Title: Spontaneous Dog Osteoarthritis –a 'one medicine' vision

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ABSTRACT

There is a growing awareness within the public and respective research communities that 'one medicine'; the mutually beneficial co-study of animals and humans, could unlock great benefits for both. It is therefore timely to explore the types of research that could be enhanced through this approach. Our review examines the proposition that suitably aligned studies of spontaneous clinical osteoarthritis (OA) in dogs can provide a wealth of research material and understanding relevant also to human, which cannot currently be obtained from rodent or experimentally-induced models.

INTRODUCTION

Osteoarthritis (OA) is the end-destination of a heterogeneous group of disease processes and its research is therefore complicated. The importance of OA as a global disease and a modern major health challenge necessitate new research strategies. In 2005, 26.9 million US adults were estimated to have OA[1] and it accounted for 2.4% of all years lived with disability (OARSI white paper 2016). OA is also a major disease burden in the dog, with an overall prevalence of 2.5% in UK veterinary primary care practice[2], rising to 80% when over 8 years of age[3]. Duration estimates calculate that affected dogs suffer with OA for around 11% of their lifespan[2].

Intriguingly, dogs show distinct, OA type-specific epidemiological patterns, notably between different breeds, as well as a clear influence of body size, obesity, sex, neuter status and age[2]. Spontaneous canine OA is generally considered to bear close resemblance to human OA, in terms of anatomic similarity, disease heterogeneity, and progression[4], appearing more informative than induced dog models. For example, changes in articular cartilage proteoglycans observed in slowly progressive spontaneous OA in dogs, regardless of their age, closely match those in human OA, and differ significantly from those seen in rapidly advancing experimental dog OA induced by anterior (cranial) cruciate ligament transection.[5]

Humans and companion canine animals both live into old age, share many environments and activities, and now often receive identical disease management, such as prolonged administration of anti-inflammatory drugs or joint replacements. Academic veterinary medicine has also developed to a point that it can provide valuable biomedical research data; referral centers are now routinely equipped with magnetic resonance and

computerized tomographic imaging, arthroscopy, and have access to immunohistochemical and molecular diagnostics. They are also starting to pilot advances in the use of anti-inflammatory and pain modulating drug therapies for OA[6-9].

This review presents a narrative synopsis of key research relating to common forms of spontaneous dog OA and places them within a framework of OA types with human disease alignment. We overview molecular genetics, methods of disease and functional outcome assessment, pain studies, and future perspectives, in the hope of highlighting potential for collaborative efforts that will expand our knowledge of dog OA for the benefit of human and veterinary patients alike.

ONE HEALTH, ONE MEDICINE & VETERINARY MEDICINE

The 'One Health' concept, which recognizes that human health is closely connected to animal health and the environment, has ancient origins dating back to Hippocrates and Aristotle. Claude Bourgelat, a key founder of 18th century veterinary medicine, advocated this intimacy, which was further emphasized by the 19th century physician Rudolf Virchow who coined the term '*zoonosis'* upon discovering that *Trichinella spiralis* in pigs caused human neurocysticercosis[10]. Despite historic recognition of this ideology, a culture of marked anthropocentricity emerged during the 1970s, shifting research emphasis to induced 'experimental' animal models.

One Health approaches regained momentum following the outbreaks of highly pathogenic H5N1 avian influenza (1996) and Corona virus-associated Severe Acute Respiratory Syndrome (2003). Distinct from One Health, *'One Medicine'* is now emerging as a holistic paradigm wherein veterinary and human medical research and clinical practice collaborate to increase their understanding of shared diseases and develop new therapies[11]. Companion animals represent a significant population, with ~70 million pet dogs in the USA alone[12]. Dogs typically live into old age, come in all shapes/sizes, from highly athletic to sedentary and overweight, and live intimately with humans. As they develop many age-related chronic diseases and co-morbidities on a foreshortened timescale (breed-influenced life expectancy of around 8-12 years) that are analogous to humans, there is a growing view that developing our understanding and treatment of dog OA could lead to breakthroughs in human OA[13].

PROBLEMS WITH EXPERIMENTALLY-INDUCED ANIMAL MODELS OF OSTEOARTHRITIS

Experimentally-induced OA models are available in many large and small species[14]. Undeniably, because they are small, easy to house, relatively inexpensive and genetic tractable, mouse models have contributed to advancing understanding of basic disease mechanisms. Regrettably, they have proved to be poor predicators of the efficacy or toxicity of new drugs in human trials[15]. Rodent model OA is usually either chemically or surgically induced. The veracity of such chemical induction with intra-articular papain or monosodium iodoacetate has however been questioned, with many concluding they have utility limited only to studies of 'joint pain' and hence, surgical joint destabilization is most frequently employed.

Early work established the clinical, biochemical and histopathological changes induced by anterior cruciate ligament (ACL) transection in dog stifle joints (Pond-Nuki model)[16,17,] or medial meniscectomy in rabbits[18]. This heralded surgically-induced OA models in smaller genetically-tractable species. Surgical medial meniscus destabilization[19], usually performed in 10-12-week old animals, is currently the most widely used model, but is by no means an ideal or 'gold standard'. Genetic modification in mice undeniably offers the advantage of allowing single gene effects to be investigated[20,21] and is used extensively; some mice, notably the STR/Ort strain, exhibit idiopathic spontaneous OA[22]. Whilst the value of these rapidly evolving murine OA models should not be underestimated, 'natural' companion animal disease may more closely reflect the complex genetic, physiological and environmental variation seen in human OA[8,23], whilst reducing the numbers of animals used for research.

ANALOGOUS CANINE AND HUMAN OSTEOARTHRITIC DISEASES

Spontaneous slowly-progressing OA occurs in various mouse strains and guinea pigs, Syrian hamsters, dogs and non-human primates, where, in general, its histopathology and pathogenesis likely more closely resemble primary human OA[24]. This similarity is prominent also in dogs with complex naturally occurring traits that share co-morbidities, such as obesity, with humans. Whilst dog OA is likely more variable, takes longer to develop and thus requires larger numbers than mouse studies to achieve appropriately powered study design, this review outlines some common naturally occurring forms in order that readers may consider their suitability as models for human equivalents.

Dysplastic hips

Hip dysplasia is a frequent risk factor for OA in both humans and dogs[25-28]. It is estimated that 25-50% of idiopathic human hip OA is due to developmental dysplasia (DDH); many later needing replacement[1,29,30]. Canine hip dysplasia (CHD) shares pathoanatomical, biochemical and clinical features with DDH and is proposed to be the best spontaneous large animal model for DDH[5,31]. Both show delayed capital ossification and an underpinning continuum of instability (detected by Ortolani test), with severe forms characterized by complete subluxation (Figure 2A-C) leading to focal cartilage overload and hip OA in untreated, or undertreated children and dogs[30,32-34]. DDH and CHD are morphologically similar; e.g. collagenous fibrils in articular cartilage of DDH patients are sparse and disordered, closely resembling TEM observations made 35 years earlier in CHD.[35] Many older dogs classified with normal hip conformation at two years (~adulthood) develop OA resembling human acetabular dysplasia and secondary OA in old age[36].

