Review Article

Update on the aetiopathogenesis of canine cranial cruciate ligament disease

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Summary
Cranial cruciate ligament disease (CCLD) is the most common cause of hindlimb lameness in the dog, being associated with and eventually leading to stifle osteoarthritis. Canine cranial cruciate ligament disease is a gradual degeneration of the ligament extracellular matrix (ECM) leading to ligament rupture. The aetiopathogenesis of this condition is still poorly understood but several risk factors have been identified such as breed, body weight, gender and conformation. Recent developments in this area include the role of genetics, stifle joint conformation, ligament ECM metabolism, and inflammation associated with immune-mediated disease within the stifle joint. A genetic mode of inheritance has been demonstrated in the Newfoundland which is predisposed to CCLD. Increased cellular metabolism within the cranial cruciate ligament has been directly associated with increased craniocaudal stifle joint laxity in dog breeds at high risk of CCLD. Conformation abnormalities, such as a narrowed distal femoral intercondylar notch, in high risk breeds have been shown to be associated with alterations in cranial cruciate ligament ultrastructure. Increased production of inflammatory cytokines, such as cathepsins and interleukins, by the stifle synovial cells may occur secondary to or may be an inciting cause of ligament degeneration. Future research endeavours will focus on the association between immune-mediated response and fibrocartilaginous metaplasia and matrix degradation within the cranial cruciate ligament, and whether this can be altered in all susceptible dogs or only certain breeds.

Epidemiology
Age
Although any breed may be affected by CCLD, increased prevalence in certain breeds of dog has been well established (3, 11, 12). A study of 1.25 million dogs over 40 years showed the five most commonly affected breeds to be Newfoundland, Rottweiler, Labrador Retriever, Bulldog and Boxer (5). Bilateral disease is common in affected breeds with 50% of Labrador Retrievers with unilateral CCL tears rupturing the contralateral CCL within one year (13). Certain Terrier breeds such as the West Highland White Terrier are over-represented (5, 14).

Recent studies have reconfirmed aetiopathogenic links between breeds and CCLD in breeds such as the Labrador Retriever, Newfoundland and Rottweiler (3, 5).
Genetic predisposition and risk factors

Breed-specific variation in incidence of CCLD has been considered strong evidence for a genetic basis to the condition (3, 4). A heritability of 0.27 for CCLD in Newfoundlands has been proposed, suggesting a possible recessive mode of inheritance (15). However in this study CCLD had a penetrance of 51% suggesting environmental factors as well as genetic factors influence the incidence of CCLD (15). A heritability estimate of 0.28 has also been identified in a cohort of Boxers (16). No statistical association of certain candidate genes (such as fibrillin, collagen type IX, and cartilage oligomeric matrix protein) with CCLD and rupture in Newfoundlands has been identified to date (17). It has also been reported that collagen type IX genes are unlikely to play a role in CCLD in Boxers (18). Genotyping for 532 microsatellite markers has been recently performed in a group of Newfoundlands with and without CCL rupture. Four microsatellite markers associated with CCL rupture were found in chromosomes 3, 5, 13 and 24 and there was significant trait association with CCLD in all of these microsatellite markers except 24. Further mapping of these regions may narrow the list of CCLD candidate genes (8).

A recent study examined Labrador Retrievers and Greyhounds with OA secondary to elbow dysplasia, hip dysplasia and CCLD for common genomic risk. Although 113 single nucleotide polymorphisms in 20 candidate genes were genotyped, no significant associations were identified (19). While this outcome would suggest that these conditions arise independently, it was proposed that the lack of accurate trait mapping significantly reduced the power of this study.

Large breed dogs at greater risk of CCLD appear to develop it at a younger age (3). Witsberger et al calculated the relative risk for age, demonstrating that dogs older than four years were significantly more likely to develop CCL rupture (5). Another study examining a population of 328 dogs found that the mean age of CCL rupture in large breeds (body weight >15 kg) was 5.5 years, whereas the smaller breeds (body weight < 15 kg) averaged 7.4 years (20). Identification of genetic associations with CCLD in predisposed dog breeds is fundamental to the progress of discovering interventions for the problem in dogs and humans.

