AETIOLOGY AND BIOMARKERS OF CANINE CCL DISEASE
John F. Innes BVSc PhD DSAS(orth) MRCVS
University of Liverpool, Liverpool, UK

Disease of the cranial cruciate ligament (CCL) is the most common condition to affect the canine stifle joint. The postulated factors involved in the pathogenesis of CCL rupture are many and include: genetics, breed, age, gender, neutering, ischaemia, obesity, immune mechanisms, tibial plateau angle, intercondylar notch, and local biomechanics. Some of these factors have been investigated, and some are currently under investigation. These investigations may lead to biomarkers for risk, diagnosis or disease progression. Such biomarkers may be based on molecular genetics, disease-associated proteins, or imaging.

Risk factors for CCL disease – age, breed (genetics), gender

CCL rupture occurs in all sizes of dogs but affects larger breed dogs more than smaller dogs, and at a younger age. Obesity is also likely to be a risk factor although further work is required in this area. Epidemiological studies have indicated an increased prevalence of CCL disease in breeds such as the Newfoundland, Rottweiler and Labrador Retriever, with infrequent occurrence in the Greyhound, Bassett Hound and Old English Sheepdog. CCL rupture occurs more commonly in neutered animals, particularly females. It is unknown if this is secondary to abnormal weight gain as certain authors have reported that 45.4% of spayed bitches are obese. It is now known that CCL disease has an inherited component in the Newfoundland and Boxer with heritability estimates of 0.27 and 0.28 respectively. One report has identified 4 chromosomal regions associated with cruciate disease in US Newfoundlands and an ongoing genome-wide association study (GWAS) in the author’s laboratory has identified three regions in UK Newfoundlands. Interestingly, there is marked genetic stratification between Newfoundlands from UK and North America.

Metabolism of the CCL

With the increased risk of CCL disease in certain breeds and neutered animals, one can ask many questions relating to the nature of the tissue in these animals. Is the CCL “normal” in these animals? For example, is the structure and turnover of the CCL normal? Is the biochemistry of the CCL normal? Does hormonal status influence CCL metabolism and function? Earlier studies examined CCL biochemistry, ultrastructure and biomechanics of CCLs from two at-risk breeds (Labrador and Golden Retriever) and compared these to a low-risk breed (Greyhound). The ultrastructure of the CCLs in these breeds was examined using transmission electron microscopy. The collagen fibril diameters were measured and it was found that the mean fibril diameter in the Labrador is significantly smaller than that of the Greyhound. This is interesting in that previous experimental work has demonstrated that following CCL transection, the collagen fibril diameter of the caudal cruciate ligament decreases. This suggests that collagen fibril diameter is a biomarker for altered loading.

Markers of collagen turnover in CCLs in these breeds of dog have also been assessed and it was demonstrated that tissue concentrations of gelatinase (matrix metalloproteinase [MMP]-2) are upregulated in the at-risk breeds compared to controls. Furthermore, using reverse zymography it was demonstrated that concentrations of tissue inhibitor of MMPs (TIMP-2) are lower in the at-risk breeds compared to control. In addition, cross-linking profiles of collagen within these tissues show more intermediate collagen cross-links compared to controls. Taken together, these data suggest that collagen turnover in CCLs from at-risk breeds is increased. This could be constituitive, or induced. The intercondylar notch has previously been investigated as an etiological factor and there is evidence that this may be an
influence on CCL biochemistry. Whilst the studies described above were of grossly normal ligaments, it is also important to study end-stage disease so that markers of disease can be determined.