Does CHD occur with sufficient predictability to provide a feasible model? CHD occurs with 75% prevalence in Golden Retrievers and Rottweilers[37]. This has heralded a need for early-stage hip laxity screening[38], as in humans, and improvement programs with novel laxity measures (distraction index, University of Pennsylvania), which allow screening at four months to identify dogs highly unlikely to develop OA by three years[36,39]. CHD resembles DDH clinically and pathologically but progresses over a compressed timeframe,

further improving its utility as a model. Many screening programs and registries employ traditional hip extended pelvic radiography (Figure 2B, C) and some have DNA banks. This highlights an opportunity to identify genetic, epigenetic, or environmental factors common to both DDH and CDH which have phenotypic characteristics similar enough to warrant simultaneous clinical and basic research, with view to augment progress in understanding, treating and preventing dog and human hip OA secondary to hip dysplasia. Recent MRI studies have explored the role of foetal movement in determining bone shape and DDH[40]. Although such MRI studies are currently difficult to undertake in dogs, it does suggest the possibility of a potential 'One Medicine' approach to advancing research in this field by careful foetal tracking of the developmental emergence of joint incongruity in DDH and CDH.

CHD and DDH treatment options consist of similar symptom management, hip reconstructions and replacement methods (Figure 2D-G). Clinical features and imaging biomarkers to identify DDH, CHD and hip OA risk at an early stage would be beneficial. Trait similarities and a truncated canine lifespan make the uncovering of common early features of end-stage hip OA likely more rapid in dogs with CHD. The dog is also an excellent model of naturally-occurring hip OA[41] and human total hip replacement[42,43]. Dogs and humans have similar bone remodelling characteristics and both require replacement for non-responsive and debilitating end-stage disease[44-46]. CHD and DDH are both followed up using similar clinical and functional measures, including validated clinical questionnaires, gait analysis and accelerometer measurements, and imaging techniques[43,47-49], therefore making for an ideal clinical model in which OA progression and the efficacy of novel therapies can be investigated.

Ruptured cruciate ligaments

Canine knees have human-like anatomy[50] and have been used in several surgical OA models, including transarticular impact[51], tibial osteotomy[52], meniscal sectioning[24], articular cartilage scarification groove model[53] and ACL transection[16]. The progressive and predictable OA changes in the ACL transection model, in particular, are often tracked in the evaluation of new therapies[54] and show molecular changes regulated by the same genes as human post-traumatic and late OA[55]. Features typical of human knee OA, including lameness and pain, effusion, osteophytes, cartilage erosion, synovitis, subchondral sclerosis and bone marrow lesions develop in each of these models.

Human ACL rupture leads to the progressive development of significant joint OA[56] and the same is true of dogs [57]. Spontaneous ACL rupture is common in dogs and certain breeds are particularly predisposed[58,59]. Analogous ACL transection is well documented to cause inflammation with cartilage and synovial reparative responses, yet ongoing instability prompts cartilage erosion and proliferation, and subchondral bone changes, mirroring spontaneous knee OA[60]. Spontaneous knee OA has ~20% prevalence in some dog breeds[61] and ~50% develop contralateral knee ACL rupture within one year, in commonly-affected breeds such as Labrador Retrievers[62]. These natural homologs of experimental ACL transection also develop early osteophytes and sclerosis[57] and end-stage OA over several years[63,64] (Figure 1H). It is, however, highly likely that the aetiology

of rupture differs, with spontaneous canine ACL rupture typically involving non-traumatic, progressive, prior degeneration and weakening at physiological loads [65] (Figure 1I, J). This contrasts to the trauma-related ACL rupture in humans, which is typically a result of non-contact sporting injury[66]. Whilst the underlying mechanisms of the canine ligament pathology remain undefined, predisposed dogs display thinner collagen fibrils in weaker ACLs, with increased expression of matrix metalloproteinase-2 (MMP2)[58]. Although rupture in young human ACL is considered truly traumatic, ~70% of macroscopically normal human ACLs have histological evidence of pathology consistent with early degeneration[67], questioning whether there may be greater homology than previously thought.

Irrespective of the route of anterior cruciate deficiency, the resultant mechanical instability and trauma in both dogs and humans, drives progressive OA and is frequently associated meniscal pathology [68], and hence dog ACL disease/OA is an excellent model of human knee OA. Data from studies of dog knee OA show that neutering increases the risk of ACL rupture as does being female and overweight[6959]. It has in fact been found that estrogen reduces ACL collagen synthesis *in vitro*[70,71] and that the risk of ACL rupture in female athletes is increased on the first and second day of their menstrual cycle[72]. Hence the *at risk* female dog may offer insight into the potential roles of hormones or post-neutering weight gain[73]. Primary knee OA incidence in post-menopausal females is also higher than in age-matched men, suggesting possible hormonal influences.[74] Additionally, the common and predictive nature of dog cruciate rupture and OA, offer unique opportunities such as rising synovial fluid concentrations of IL-8 predicting contralateral cruciate ligament failure[75]. It remains to be seen if these are recapitulated in humans.

Osteochondrosis lesions

Canine shoulders not only develop age-related primary OA[76] but also most-commonly osteochondrosis, with osteochondritis dessicans lesions (OCD; Figure 1A-C)[77]. Osteochondrosis occurs in many animals[78,79] and humans and is characterised by disordered endochondral ossification, superimposed upon previously normal growth[80]. This accepted pattern of pathogenesis emanates from work in pigs, but data from other species lacks consistency. The location, radiographic and macroscopic appearance of lesions in femoral and humeral condyles and trochlear talus does however point strongly to shared aetiology[81]. In dogs, osteochondrosis predominates in medium/large breeds, affects males more than females and is often bilateral and site-specific[82]. Intriguingly, human males are also more frequently affected and bilateral disease is common[77,83,84].

Most histological human osteochondrosis studies use samples from end-stage disease, thus limiting scope to elucidate factors influencing onset. Some, nonetheless, have shown evidence of fibrocartilage at the junction between osteochondral ossification and opposed parent bone, resembling delayed or ununited fracture tissue[85]. This contrasts completely with reports of absence of calcified tissues in human and animal tissues, and suggests that osteochondrosis does not originate in subchondral bone[86,87]. Unilateral osteochondrosis in young dogs allows for sampling of early contralateral lesions and for arthroscopic autologous or biomaterial articular resurfacing[88,89]. There are strong links established between aberrant re-induction of endochondral ossification

processes in both human and mouse OA articular cartilage[90,91]. It is therefore intriguing that the canine shoulder is targeted in this particular way, much more so than the human. Future studies might focus on the role of the mechanical environment in the canine shoulder as a stimulus for the re-induction of these aberrant endochondral-like processes.

Dogs exhibit astounding, several-fold size variation and clear inter-breed divergence in growth rate and/or physeal closure at puberty. Earlier physeal closure in small breeds is consistent with more rapid growth and likely OCD predisposition. Growth plates in larger Great Dane breeds have a larger hypertrophic region and more active BMP2/BMP6 signaling than miniature breeds[92], suggesting that studies of OCD may give unique insight into the role of longitudinal bone growth in this form of OA. Another possible connection emerges from studies that establish a direct linkage between genetic selection for high growth rates, failure in mechano-adaptive bone changes and predisposition to skeletal diseases, as seen in chickens[93,94]. Whether similar relationships persist in dogs and humans has yet to be explored.