Gender, neuter status and hormonal influence

The prevalence of CCL rupture is higher in neutered dogs, particularly females (21–23). Neutered female dogs have the highest occurrence of CCLD according to recent studies, and they may experience increased weight gain and body fat composition (24). In women, increased incidence of anterior cruciate ligament rupture is associated with elevated oestrogen levels in the pre-ovulatory phase of the menstrual cycle (1). However, as ovariectomy in dogs is associated with persistent hypo-oestrogenaemia, oestrogen may reduce the incidence of CCLD in the dog (25). There have been not any conclusive studies determining the effect of systemic or local hormonal status on CCLD in dogs or humans.

In many species, including the dog, white adipose tissue has been shown to be an important endocrine organ, elaborating an array of chemical mediators (adipokines) which may be pro-inflammatory (26). In humans, adipokine (adiponectin, leptin and resistin) release from intra-articular adipose and other joint tissues has been demonstrated (27). It is hypothesized that the adipose tissue of obese individuals is in a state of chronic inflammation, suggesting systemic or local adipokine elaboration, or a combination of both could be involved in the aetiopathogenesis of connective tissue pathology (28, 29). However, key pro-inflammatory adipokines have not been assessed and, a possible role for systemic adipose tissue has not been examined in CCLD and requires further investigation.

Exercise

An ability to sense and respond to mechanical force is a fundamental feature of all living tissues, including tendons and ligaments (30). The CCL fibroblasts appear to respond to their mechanical environment, and mechanical force may be required for cell arrangement (31, 32). Overstimulation of tendon cells through repetitive loading may initiate a degenerative cascade leading to tendinopathy (33, 34). However, under-stimulation of tendon cells can also produce a pattern of catabolic gene expression that results in loss of tendon material properties (35, 36). Such under-stimulation may arise from alterations to the cell–matrix interaction resulting from fibril damage at extremes of physiologic loading, and has been proposed as a mechanism for tendinopathy (37). In the CCL, compromise of cell-matrix coupling through fibril microdamage or cell morphology changes may lead to under-stimulation of fibroblasts and a degenerative cascade. There is a paucity of research examining the effect of exercise on the biochemical properties of the canine CCL. An experimental study examining the effect of regular consistent exercise on canine stifle joints, its associated ligaments and cartilage did not find any injuries in these tissues or any evidence of erosions or osteophytes in the joints after lifetime exercise (38).

The results of recent studies suggest that inactivity may predispose tendon and ligaments to altered and detrimental homeostasis. However it does not appear that exercise influences articular and periartricular tissue damage of canine stifle joints, and therefore it should be recommended in a regular and consistent regime.

Cruciate ligament factors

The cruciate ligaments are comprised of cells and ECM, and are mainly (60–80%) water. Of the dry weight of cruciate ligaments, 90% is collagen (mostly type 1), with smaller amounts of elastin, proteoglycans, glycoproteins and lipoproteins (39).

Cells and blood supply

Changes in cell phenotype and reduction in density preceding CCL rupture have been described previously (11, 40). Such changes are more common in the middle of the
CCL; the common site of rupture (41). Also described is decreased cellularity in the core of ruptured CCL, with a chondroid transformation of cells and extensive disruption of the ligamentous matrix (42). Loss of cells, whether through apoptosis or necrosis, may result in failure to maintain ECM integrity if they are not replaced. Using caspase-3 as a marker for apoptosis, significantly more apoptotic cells were seen in ruptured CCL compared to intact CCL (43). Another study using combined markers for caspase-3 and poly (ADP-ribose) polymerase did not find any difference in the amount of apoptotic cells between the intact areas of partially ruptured CCL, torn portion of partially ruptured CCL, and completely ruptured CCL (43). In addition, there was not a significant correlation between the degree of synovitis or osteophyte production and apoptotic cells (44). These studies suggest that apoptosis may be an intrinsic aetio-pathogenic factor leading to CCLD rather than a consequence of acute rupture of the ligament. It remains unclear whether apoptosis observed in this study is induced extracellularly by extrinsic inducers such as inflammatory mediators and nitric oxide, or intracellularly by intrinsic inducers such as hypoxia and nutrient deprivation. Therefore intrinsic or extrinsic inducers of cruciate ligament cell apoptosis may contribute to diminished cell communication, altered metabolic response, and ultimate ligament degeneration. Recently, however, it has been shown that apoptosis of ligamentocytes in the canine CCL was not influenced by increased nitric oxide production within the stifle joint (45).