Recent work has focussed on the cell morphology and role of elastin within the CCL. Confocal microscopy techniques were used to investigate cellular processes and the distribution and possible role of elastin. Studies have shown that the cells of the epiligament form a dense meshwork of short, branched processes. A dense meshwork of cells was seen in the interfascicular regions of the ligament with thick, branching processes of widely varying length, and round nuclei. Cells of the fascicular regions were of three broad types: one group of cells within the ligament had long, thin cytoplasmic processes extending mainly parallel to collagen bundles, long narrow nuclei and some shorter transverse processes; a second group had shorter, thicker, frequently branching processes, with rounder nuclei; the third group were isolated, with rounded nuclei and no cytoplasmic processes. Cell processes were seen to penetrate the interior of collagen bundles. The majority of each ligament comprised either a mixed population of groups one and two, or of group three alone, although juxtaposition of all cell morphologies was also seen. In the midsubstance of the CLs, a decrease in cell density with an increase in the relative proportion of group three cells compared to proximal and distal CLs was noted. Marked local variation in cell density existed, with small areas completely devoid of nuclear or cytoskeletal staining. Tension appears important in maintaining cellular processes and a lack of processes and rounded nuclei may be an adaptation to ligament mechanics or physiology. Local variation in morphology may indicate differences in mechanics between adjacent collagen bundles.

Alterations in cell morphology may alter the ability of cells to produce healthy matrix and repair damage through disruption of collagen production. Long cytoplasmic processes provide a mechanism whereby cells apparently distant from blood vessels may acquire nutrients. In the midsubstance, the mechanical environment may make sustainability of cell processes impossible, so the observed decrease in cell density in the midsubstance may reflect lower available nutrition. Areas devoid of nuclear or cytoskeletal staining may mark the initiating event in fibrocartilaginous change. There is marked regional variation in the cell morphology of the canine CL complex. The possibility of a three dimensional network of cells has ramifications for cell nutrition, mechanical sensing and coordinated response to injury in the CL complex.

**CCL disease – endocrinological perspectives**

Connective tissue metabolism has been shown to be influenced by the endocrine system. Female human athletes are more prone to anterior cruciate ligament rupture compared to male athletes. In rabbits, estrogen has been shown to downregulate metabolism of CCL cells in vitro. Furthermore, estrogen has been reported to decrease collagen synthesis in the human anterior cruciate ligament and an increased incidence of rupture has been recognised at certain stages in the female menstrual cycle. Estrogen receptors have been demonstrated on the surface of ACL cells. Neutering in dogs has been shown to increase in risk of CCL disease and this may relate to change in hormonal status. However, it is also possible that this increased risk relates to increased bodyweight caused by obesity induced by neutering. Conversely, one should also consider the hormonal output of white adipose tissue and the possibility that adipokines (e.g. leptin, TNF-α) may affect connective tissue metabolism. The long form of the leptin receptor has been identified on canine CCL cells but its physiological role in these cells is yet to be elucidated; TNF-α is another cytokine/adipokine that is able to upregulate protease expression in canine CCL cells in vitro.
CCL disease – biomechanical perspectives

In recent years, the tibial plateau has been a subject of much debate. Does an excessive slope to the tibial plateau contribute to the incidence of CCL disease? One study suggests that dogs with CCL rupture do have an excessive slope to the tibial plateau. However, other studies have failed to substantiate these data. Wilke and colleagues measured traditional and standing TPAs in affected and unaffected Labradors and unaffected Greyhounds. The authors concluded that although TPA may be associated with damage to the cruciate ligaments, many dogs with a steep TPA do not develop cruciate ligament disease. More recent studies have also failed to associate TPA with subsequent contralateral CCL disease. Interestingly, one recent study has raised the possibility of tibial tuberosity conformation being a risk factor for CCL disease. Another study looked at a variety of conformational variables and suggested that cranial angulation of the proximal portion of the tibia, excessive steepness of the tibial plateau, and distal femoral torsion appeared more likely to be associated with CCL deficiency than femoral angulation, tibial torsion, intercondylar notch stenosis, and increased inclination of the patellar ligament. The analysis of conformational variables is likely to be assisted by advanced imaging techniques such as CT alongside computer simulation of canine gait.

References