COMPARATIVE GENETICS OF OSTEOARTHRITIS IN DOG BREEDS

The Victorians (1837-1901) engendered immense pressure on canine evolution. Nearly all ~400 recognised dog breeds were stringently selected to create huge intra-specific phenotypic and behavioral variation; further reinforced by rigorous Kennel Club requirements. Broad linkage disequilibrium is therefore a characteristic of many breeds due to founder events and selection bottlenecks. Many breeds are, in essence, a homolog of the rare isolated human populations much coveted by geneticists. This selection concomitantly created significant naturally occurring, polygenetic disease predilection in some breeds. Intra-specific comparison of dogs (affected vs. unaffected) offers scope to identify candidate disease genes from these polygenetic conditions. Frequently, an argument is made for comparing pure breed dogs to mongrel or crossbreeds. We would argue that the advent of designer crossbreeds such as the ubiquitous Labradoodle (Poodle x Labrador) and the difficulty of defining the source breeds in most mongrels and crossbreeds, that this type of comparison is best avoided. Instead, it is more informative to compare high disease prevalence, pure breed dogs to low prevalence pure breeds, such as Labradors vs Greyhounds for hip dysplasia or cruciate rupture. As the canine genome is sequenced[95], identification of genome-wide associations with fewer markers in dog breeds than is needed in outbred human populations offers significant opportunities; a few from hip and knee that have significant potential are focused upon in the next section.

Hip Dyplasia: Whilst a genetic basis of DDH is almost certain[96,97] this is undisputed in CHD[98]. DDH occurs in 1-20/1,000 live births, across all races and predisposing factors include familial history, being first-born and breech birth position[99,100]. CHD frequency in different breeds varies much more markedly, reaching ~75% and, in contrast to DDH, shows no sex predilection in most breeds; female Polish Tatra Sheepdogs however have >3-fold risk over males[101]. This greater intra-/inter-breed variation may yet prove valuable in identifying the genetic basis of hip dysplasia. Demographics of CHD more closely mirror DDH in late onset acetabular

dysplasia[101]. Familial segregation studies suggest human DDH has a multifactorial genetic basis, but statistical support for this varies across populations and nationalities. It was recently reported that recurrent risk among siblings of affected families was ~10-fold greater than in controls, with high heritability (~85%)[102]. Dig CHD heritability estimates range from 20-60%[103]. Multipoint linkage and GWAS (genome wide association studies) suggest that 5-10 quantitative trait nucleotides (QTN) of modest effect, control CHD expression[104]. These findings are however not always replicated in different countries with different breeds[105].

Some 15 genes with known roles in embryonic patterning, ECM structure and remodeling, are now associated with DDH predominantly via screening for candidate polymorphisms[106]. Many lack replication, except CX3 chemokine receptor 1 (CX3CR1, aka fractalkine, G-protein receptor) that was first identified by linkage and exome sequencing[107,108] and recently a polymorphism independently linked with DDH[109]. CX3CR1 serves roles in mesenchymal stem cell recruitment and CX3CR1-deficient mice develop acetabular dysplasia[110].

Bernese Mountain dogs also possess a canine chromosome (CFA) 37 locus with significant CHD association (near *FN1* gene associated with human DDH)[111]. Alternative CHD-associated loci identified by GWAS in UK Labrador Retrievers (>1,000), include those on CFA01 and CFA21[105]. Another across-breed mapping study identified a *CTBP2* SNP on CFA28, linked to CHD, specifically the Norberg angle[41]. This and two more loci nearest *TRIM2* and *DPP4*, were later associated with CHD by analysis of the same data by a novel iterative mixed model approach[112]. Intriguingly, Feldman *et al*. found that three patients severely affected by sporadic DDH shared an identical frameshift ZRANB1 mutation[108]; notable, as *ZRANB1* is in the same canine linkage disequilibrium interval as the *CTBP2* polymorphism on CFA28[41]. Similarities in DDH and CHD genetics indicate that studying CHD in these selected breeds will yield novel mechanistic insights into hip dysplasia aetiopathology in these, and potentially other species (Figure 3).

Like DDH genetic studies, dog GWAS have also resisted replication across breeds and laboratories. It is well to consider that structural variants (deletions, duplications, inversions and translocations) are estimated to produce ~30% of causal variants, fine-mapped in dogs. These are often not detected using genome wide SNP arrays. In 4,200 genotyped dogs, most variants were poorly tagged by markers in a high-density mapping array of over 180,000 markers. Thus, previous canine GWAS are likely to have missed most causal variant mutations. An intronic deletion in *FBN2* was associated with CHD in a linkage analysis of a direct hip laxity trait (distraction index) and also showed upregulation in samples from dysplastic dog joints[113]. Although there is strong evidence that the phenotype and progression of secondary OA are similar in dysplastic human and dog joints, joint genomic, transcriptomic, biomarker, and methylomic analyses are likely to be highly informative. Fresh samples can be retrieved readily from dogs undergoing joint salvage procedures and may facilitate candidate gene screening to overcome the replication barrier, as genetic links are likely to have been missed previously. Whole genome sequencing and genotype imputation is likely necessary to capture all causal mutations in canine GWAS.

Legg Calve Perthes Disease (LCPD): characterized by slow femoral head destruction in children (and adolescent avascular necrosis of the femoral head (ANFH), has an ortholog in small breed dogs. Radiographs exhibit a continuum from mild disease with subchondral and epiphyseal osteolysis, to complete femoral head

obliteration (Figure 1F, G). Hip coxa plana (coxa vara and elevated femoral greater trochanter) deformity and premature OA are typical LCPD features in children and small breed dogs. Bilateral hip OA is common in human LCPD, peaks between 4-8 years of age[114] and occurs ~4 times more often in boys (~1:3,000). Dogs, in contrast, show no sex predilection.

LCPD and ANFH symptoms include hip pain, limping and differing limb length. Clinical signs appear in Yorkshire Terriers, Maltese, Miniature Poodles and Chihuahua during early life (~3-11 months) and peak at skeletal maturity (6-7 months). Histologic findings suggest obstructed blood supply and necrosis of the femoral capital epiphyseal bone. Vascular studies also demonstrate greater vulnerability to trauma in the femoral epiphyseal blood supply in susceptible small breed dogs when compared to non-susceptible, mixed breeds[115]. Interrupted blood supply and local hypoxic injury are thus common in both LCPD pathogenesis in both children and young dogs[116]. Human LCPD patients exhibit elevated Factor V Leiden serum levels[117], polymorphisms in endothelial nitric oxide synthase[118], abnormal complement and coagulation cascades, and lipid metabolism[119]. Whilst raised serum levels of coagulation cascade proteins were not seen in 18 LCPD-affected dogs[120] it is evident that there are phenotypic, demographic, and hormonal similarities to human LCPD, including low circulating insulin-like growth factor-1 levels, reduced arterial caliber and function, and a hyperactive personality[121,122] (Figure 3).

Familial and isolated LCPD occurs in humans[123,124], with an estimated ~0.84 heritability in relatives of probands (first affected family member)[125] as well as links to environmental and demographic factor(s)[126]. Such heritability was found in a pedigree of experimental Manchester Terriers[127]. Odds ratios for LCPD ranged from 4-191 in small pure breeds compared to a mixed breed population[128]. A *COL2A1* mutation associated with LCPD in isolated human families[23,129,130] has been excluded as a candidate in dogs[122] and in humans with associations with apoptosis-related genes[131]. A major canine genetic locus with incomplete penetrance and autosomal recessive inheritance has also been proposed[132]. Human methylomic studies[133] and others have however concluded that even familial LCPD clustering may not have a strong genetic component, since co-twin and even monozygotic twins of an affected individual have low absolute LCPD risk[134]. This however, does not exclude canine studies as a means of revealing common aetiopathologic pathways in non-COL2A1 associated canine and human LCPD.