Cells subjected to tensile load have cytoplasmic processes which may be long and extend in all directions through the collagen fibres (46, 47). The detection of gap junctions in association with these processes suggests the potential for cell-to-cell communication (46). This three-dimensional structure has been termed the cellular matrix, and has been described in many tissues including the ovine CCL (47). Recent unpublished observations describe cells of similar morphology in intact cruciate ligaments of Greyhounds and Labrador retrievers (K. D. Smith unpublished data, 2010). Cells frequently had cytoplasmic processes which varied in length and were often seen to exceed 100 μm. Processes were frequently shown to contact other cells, extending longitudinally and transversely through the cruciate ligaments. Cells with long processes were more commonly noted in the cruciate ligaments of the Greyhound than the Labrador Retriever. These findings suggest the possibility of communication between cells (particularly in the Greyhound) which, if disrupted or absent, may result in altered metabolism of the ECM, ultimately leading to CCLD as seen in high risk dog breeds such as the Labrador Retriever. In summary, alterations in cruciate ligament cell morphology may play a role in CCLD aetiology. Disruption or lack of development of cruciate ligament cell cytoplasmic processes with rounding of cell nuclei (‘chondrocytic change’) may alter communication between cells, possibly affecting ECM metabolism. Although the CCL has been considered extra-articular due to the enveloping synovial epi-ligament, recently it has been suggested that free passage of macromolecule markers from synovial fluid to the substance of the CCL and blood occurs (48). As such free movement exists between the synovial fluid and CCL substance, therefore, reduced intra-articular osmotic pressure, which may occur in OA, may affect blood flow to the CCL.

### Extracellular matrix

The ECM changes in ruptured CCL have been well documented (49, 50). More recently, these changes have been further characterised biochemically, revealing that ruptured CCL have significantly higher amounts of immature collagen cross-links, total and sulphated glycosaminoglycans, water content and concentration of matrix metalloproteinase-2, compared with intact ligaments (51). These findings suggest increased ECM turnover in ruptured CCL. Although the ECM changes may have occurred before ligament rupture, it is possible that these observed changes may be part of a reparative process after rupture. With regard to normal CCL, it has been shown recently that macroscopically, normal CCL from dog breeds at a high risk of CCLD (Labrador Retrievers) compared to those from dog breeds at a low risk (Greyhounds) have altered ECM collagen turnover (increased pro-MMP-2) and structural properties (decreased integrity of the collagen triple helices) (52). This study also suggested that the different metabolism of the collagenous matrix in the CCL from a high risk breed may be related to greater stifle joint laxity and sub-optimal ligament material properties (52).

An ultrastructural study of normal CCL in these two breeds of dog revealed that the collagen fibril diameters of Greyhounds were larger than those of Labrador Retrievers, reflecting relatively enhanced ligament maturity and mechanical properties (10). The same study also revealed increased collagen turnover in the Labrador Retriever CCL (10). There were, however, fibrocartilaginous areas discovered within the CCL ultrastructure for both breeds manifested as rounding of cell nuclei and increased staining for proteoglycans. Cartilage-like tissue is more vulnerable to disruption under normal tensile forces, therefore any fibrocartilaginous transformation may predispose to CCLD (53). The formation of fibrocartilage does not appear to be disadvantageous to healthy racing Greyhounds as they rarely rupture their CCL, and therefore this cannot be regarded as a purely pathological degeneration in this breed. Therefore it is currently unclear whether fibrocartilaginous change is a adaptive condition in exercising and low risk breeds to CCLD, or a pathologic change (with the fibrocartilaginous change inducing an inflammatory response) in high risk breeds resulting in eventual CCLD.

