteochondrodysplastic phenotype (15).

In veterinary medicine, there is a scarcity of information about the skeletal alterations in animals and no classification like the International Classification of Osteochondrodysplasias in humans. For these reasons, many skeletal dysplasias in animals are named according to their morphologic similarities with the human skeletal dysplasias even though the molecular origins could be different (10,16). The objective of our study was to describe the radiologic characteristics of the long bones and the histopathologic features of their growth plate in the basset hound. For comparison, we also studied Doberman pinschers.

To characterize the form and size of the long bones, we used 3 puppies 2 mo of age and 3 dogs 1 y of age of each breed. Radiographs were made with craniocaudal and medial-lateral views of the humerus, radius, ulna, femur, and tibia. To histologically analyze the growth plate, we euthanized 1 puppy 2 mo of age of each breed by the intravenous administration of sodium pentobarbital, 70 mg/kg. Distal ends of thoracic and pelvic limbs were obtained by cutting 5 to 10 mm proximal to the growth plate on each bone. Samples were cleaned in 1X phosphate-buffered saline and fixed in 4% paraformaldehyde. After 24 h, the samples were decalcified in 5% trichloroacetic acid for 8 d. Longitudinal growth-plate slices \(1 \times 1 \times 3\) mm containing epiphyseal and metaphyseal bone ends were obtained. Tissues from the humeral and femoral growth plates were embedded in paraffin and cut in 5-\(\mu\text{m}\)-wide slices. After being stained with hematoxylin and eosin, the slices were analyzed by light microscopy.
The 2 breeds showed remarkable radiologic differences in the long bones of both puppies and adults. The basset hound humerus, in comparison with the Doberman humerus, showed a pronounced curvature at the mid-diaphysis, with shortening and widening at the proximal end. The proximal epiphysis was considerably curved, and its caudal end showed a beak-like protrusion. The proximal and distal metaphysis showed considerable widening that diminished towards the medial diaphysis, in a truncated cone fashion. The opacity of the caudal cortical bone was increased; this was most evident in the medial third of the diaphysis (Figures 1A and 1B).

The radius and ulna in the basset hounds were short and wide, with a broad interosseous space owing to the pronounced curvature at the medial third of the radial diaphysis. The distal metaphysis of the radius was severely widened and denser than that of the Dobermans. The ulna was wider than in the Dobermans and had prominent protuberances; its diaphysis was slightly widened in the distal third, as was the proximal part, between the anconeus and olecranon processes (Figures 1C and 1D).

The femur in the basset hounds appeared shortened and widened at the proximal and distal metaphyses, with increased opacity. The trochelear and condylar regions were wider than in the Dobermans, although without other anatomic changes. The neck of the femoral head was shorter, and its epiphysis was cap-like, with a salient rim. The major trocanter was widened at its borders, and the minor trocanter protruded mediadally. The head of the femur was in good proportion to the acetabulum; thus, the coxofemoral articulation was correct (Figures 2A and 2B).

The tibia in the basset hounds appeared shortened and widened, with notably increased opacity and widening of the proximal metaphyses. The proximal third of the diaphysis was concave, more so at the cranial line. The fibula was not parallel to the tibia; thus, the interbone space was larger in the basset hounds than in the Dobermans (Figures 2C and 2D).

Histologically, the height of the entire growth plate was markedly greater in the Doberman than in the basset hound (Figures 3A and 3B). In the resting zone, the shape of the cells was similar in the 2 breeds, but in the basset hound the zone was larger, and the extracellular matrix was abundant. The proliferating zone in the Doberman was organized in typical symmetric columns formed by single cells stacked on one another. In contrast, in the basset hound this zone contained disorganized columns: some were short and thinly formed by piling up of a single cell, whereas others were formed by piling up of more than 1 chondrocyte; the columns were not symmetric and were separated by wide areas of cartilage matrix. In the Doberman, the growth-plate cells near the diaphysis were hypertrophic and mostly of consistent size, although shrunken cells were also observed; the columns stayed organized and separated by a small amount of cartilage matrix. In contrast, in the growth plate of the basset hound, the hypertrophic zone was greatly diminished.
and composed of columns of cells of variable size. Next to the hypertrophic chondrocytes in the growth plate of the Doberman, we observed a large quantity of chondrocyte lacunae, with a few shrunken cells and scarce cellular matrix; in the transition zone were osteoblasts, osteoclasts, erythrocytes, and vascular ingrowth, as well as cartilage areas occupied by osteoblasts but no primary bone formation. In the growth plate of the basset hound cellular lacunae were sparse, and in the transition zone we observed early vascular ingrowth, a great quantity of osteoblasts, and abundant primary bone formation on the cartilage matrix. These characteristics in the basset hound suggest an alteration in chondrocyte proliferation and premature ossification.

Our study revealed that basset hounds have curved long bones that shorten and bow the thoracic and pelvic limbs. In addition, the metaphyses are wide and the epiphyses irregular. However, the dogs do not have the craniofacial and vertebral alterations that affect achondroplastic humans. For this reason, the basset hound skeleton is not typical of human achondroplasia. Other osteochondrodysplasias, such as hypochondroplasia, pseudoachondroplasia, and metaphyseal dysplasia, do not present craniofacial alterations. We found, by histopathologic analysis, that the abnormalities of the growth plate in the basset hound are similar to those in humans with achondroplasia and mice with experimentally induced achondroplasia (17). However, these histologic findings are also similar to those observed in humans and mice with metaphyseal dysplasia (18). Therefore, the histologic characteristics of the growth plate alone are insufficient to classify the type of osteochondrodysplasia that basset hounds have.

At present, osteochondrodysplasias in humans are classified on the basis of molecular diagnostics (7). In veterinary medicine, the data are too sparse for a similar classification in animals. We previously reported that basset hounds do not have the mutation usually found in humans with achondroplasia (15). We suspect that the basset hound be identified as osteochondrodysplastic, since osteochondrodysplasia is a term widely used to describe the alterations in cartilage and bone. Studies on the pathological mechanisms in the growth plate of the basset hound and the search for mutations in genes related to skeletal development in these dogs will help us define the type of osteochondrodysplasia. We propose 2 candidate genes that might be associated with the typical osteochondrodysplasia of the basset hound: the FGFR3 gene, whose complete sequence could be obtained to eliminate the possibility of a mutation in a region other than the transmembrane domain; and the collagen X gene, which is expressed exclusively in hypertrophic chondrocytes in the growth plate and is responsible for the Schmid type of metaphyseal dysplasia in humans, in which hypertrophic cartilage is absent (19).

Identifying the gene responsible for canine osteochondrodysplasias and studying its mechanisms of action will provide clues to the development of the skeletal system in mammals.

Acknowledgments

Simón Martínez and Raúl Fajardo were supported by grants from Universidad Autónoma del Estado de México, projects 1750/2003 and 1800/2004.

References