Anterior (Cranial) Cruciate Ligament Rupture: Non-contact rupture of human ACL has a complex etiology and >50% of operated patients have pain and secondary OA at 10-year follow-up. As in the dog, variation in outcome is influenced by age, sex, genetics, obesity, muscle strength, activity and re-injury[68]. Young female athletes have 3-6 fold elevated risk of ACL injury[135]. This doubles in those with similarly-affected relatives[136] and is raised further in Caucasians[137], suggesting gender- and genetically-linked human determinants. Five year-old dogs consistently show degenerative microscopic and material changes in the cranial cruciate ligament (CCL human anterior equivalent) (Figure 1K). Susceptibility to CCL rupture is increased in Labradors and Golden Retrievers and their CCLs have elevated collagen turnover, decreased stiffness, and less mature collagen crosslinks than those of relatively rupture- resistant Greyhounds[58]. The genetics of dog CCL rupture are complex, with a 0.15-0.27 heritability in the Newfoundland which have 4 putative QTL by linkage analysis[138], but non-overlapping association on CFA1, 10 and 33 by GWAS[139]. A case: control comparison

across four breeds, revealed SNPs key to ligament ECM composition and strength associated with CCL rupture susceptibility[140]. Huang et al., later reported associations on CFA7-9[112] and Baker et al., on CFA24[141] that reached genome wide significance for CCL rupture in Labrador Retrievers. This lack of replication is likely due to similar limitations that apply to CHD (see above).

Gene polymorphisms in *FBN2*[142], *VEGFA*, *KDR*[143], *COL1A1*[144], *DCN*, *ACN*, *BGN*, and LUM[9], *COL5A1*[145], and interactions between *COL5A1* and *COL12A1* are linked to human ACL rupture; many encoding ECM proteins and growth factors. Kim *et al*[146] and Kaynak et al[147] elegantly reviewed genetic associations with human ACL rupture and describe a *COL1A1* polymorphism that replicated in several studies[148-151]. The former followed with a GWAS screen, which failed to unveil ACL rupture associated polymorphisms, highlighting that replication and cross-species overlap are vital in complex traits. Functional studies based on relevant temporal tissue samples that identify expression QTL which overlap with genomic QTL and, with induction of phenotype in other species will be necessary to establish causation. (Figure 3).

FUNCTIONAL OUTCOME ASSESSMENT IN OA

Pain is a cardinal symptom of OA, and symptomatic management with a limited repertoire of drug groups, in particular analgesics and anti-inflammatory drugs plays a central role in veterinary[152] and human treatment. This empirical and limited approach severely hampers any useful information gathering. A clear distinction between dogs and humans however, is the ability to self-report pain. Although many veterinary studies have used visual lameness and clinical pain assessments, which report only single outcome measures, force plate and radiography are most commonly used [153]. This has led to objective force plate outcome measurement, becoming a common 'gold standard' for functional assessment in dog research. Kinetic gait analyses using force plates and pressure mats provide objective snap-shots of impairment[154,155], and the size and amenable nature of dogs make them suitable for such assessments[155,156].

As subjective measures of pain can be readily quantified in humans, similar objective data has only had limited use[157]. Instead, clinical metrology instruments and a patient-centred approach to outcome assessment has become a mainstay in human OA assessment. The patient-centered approach has now been appropriated into veterinary assessments. In dogs, clinical metrology instruments or validated outcome questionnaires are also used to capture pain-related behavior over prolonged periods in home environments[158], with pet owners providing proxy assessments just as parents or care-givers would[159,160]. Although this methodology is significantly more available than objective assessment, the proxy reporting remains an issue for their relevancy. Nonetheless, these instruments are validated, cheap and straightforward to manage and analyse, potentially expanding the ability to gain additional outcome assessments from veterinary trials. Examples include the Canine Brief Pain Inventory (CBPI) [161] that is analogous to the human Brief Pain Inventory (BPI)[48,160]. Such inventories, including the Liverpool OA in Dogs index[162], have to: i) be valid, reliable and responsive to clinical change, ii) measure what they seek and, be validated against a gold-standard, such as force plate analysis, and iii) demonstrate reliability to generate the same outcome whenever an unchanged subject is re-assessed[160]. Their power in showing disturbed sleep in dogs with OA verifies their utility [163].

New miniaturised data recording technology make telemetric accelerometry or activity monitors practical in the clinical setting. These objective assessments are cheaper, less complicated than force plates and offer easier longitudinal assessments for OA interventions and disease progression[156,164-166]. Many other tests are useful in OA monitoring, including thermal imaging and mechanical nociceptive threshold testing[167,168]. Functional activity monitoring, force plate analysis, and advanced MRI are performed in dogs in a manner that mirrors human patients. Brain imaging in conscious pet dogs is also reliable and practical, with obvious potential for comparative neuroscience studies[169,170].

Pain models: US Food and Drug Administration (FDA) guidelines for OA drugs, devices and biological treatment are available but, as they note, pre-clinical research advances are not being translated into effective new drugs in clinical practice, leading to questions regarding the predictive utility of current animal models (http://www.fda.gov/downloads/Drugs/GuidanceCmplianceRegulatoryInformation/Guidances/ucm071577.pdf) [17115,172]. Do current animal models effectively mimic OA stage, with measurable and translatable outcomes? Similarities in neurophysiology across mammals strongly suggest that pain, experienced in humans and animals is identical[172]. However, pain experience in OA is complicated and involves peripheral nociceptive sensitization, structural changes in joint innervation, central nervous system sensitization and neuropathic changes and a host of mediators as well as simple nociceptive input from damaged joint tissues[173]. Pain severity often shows poor correlation with radiographic human[174] or dog OA[175] or visible structural joint changes alone. New OA pain therapies thus require effective models that recapitulate OA joint changes as well as clinical symptomatology.

OA pain levels are influenced by synovitis, osteochondral pathology and sensitization, not accounted for by structural radiographic change[173,176]. Good OA models need to reflect the natural longitudinal history of human OA and, hence, studies of spontaneous dog OA phenotypes with advanced non-invasive imaging may best resemble progression in some human OA phenotypes[177,178]. Semi-quantitative MRI is powerful for imaging hitherto unobserved OA processes; it is reliable, validated and has already been used in multicenter clinical trials[179,180].

Defined by characteristic MRI signal intensity changes, the presence, number and size of recently identified bone marrow lesions (BML) have been linked intimately with human OA pain severity[181]. Natural animal BML models are clearly required and their potential has now been demonstrated in studies linking BML-like structures with focal articular cartilage change and disability in the dog ACL transection model and dog CCL rupture with OA[182]. The search for model species for human pain needs also to carefully consider the evolutionary role of pain responses. As prey, rodents are thought to show less overt pain signs than predators, like humans and dogs. As these 'responses' are common end-points for measuring pain, it is pertinent that they are evolutionarily intertwined. Thus, fellow predator species, like dogs, are likely to more accurately represent human pain physiology than rodents.

Quantitative Sensory Testing (QST) has been used in laboratory settings and humans to quantify pain. QST-assessed central sensitization has been demonstrated in human OA [183,184], in experimental dog OA[185] and recently in spontaneous dog OA with increased mechanical and thermal allodynia[186]. QST efficacy has also been demonstrated in dog total hip replacements where, as in humans [187], hyperalgesia was reversed[188]. Clinically-affected dogs could therefore be optimal for testing anti-hyperalgesia therapies and, at the same time, realize the potential benefit. Overall, there is compelling evidence that studies in companion dogs with OA and chronic pain may reliably predict treatment efficacy in humans through randomised controlled veterinary trials (RCVTs)[166,189,190]. Parallel drug intervention dog studies are thus appropriate to accelerate drug trials designed to treat human pain and may speed off-license pain treatment to improve the welfare of dogs as well.