### Stifle joint factors

#### Mechanics

The material and structural characteristics of the canine CCL have been reported with a view to development of optimal methodologies of surgical intervention in CCLD (54, 55). However until recently, few studies had compared canine stifle joint stability (craniocaudal laxity) in normal dogs at a high and low risk to CCLD. Wingfield and
others compared stifle joint laxity in Rottweilers (high risk) and Greyhounds (low risk), reporting greater craniocaudal joint laxity in the Rottweiler stifle joint compared to that of the Greyhound at stifle joint angles between 150 degrees and 110 degrees (56). In a related study, structural (load to failure) and material (ultimate stress and tangent modulus) properties were shown to be significantly higher for Greyhound stifle joints during tibial loading compared with those in the Rottweiler (57).

These findings were confirmed in another study examining intact CCL in two differing dog breeds at risk to CCLD (52). Craniocaudal laxity was significantly greater in the normal Labrador Retriever (high risk breed) stifle joints compared with Greyhounds (low risk breed) and this appeared to be related to altered CCL ECM ligament composition as indicated previously. In summary, these findings suggest that altered mechanical properties of high risk CCL occur prior to ligament rupture, contributing to increased stifle joint cranio-caudal laxity and ultimate joint instability.

Proprioception

The proprioceptive functions of the normal CCL are undoubtedly important for prevention of joint damage as well as for postoperative rehabilitation (58). Denervation impairs healing in the rabbit medial collateral ligament and alters expression of repair-associated gene mRNA (59, 60). Alterations in neuromuscular coordination have been implicated in increased ACL rupture rates in women (61). However the contribution of these to CCLD and rupture in the dog is not understood.

Conformational variation

Conformational variation of canine hindlimbs such as a straight stifle joint angle, narrow distal femoral intercondylar notch, and steep tibial plateau slope have been associated with CCLD (62–64). The presence of a straight (hyperextended) stifle joint and narrow intercondylar notch may inflict constant or intermittent impingement and abnormal compression of the CCL against the intercondylar notch (62). A steep tibial plateau slope, or excessive internal rotation ofibia associated with medial patella luxation together with genu varum may also cause increased internal stress and micro-injury of the CCL, leading to CCLD (64). Altered gait as demonstrated by stifle joint kinematics may also contribute to abnormal loads on the CCL of dogs with a high risk of CCLD (6) and differences (lower thigh and crus mass and thigh moment of inertia in CCL- deficient limbs compared to normal limbs) in body segment parameters (e.g. mass, centre of mass and moment of inertia) have been noted in Labrador retrievers with and without CCLD (65). More recently, the morphometric characteristics of the pelvic limbs of Labrador retrievers with and without CCL deficiency have been described, suggesting that cranial angulation of the proximal tibia, excessive tibial plateau angle (TPA), and distal femoral torsion appeared more likely to be associated with CCLD than femoral angulation, tibial torsion, and intercondylar notch stenosis (66).

There have been numerous studies evaluating the association of TPA and CCL rupture (14, 64, 67–70). Although anatomical differences in the shape of the proximal tibia have been documented in dogs with CCL rupture, its role in CCLD is unclear (70). The mean TPA in dogs varies between 23° and 25°, while a wide range of TPA has been reported (13° to 34°) in some studies (70). Although the correlation between CCL rupture and excessive increases in TPA (TPA >55°), possibly secondary to growth plate injuries, seems established, the association between TPA in the aetiology of CCLD in normal canine population remains controversial (64). Studies have shown that the TPA are not significantly different in Labrador Retrievers with and without CCLD, or between Greyhounds and Labrador Retrievers (69, 70). The true effect of TPA on CCL stresses in vivo is currently unknown, because muscular force, body size, obesity, rapid weight gain, relative inactivity, and exercise can influence the amount of stress sustained by the CCL, in addition to the TPA (6).