A SHARED ENVIRONMENT, DIET AND OBESITY

Obesity is becoming a health crisis for both humans and their pets. Thus, >40% of USA adults were obese in 2015-16[191] and, similarly, prevalence of dog obesity was 24% in the 1980s, rose to 41% by 2005[73,192] and has likely increased further. Obesity is a known risk factor for human[193] and dog OA[194], yet evaluating its independent influence in humans is difficult. Work with inbred experimental dog colonies, however, has clearly shown that dietary restriction reduces OA. Six week-old gender- and body weight-matched Labrador retriever pairs from closed, inbred colonies were either 'control-fed' (*ad libitum*) or 'diet-restricted' (75% of control-fed). Radiographic hip OA was found in 42% of control-fed dogs by 2 years (4% in diet-restricted), which increased to 52% (vs. 13%) by five and reached 83% at 15 years (50% in diet-restricted). Intriguingly, diet-restriction also increased longevity[195] and weight only moderately correlated with OA severity, suggesting that other factors, related to increased food intake, exert influence[196]. Diet restriction also reduces severity and prevalence of shoulder[76] and elbow OA[197]. Whilst the aetiology of obesity-related OA remains unclear, mechanical joint impact from excessive mass overloading has been proposed; this is despite the predisposition extending to hand OA in obese humans which suggests that this form of OA incitement is more systemic. A humoral role for adipose tissue in driving systemic low-grade inflammation, with increased adipokines has instead been implicated [198]. Dog adipocytes have also been shown to express key adipokines and overweight dogs are commonplace, much like their owners.

APPLICATIONS AND PROSPECTS

There has been a growing drive to view OA not as one disease but as a syndrome encompassing heterogeneous, stratified groups of different associated populations and characteristic etiologies. This has led to a recent growth in the appreciation that new targeted therapeutic approaches might be accelerated by OA stratification, based on phenotype (or endotype), which may also lead to better alignment with preclinical animal models. We conjecture that the common dog OA types we have highlighted in this review provide models for ready alignment based upon anatomy, aetiology and pathophysiology and propose a system for their use with view

to analogous human OA (Table 1)

Human disease stratification, based on phenotype has previously identified five OA subdivisions based upon joint involvement, muscle strength, obesity and psychological depression[199], whilst a systematic review by Dell'Isola[200] identified six groups with either central chronic pain sensitization, inflammatory, systemic metabolism, bone/cartilage remodeling, mechanical overload and minimally symptomatic OA phenotypes. Osteoarthritis Research Society International (OARSI) recommends five phenotypes based on clinical presentation criteria[201] and another systematic review of knee OA identified gender, obesity and other metabolic abnormalities, cartilage damage patterns, and inflammation variables upon which distinct structural OA phenotypes might be delineated[202].

What are the prospects that the study of dog OA in such a One Medicine approach might therefore accelerate new developments? Currently, cancer research demonstrates the most readily adopted application of the One Medicine approach. Cancers account for >50% of dog mortalities and, like OA, its multifactorial and complex aetiology reduces the predictive value of rodent models. The Canine Comparative Oncology Genomics Consortium (National Cancer Institute, 2007) initiated an extensive, naturally-occurring canine cancer tissue bio-repository. Partnerships between veterinary/human oncologists and biologists later generated a Comparative Oncology Trials Consortium[8], which rapidly revealed new facets of carcinogenesis [203,204], translated to human trials[205]. From the examples highlighted in this review, the authors identify four clear opportunities to take this approach forward in OA research:

1. A source of natural diseased tissue for research

From the examples provided in the proposed categorization of OA types, researchers could identify a potential clinical dog syndrome and then perform studies to verify the validity of the alignment we propose (in Table 1). This could, for example, involve exploring whether there are in dogs as in humans, two distinct subgroups of symptomatic knee OA patients based upon inflammatory gene expression profiles in peripheral blood leucocytes[206] or whether dogs exhibit the alternative metabolic or cell senescent 'mechanistic' human OA phenotypes[202]. In addition, clinical sample retrievals such as OCD fragments, resected ruptured anterior cruciate, excised damaged meniscus, plasma or urinary or synovial fluid sampling for biomarker assessment, or resected osteoarthritic femoral heads from hip replacement procedures would facilitate greater understanding of OA mechanisms, and perhaps enhance diagnostic and prognostic criteria.

It would also be possible to correlate arthroscopic, surgical and advanced imaging data with stage-specific changes in samples taken from dogs with specific OA phenotypes (Figure 1C, 1M). Examples include CCL transection and synovial fluid and serum sample analysis along with correlation with joint scores which has been performed in experimental models previously but could be evaluated in spontaneous dog OA[207] with appropriate OA staging[207-209] and radiographic scoring[210]. Compared with rodent models, in which such evaluations are not routine or even technically feasible, larger dog joints permit longitudinal study with modern imaging and tissue sampling, and potential for revealing additional insights into early and later stage OA. Indeed sampling could begin as part of a clinical trial, as soon as clinical, radiographic, CT or MRI evidence

of abnormal joint architecture is identified. For CCL rupture, dogs with unilateral CCL disease often have premonitory radiographic and clinical signs of synovial effusion. Further partial CCL tears are often associated with painful lameness in affected dogs even though instability is minimal. Measuring soluble biomarkers in biological fluids might facilitate early diagnosis or evaluation of interventions[211]. Getting usable samples of sufficient quantity is a practical possibility when working with large animal dog OA models (e.g. Cornell Veterinary Biobank; https://www2.vet.cornell.edu/departments/centers/cornell-veterinary-biobank).

2. A means to identify the genetic underpinnings of homologous disease

Inbred dogs lend themselves to genetic analysis of complex diseases; hip dysplasia, OCD and Legg Calve Perthes are excellent examples. Dogs with OA, with blood collected for routine haematology/biochemistry (for clinical management) could potentially have any residual blood directed into research. Tissues removed as part of clinical disease management could also be utilised. Making use of the broad linkage disequilibrium introduced by selective breeding with high predilection breeds versus low predilection pure breeds will help to identify candidate disease genes in these polygenetic conditions.

3. An intermediary between rodent and human clinical trials with natural disease

Dog OA is ideally suited for veterinary Randomised Controlled Trials (V-RCTs), because of the rigor of the functional outcome measures. Recent work comparing peak vertical force (PVF) and accelerometer data to continuously track activity at home, in spontaneous ACL disease showed excellent between-session reliability, well-aligned with locomotor activity. This indicates that PVF is a robust, reliable and reproducible non-invasive tool for monitoring and assessing the effectiveness of new therapies in natural knee OA[212]. Such studies are free from the ethical objections associated with the use of experimental dog models and are absolutely aligned with the 3Rs agenda[213]. They are also cheaper and increase the possibility of biological sampling without using additional dogs. Examples demonstrating this utility include the study of anti-nerve growth factor treatment in dogs[166] and humans[214] and also the use of a novel anti-inflammatory agents, licofelone and doxycycline, each of which was similarly effective in spontaneous dog OA[215,216] and human OA patients in Phase III trials[217,218]. Intra-articular hyaluronan injection in humans[219] and in dogs with CHD[220] also showed comparable short-term symptomatic benefit without structure modifying efficacy.

An example, where dog OA studies have primacy in the One Medicine approach include trials of stem cell therapy, which have advanced more rapidly in canine OA, than in humans. Allogenic mesenchymal stem cells harvested from visceral adipose dog surgical waste (from ovariectomy) have been combined with hyaluronan and injected intra-articularly into dysplastic dog elbow OA joints, with reports of reduced lameness and hyaline-type cartilage regeneration[221]. Measurement of PVF and vertical impulse using force platforms suggested transitory improvement in severe hip OA following intra-articular adipose-derived mesenchymal stem cell administration[222].