Intercondylar notch stenosis and its association with CCL and ACL rupture in dogs and humans respectively has been well described (71–73). The intercondylar notch width indices were found to be greater in the stifle joints of low risk dogs (Greyhounds) compared to those of high risk (Labrador and Golden Retrievers) (62). The authors concluded that impingement by the intercondylar notch on the CCL of high risk breeds may result in altered biochemical composition and reduced structural integrity of the ligament, predisposing the ligament to increased laxity and degeneration. Guerrero et al. evaluated the effect of conformation of the distal portion of the femur and proximal portion of the tibia on CCL rupture, and reported that no anatomical differences were detected in the distal portion of the femur between dogs with and without CCL rupture (74). The authors proposed that development of the tibial tuberosity and shape (convexity) of tibial condyles may be relevant in the pathogenesis of CCL rupture. Tibial tuberosity conformation may also be a risk factor for CCL rupture, with a recent study reporting that smaller tibial tuberosity widths were associated with increased cranial tibial thrust, which yielded propensity for more rapid development of CCLD, thus leading to rupture in a younger population of dogs (75).

Subchondral bone pathology

Increased femoral and tibial subchondral bone stiffness may be involved in the pathogenesis of OA (76). However, published and preliminary data suggests that subchondral bone stiffness preceding knee joint laxity and OA does not occur in guinea pigs (77, 78). Altered joint balance, as reflected in increased tibial subchondral bone mineral density, has been demonstrated in breeds deemed at high risk of CCLD compared to dogs at low risk to CCLD (E. J. Comerford 2010, unpublished data). Gait asymmetry and “handedness” in the dog may lead to this imbalance in high risk dogs contributing to increased subchondral bone mineral density and joint degeneration (E. J. Comerford 2010, unpublished data). Recently, magnetic resonance imaging studies suggested the significant role of subchondral bone lesions in CCLD mechanisms (79, 80). It is therefore
currently unclear whether altered subchondral bone mineral density plays an aetiopathogenic role in CCLD or may be intrinsic to evolution of the disease process in dogs or in humans.

Inflammation and immunity

The hostile intra-articular environment created by arthropathies such as immune-mediated arthritis, immune synovitis and joint sepsis may result in CCLD (81–84). Some authors have suggested that there is an immunologic component to rupture of the CCL, because of the demonstration of immune-complexes in synovial fluid and sera and immunoglobulin (mainly IgM) in the synovial membrane (83–85). A recent study evaluated anti-collagen type I antibodies in synovial fluid of the affected stifle joint and the contralateral stifle joint of dogs with unilateral CCL rupture, and concluded that synovial fluid antibodies against collagen type I alone do not initiate CCL rupture (86).

There is also considerable interest in the pro-inflammatory cytokines and their role in the catabolic processes occurring in pathological connective tissues, but their role in the pathogenesis of CCLD is still unclear (85–87). The expression of mRNA of cytokines in synovial fluid cells from multiple joints in dogs with unilateral CCL rupture was measured and this revealed that that IL-8 expression tended to be higher in stifle joints that will sustain rupture of the CCL during the subsequent six months by comparison with those joints where rupture will not occur (87). Collagenolytic enzyme expression has been found in the ruptured CCL and synovial fluid, and synovial macrophage-like cells that produce matrix-degrading enzymes have been identified (50, 88, 89). These findings suggest that inflammatory process may predispose to CCLD, by release of inflammatory mediators and proteolytic enzymes during inflammatory process. Release of collagenolytic proteases from the synovium into the stifle synovial fluid can significantly degrade the structural properties of the CCL and increase the likelihood of a pathological rupture (50, 88, 89).

In a recent study, a rat model of ACL injury demonstrated increased collagenolytic enzymes in synovial fluid (90). Of all tissues within the joint, the synovium contributed most to this increase. Thus inflammation of the epiligament may have an influence on the physiology of the cruciate ligaments through release and mediation of growth factors and cytokines and release of collagenolytic enzymes. Furthermore, as the epiligament does not appear to form a barrier to macromolecule passage between synovium and CCL, these molecules may act directly on the ligament (48).