469

470 ***4. Piloting of new technologies or surgical therapies***

471 A large animal with natural disease and compressed life-times has particular benefits; human scale implants
472 and instruments can be used, such as arthroscopic treatment; therapies are piloted in a natural rather than
473 induced disease model; and the relatively short dog lifespan allows for end of life retrieval studies. Although
474 this may last several years (dog lifespan ~8-12 years), these durations are much longer than most, purely
475 research, studies would entertain, and yet not be so long to be prohibitive. Total hip replacements (THR) for
476 example, have been in veterinary clinical usage since 1976. Outcomes are good, with <20% complication rates
477 for cementless replacement after four years[42] (Figure 2G). Development of the implants for humans, including
478 resurfacing hip replacements[223], porous implants[224] and hydroxyapatite coated prostheses, all relied heavily on
479 testing in experimental dogs, and current veterinary modular hip replacements include both cemented and
480 uncemented osseointegrative replacements. Similar complications such as aseptic loosening, bone remodeling
481 and implant infection are seen in dogs as in humans. Post-mortem retrieval of implant material from veterinary
482 patients, several years later is relatively cheap and easy, and could provide researchers with insights that are
483 currently lacking. Such samples have been used to examine the mechanical, histomorphologic and
484 radiographic features of aseptic loosening, which is a particular concern in human THR in the under 50s. These
485 studies pointed to failure initiated by PMMA-debonding from the metal implant [225]. Improved designs for new
486 implants, if appropriately and ethically managed, could be piloted in dogs as they offer a comparatively short
487 time-frame for retrieval when compared with a human clinical trial.

488 Other than implants, surgical treatment of articular cartilage defects in dogs and humans has included
489 osteochondral grafts and autologous chondrocyte implantation . Mosaicoplasty or osteochondral autologous
490 transplantation is used in humans for full-thickness lesion repair and in dogs for treating OCD[226-228].

491 **CONCLUSION**

492 This review has sought to highlight the potential benefits for dog and human health that could follow the
493 adoption of '*One Medicine'* approaches to basic and clinical research and practice for OA. Human and dog
494 OA are heterogeneous and spontaneous with many homologies, similar co-morbidities and known distinctions
495 (Figures 2 and 3). There is much to be gained from studying a large animal with spontaneous OA, and
496 understanding the reasons for differences may be just as informative as the similarities.

497 A key current issue is that publication of veterinary research findings is usually restricted to veterinary-focused
498 journals. We have consequently sought also to increase awareness of: i) V-RCTs, with a database (being
499 developed by American Veterinary Medical Association), ii) national repositories of canine OA samples, iii)
500 national retrieval banks for implants and, iv) clear V-RCT guidelines with standardized outcome assessments
501 in order to allow their amalgamation into an OA '*One Medicine*' paradigm. We emphasise that these resources
502 have barely exploited in OA research and that their integration could generate breakthroughs in OA treatment
503 in dogs and humans and in understanding how genetics, epigenetics, biomechanics and lifestyle impact OA
504 aetiology and pathogenesis.

KEY POINTS

- Dog OA types offer a potential stratification rationale for etiological differences and alignment to homologous human OA phenotypes
- Relatively compressed time-course of spontaneous dog OA offers more ideal longitudinal research opportunities
- Genetic inbreeding and dog breed OA predisposition allow for easier candidate genes identification than in outbred humans
- Collaboration with veterinary researchers can provide OA samples from early stage disease
- Opportunities to evaluate and translate new therapeutics into a spontaneous disease model
- Comparative OA studies provide insights from different mechanical environments linked with weight-bearing and non weight-bearing in quadrupedal dogs and bipedal human joints

Figure 1. Canine OA locations and types.

A-C. Shoulder OCD lesions in adolescent dog: (A) lateral radiograph (arrow marks the flap); (B) transverse CT and (C) arthroscopic removal. D-E. Hip CDH: (D) Transverse CT showing subchondral lesions and peripheral new bone formation associated with (E) OA eburnated explanted femoral head. F-G. Hip LCPD: (F) Excised femoral head with central dark line showing articular surface defect and (G) radiograph with typical LCPD focal lucencies. H-J. Knee OA: (H) Lateral radiograph of OA canine knee with ACL rupture; (I) knee with healthy ACL and (J) spontaneously degenerate ACL (arrow shows anteriomedial band damage). K-M. Canine elbow OA: (K1-4) Anterio-posterior radiographs showing progressively increasing OA change; (L) Transverse CT of dysplastic elbow with OA and (M) Outerbridge grade III cartilage degeneration on arthroscopic examination.

Figure 2. Comparative canine and human diagnostic imaging.

Radiographic images of (A) dysplastic human infant luxated left hip (with permission R. Loder); (B) bilateral dysplastic and luxated hips of 3-month-old dog imaged in supine quadrupedal weight-bearing position; (C) an adult dog with severe hip dysplasia and luxoid hips imaged in a dorsolateral extended-hip position, and OA hip joints from (D) middle aged male human and (E) middle aged large breed dog, both with advanced remodeled new bone formation and sclerosis. Radiographic images of (F) a human total hip replacement, uncemented stem and cup, and (G) canine total hip replacement (cemented stem, uncemented cup). (H) T1-weighted sagittal MRI of healthy canine knee. (I) Proton density turbo spin echo sequence (PD TSE) sagittal MRI human knee (Courtesy Karyn Chappell).

Figure 3. Diagrammatic representation of three canine forms of OA (hip LCPD, hip CHD and knee ACL) with relationships to human homologs highlighted where applicable. Similarities to aetiopathology in canine and human OA forms of each is demonstrated.

Table 1. Proposed system for stratification of the common dog OA types with corresponding alignment to analogous human OA, based upon anatomy, aetiology and pathophysiology. * potential to classify an adult form of DDH (with acetabular dysplasia) with late onset hip OA in aged dogs that are otherwise 'normal' upon screening at 2 years of age.

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Canine OA Type	*Canine disease*	*Canine Epidemiology*	*Human analogy*
Acquired juvenile instability	Hip dysplasia	Juvenile large/giant breeds (prevalent in Retrievers, Rottweilers, German Shepherds; extremely rare in Greyhounds and Borzois) Adolescent dogs, 3-12months old* Progression to OA 1 year	Developmental dysplasia of the hip Infants, female prevalent Progression to OA 30 years
Acquired adult instability	Anterior cruciate rupture	Young adults (2 years) and older medium/large breeds (Rottweiler, Retrievers, Staffordshire Bull Terriers). Middle-aged to geriatric in small breeds (>6 years, Yorkshire Terriers, West Highland White Terriers). ~50% develop contralateral disease in <2 years. <50% with meniscal (mostly medial) pathology. Neutered females increased risk	Anterior cruciate rupture and meniscal injuries Active adults Menstrual cycle influence
Developmental Vascular	Legg Calve Perthes	Small breeds (Toy/Terriers – Miniature Poodles and West Highland White Terriers autosomal recessive trait. Adolescent (4-11 months old)	Adolescent avascular necrosis of the femoral head
Developmental endochondral	Shoulder OCD, knee OCD	Large/giant breeds (Great Dane, Retrievers, Rottweilers) Adolescent to young adult (5 months – 1.5 years) Increased in males, often bilateral	Children, adolescents, young adults Familiar history Increased in males often bilateral
Environmental: obesity-related	Elbow, hip, shoulder	Any breed, notably Labrador retriever Adult 4-8 years old	Middle aged and older, multiple joints affected
Environmental: athletic/trauma-related	Hip, elbow, hock (ankle), carpus, digits	Racing Greyhound, 4-8 years old, digital osteoarthritis, carpal sprains leading to OA	Athletic individuals, often middle aged

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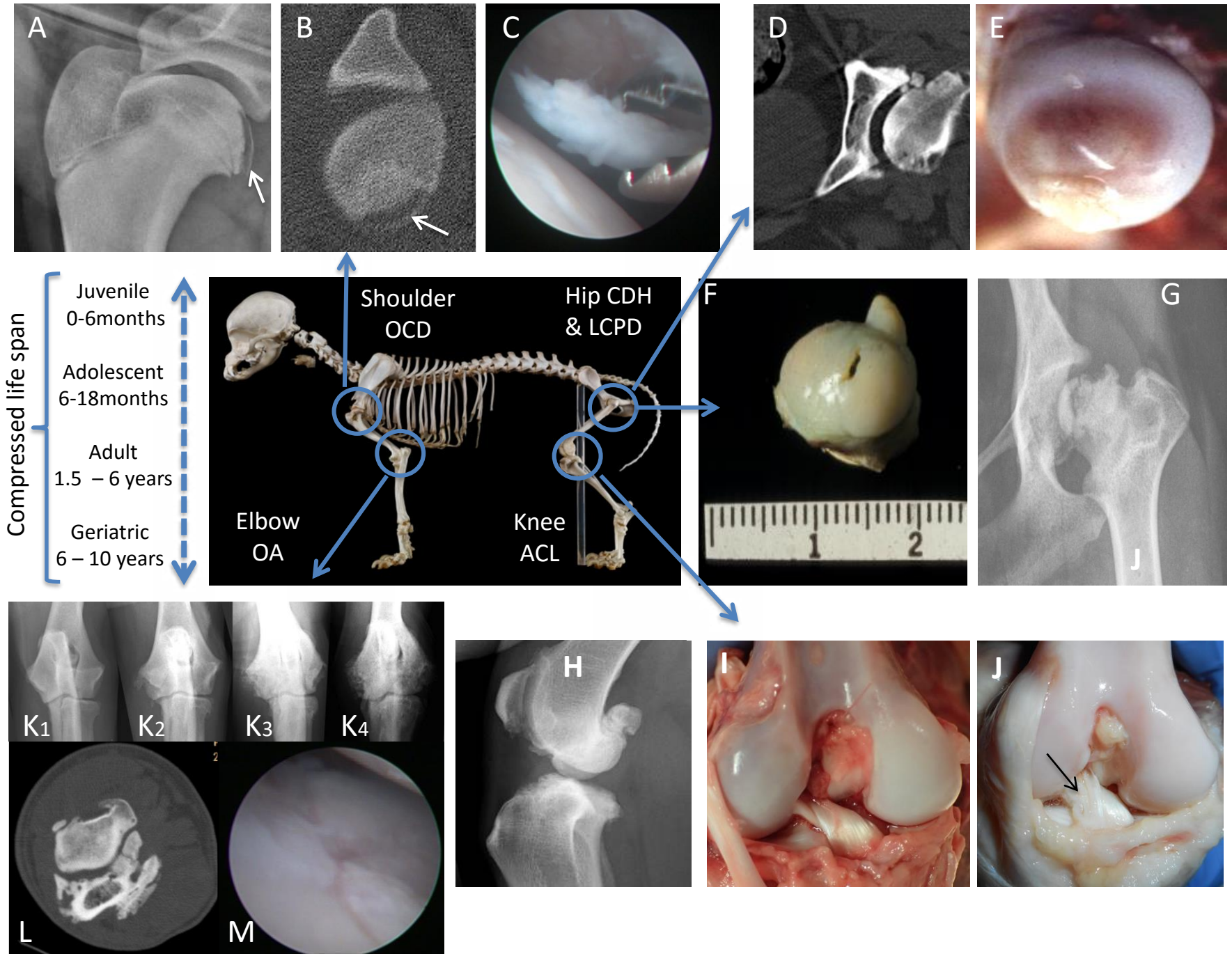
Fig. 1


A
B
C
D
E
Compressed life span
Juvenile
0-6months
Adolescent
6-18months
Adult
1.5 – 6 years
Geriatric
6 – 10 years
Shoulder
OCD
Hip CDH
& LCPD
Elbow
OA
Knee
ACL
F
G
J
K1
K2
K3
K4
H
I
J
L
M