The role of bacteria and associated synovial inflammation in the aetiopathogenesis of CCLD has also been speculated (82). Muir and others investigated the role of mixtures of bacterial nucleic acids in the pathogenesis of arthritis in naturally occurring CCLD using PCR, compared with that of normal stifles and stifles with experimentally induced CCL rupture (91). The presence of bacterial DNA within the synovium was significantly associated with naturally occurring CCL rupture and DNA from environmental bacteria was only found in dogs with the naturally occurring CCL rupture. The authors hypothesise that mixtures of bacterial DNA are an important causative factor in the pathogenesis CCL rupture. In addition, Muir et al. determined expression of a panel of immune response genes and matrix turnover genes in synovial fluid collected from dogs with CCL rupture, and suggest that antigen-specific immune responses within the stifle joint may be involved in the pathogenesis of persistent synovitis and associated joint degradation in dogs with degenerative CCL rupture (82).

Despite these recent findings, it still remains controversial whether inflammatory changes are primary contributors or are secondary phenomena in response to the tissue damage during CCL rupture, stifle instability and OA. To date little work has investigated for evidence of these changes in normal CCL from dogs at a high risk to CCLD.

Diminished healing capacity

Poor healing in the CCL has been well documented (39, 92). The canine CCL, in comparison with the medial collateral ligament, demonstrated the lack of provisional scaffold (fibrin-platelet plug) formation as well as reduced levels of key ECM proteins and cytokines within the wound (93, 94). The failure of the provisional scaffold to form in the CCL may result from increased urokinase plasminogen activator within the joint following trauma, a response thought to prevent arthrofibrosis in humans (95). This premature loss of the provisional scaffold has been proposed as a mechanism for failure of the CCL to heal (96).

The inorganic free radical nitric oxide has also been a topic of recent research. Tissue concentrations have been shown to increase in CCL compared to other articular stifle ligaments and nitric oxide has also been demonstrated in ruptured CCL (97, 98). Nitric oxide may inhibit collagen and PG production, and thus contribute to poor healing in humans (99). The canine CCL has been shown to produce more nitric oxide in vitro when exposed to an inducible nitric oxide synthase cocktail than explants of the canine medial collateral ligament or ligament of femoral head (100). However, in the same study a correlation between matrix metalloproteinase production and nitric oxide was not demonstrated, and its role in CCL physiology is largely unknown.

Other mechanisms proposed for poor CCL healing include deficiencies in stimulation or intrinsic deficiencies of cell migration and proliferation (101–104). Inadequate blood supply may also contribute to the development of CCL rupture (11, 41, 49, 92, 105, 106). An in vitro model of CCL injury showed increased matrix metalloproteinase expression, and a fold increase in matrix metalloproteinase-2 activity in the CCL compared to the medial collateral ligament. This may contribute to poor healing of the CCL by comparison with the medial collateral ligament which heals readily (90).

Conclusion

In summary, the aetiopathogenesis of canine CCLD is unknown, but it certainly appears to be a multifactorial condition re-
sulting in overall stifle joint ‘organ’ failure (107). One working hypothesis is that genetic and breed factors may contribute to abnormal stifle conformation, kinematics and gait, which then in turn may lead to cytokine and protease release from stifle joint synovial fluid and membrane, and possibly the CCL itself. However abnormal mechanics such as altered periarticular stifle joint conformation may also result in CCL compression at extremes of joint motion leading to structural changes within the ligament ECM (abnormal biology) which in turn can contribute to ligament laxity and joint instability. The ECM compositional changes (such as increased fibrocartilage) may in turn induce an inflammatory reaction, switching on an inflammatory cascade within the stifle joint contributing to CCLD and ligament rupture. Whether abnormal biology or mechanics initiate CCLD as has been discussed by Cook, the authors of this review agree that both factors play a significant overlapping role in the progression of stifle joint OA and ultimate joint failure (107).

References

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