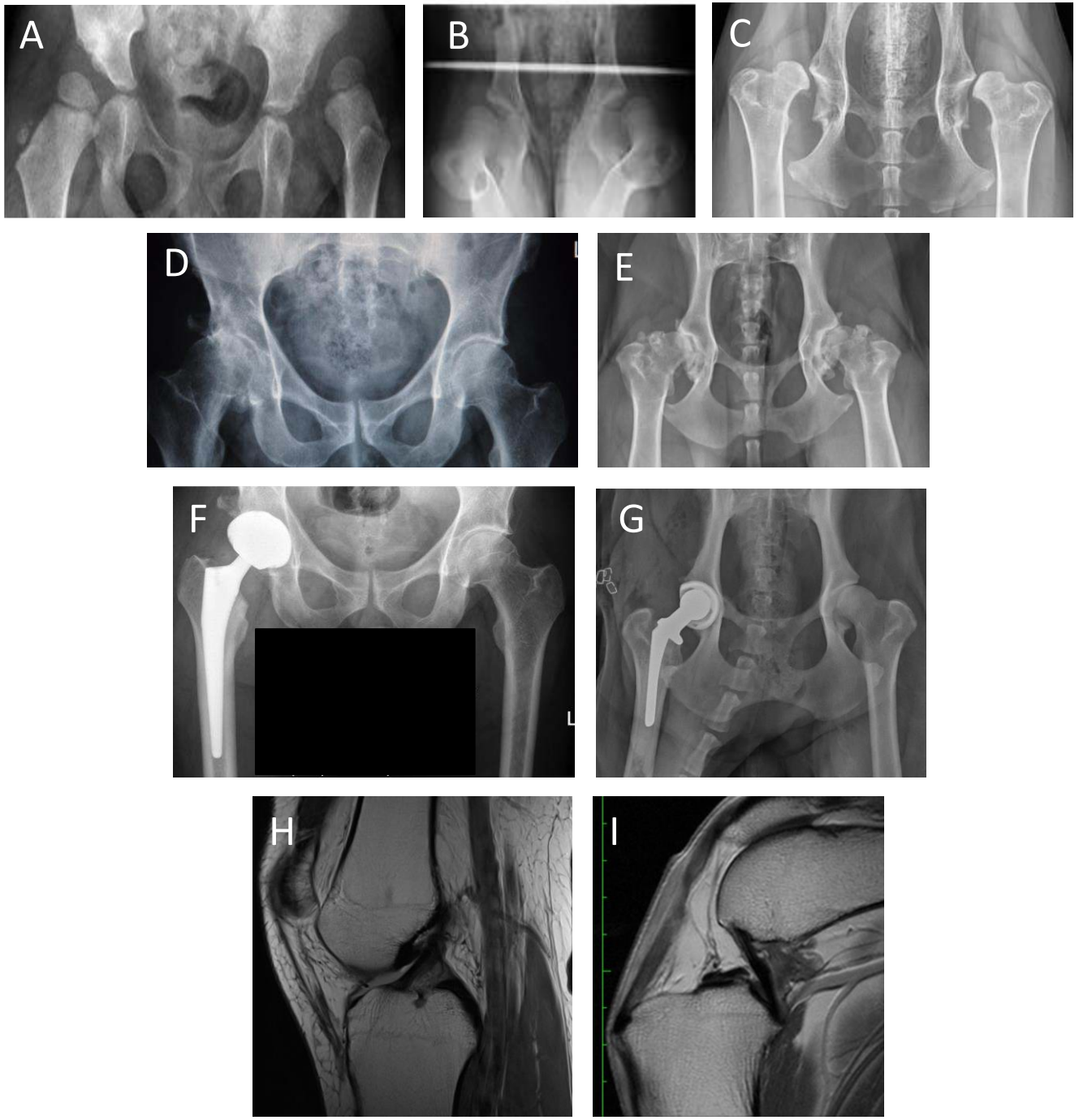


Fig. 2

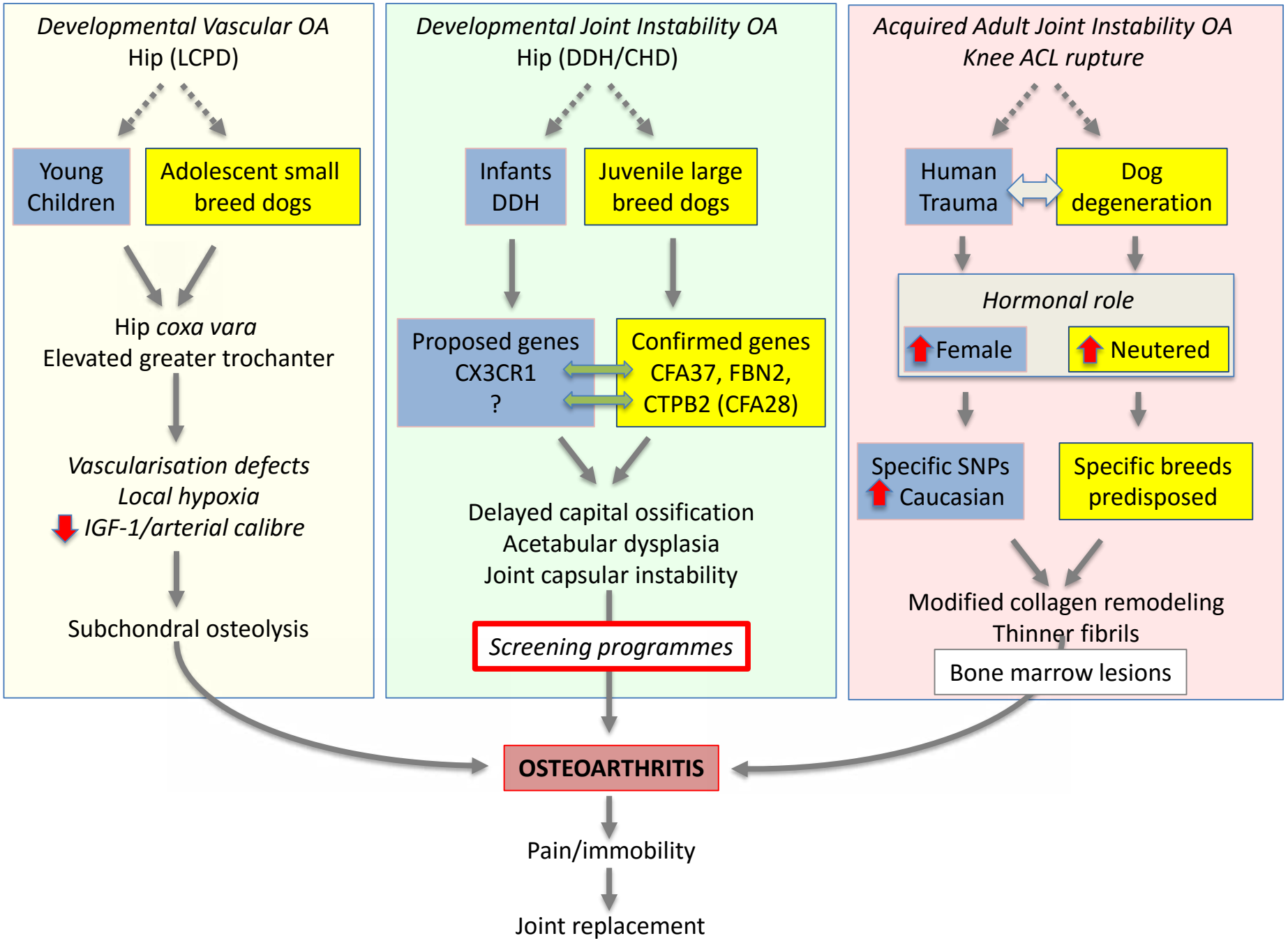

Developmental Vascular OA
Hip (LCPD)
Young Children
Adolescent small breed dogs
Hip coxa vara
Elevated greater trochanter
Vascularisation defects
Local hypoxia
IGF-1/arterial calibre
Subchondral osteolysis
Developmental Joint Instability OA
Hip (DDH/CHD)
Infants DDH
Juvenile large breed dogs
Proposed genes
CX3CR1
?
Confirmed genes
CFA37, FBN2,
CTPB2 (CFA28)
Delayed capital ossification
Acetabular dysplasia
Joint capsular instability
Screening programmes
Acquired Adult Joint Instability OA
Knee ACL rupture
Human Trauma
Dog degeneration
Hormonal role
Female
Neutered
Specific SNPs
Caucasian
Specific breeds predisposed
Modified collagen remodeling
Thinner fibrils
Bone marrow lesions
OSTEOARTHRITIS
Pain/immobility
Joint replacement

Fig. 3

Figure 1. Canine OA locations and types.

A-C. Shoulder OCD lesions in adolescent dog: (A) lateral radiograph (arrow marks the flap); (B) transverse CT and (C) arthroscopic removal. **D-E**. Hip CDH: (D) Transverse CT showing subchondral lesions and peripheral new bone formation associated with (E) OA eburnated explanted femoral head. **F-G**. Hip LCPD: (F) Excised femoral head with central dark line showing articular surface defect and (G) radiograph with typical LCPD focal lucencies. **H-J.** Knee OA: (H) Lateral radiograph of OA canine knee with ACL rupture; (I) knee with healthy ACL and (J) spontaneously degenerate ACL (arrow shows anteriomedial band damage). **K-M**. Canine elbow OA: (K1-4) Anterio-posterior radiographs showing progressively increasing OA change; (L) Transverse CT of dysplastic elbow with OA and (M) Outerbridge grade III cartilage degeneration on arthroscopic examination.

Figure 2. Comparative canine and human diagnostic imaging.

Radiographic images of (A) dysplastic human infant luxated left hip (with permission R. Loder); (B) bilateral dysplastic and luxated hips of 3-month-old dog imaged in supine quadrupedal weight-bearing position; (C) an adult dog with severe hip dysplasia and luxoid hips imaged in a dorsolateral extended-hip position, and OA hip joints from (D) middle aged male human and (E) middle aged large breed dog, both with advanced remodeled new bone formation and sclerosis. Radiographic images of (F) a human total hip replacement, uncemented stem and cup, and (G) canine total hip replacement (cemented stem, uncemented cup). (H) T1-weighted sagittal MRI of healthy canine knee. (I) Proton density turbo spin echo sequence (PD TSE) sagittal MRI human knee (Courtesy Karyn Chappell).

Figure 3. Diagrammatic representation of three canine forms of OA (hip LCPD, hip CHD and knee ACL) with relationships to human homologs highlighted where applicable. Similarities to aetiopathology in canine and human OA forms of each is demonstrated.