Question 6. What are the major strain and sex differences in animal models of aging associated OA and how should the field manage these differences?

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Response/Recommendation: Most studies of age-related OA have utilized male C57BL/6 mice, which limits the field's understanding of how strain and sex affect disease pathogenesis and potential therapeutic interventions. Investigators should continue recent trends of clarifying the complete strain information (e.g. C57BL/6N or C57BL/6J), improved inclusion of male and female mice, and expanded measurements of the OA phenotype that include functional measures such as pain. Meaningful advances regarding the role of strain (genetics) in murine OA would require large-scale studies using resources such as the Collaborative Cross to specifically test the interaction of strain and sex. The mismatch of higher OA in female humans (as compared to males) but lower OA in female mice (as compared to males) is a major concern. The field should expand the use of alternate animal models with distinct trajectories of sex-specific aging changes and joint degeneration. Finally, murine or other animal studies should continue to pursue defined perturbations such as induced menopause to test plausible mechanisms related to sex differences in age-related OA.

Level of evidence: Moderate

Scope: The primary focus is on assessing the evidence for an interaction of strain and sex in animal models of age-related OA. Papers exploring this question in murine and non-murine models are considered. Models of accelerated aging or specific genetic perturbations (i.e. knockout mice) are not considered as they are addressed by other questions.

Rationale: Genetics, sex, and aging are three of the most critical risk factors for the development of OA in humans. The complex interactions between these risk factors has been challenging to dissect in human studies, which limits mechanistic insight and the potential to develop targeted interventions[1,2]. Animal models have a critical role to play in filling these knowledge gaps, but to do so need to incorporate sufficient genetic diversity, appropriate species, and rigorous evaluation of OA as a multi-faceted disease.

Assessment of evidence: One of the first systematic studies on the potential interaction of mouse strain and sex in age-related OA was performed by Jay & Sokoloff in 1956[3], with a simple scoring system for 670 mice across 18 strains/substrains aged 12-22 months showing consistently higher OA in males as compared to females. Despite this initial interest, the explosion of genetically-engineered mouse models titled the playing field towards widespread use of C57BL/6, and in this strain the higher incidence of both age-related and injury-induced OA in males made it attractive to use only males from a power analysis and cost point of view. Of the 41 studies on age-related OA evaluated by a 2022 meta-analysis[4], only 7 of those included both male and female mice[5–11] and only 2 of these 7 used a strain other than C57BL/6[10,11]. It is important to note that C57BL/6J and C57BL/6N substrains differ in ways that affect physiology relevant to aging[12], but as of now the possible effects on age-related OA have not been comprehensively evaluated in direct comparison studies. Recent studies have sought to improve genetic diversity[13,14] and evaluate both male and female aged mice[6,15,16]. The increased prevalence of OA in males was confirmed in UM-HET3 mice derived from four founder strains, which showed that osteophyte formation and subchondral bone changes were more highly correlated with articular cartilage degradation in female mice[17]. The Kenny *et al.* study shows both the promise and challenge of using murine resources such as the Collaborative Cross (CC) to directly study strain effects in age-related OA[14]. They evaluated >200 mice across 43 strains that were derived from the 8 CC founders and identified a contribution from the *Glis3* locus that aligns with human GWAS, but even larger studies would be needed to distinguish sex effects at several timepoints of aging. The JAX Diversity Outbred resource starts from the same 8 CC founder strains, but provides greater genetic diversity through outbreeding and can be helpful as companion studies to human GWAS as has been done in blood cells[18]. Identifying the specific loci responsible for phenotypes in these advanced crosses could be supported by recent developments in high resolution genotyping[19], which can also be useful for assessing genetic drift in colonies.

Emerging studies have also expanded the breadth of OA analysis used to evaluate age-related tissue changes and OA in male and female rodents. For example, Geraghty *et al.* showed that some features of OA showed a stronger phenotype in males (cartilage degradation, osteophytes, etc.) whereas other features of OA showed a stronger phenotype in females (increase in synovial fibrosis over baseline, hyperalgesia and allodynia by withdrawal thresholds, etc.)[16]. A study in rats showed that aged females were the most susceptible to mechanical hyperalgesia after intra-articular injection of noxious stimuli[20]. The inclusion of gene expression and other omics approaches alongside measures of OA have shown promise for assessing whether the signaling pathways and mechanisms of age-related OA are similar in male and female mice. For example, there is greater involvement of signaling that results from advanced glycation end products in male mice[6]. Of note, single-cell methods that have been successfully implemented in murine joint tissues after injury[21] may be useful in overcoming the challenge of isolating large numbers of pure joint cells from aged mice that were required for previous technologies.

Mice have often been considered to be in the “goldilocks zone” for aging research; they age slowly enough to test mechanisms and interventions, but undergo physiological age-related changes relevant to humans in a somewhat tractable experimental time frame. However, it is important for the field to consider the relationship between strain and sex in other species as well. Rats are rarely used for age-related OA due a perceived lack of disease without joint injury, which may be true for Lewis, Sprague-Dawley, and Wistar strains that have been primarily been used in these models[22]. However, an early study showed that Fischer 344 (F344) have detectable cartilage lesions in all rats at 24 months of age with features such as chondrocyte cloning and full depth loss of toluidine blue stain[23]. The National Institute on Aging Aged Rodent Colony provides F334, Brown Norway, and hybrid rats, which enabled studies on redox regulation with aging along with showing increased (but still moderate) OA in male F334BN rats at 30 months as compared to 10 months[24]. Work with the Dunkin Hartley strain as compared to controls has demonstrated consistent spontaneous OA, with faster progression in males (moderate to severe OA typically emerging at 3-9 months of age) as compared to females (moderate to severe OA typically emerging at 6-12 months of age)[25]. A 2024 review identified only 11 studies that evaluated both sex and age in large animal models of OA such as dogs, pigs, sheep, and horses; few of these studies focused on spontaneous OA and those that did studies provided only minimal analysis or were underpowered to evaluate the interaction of sex and age[26]. Primates such as baboons show greater age-related OA in females as compared to males[27], but are not good candidates for mechanistic or intervention studies. Marmosets may provide an alternative primate model to study the role of sex and OA, as they showed progressive OA over the ages of 2.5 – 16.5 years of age, with a significantly increased relationship between histological OA and age in females as compared to males[28]. The balance between cost and additional utility of non-rodent models may depend on the specific research question.

Perturbations to sex hormones in animal models is a promising approach to understand the mechanisms that drive sex differences in OA. Ovariectomy is the most common method and a meta-analysis identified that loss of estrogen through this method leads to more severe OA in mice and rats[29], although it should be noted that nearly all studies reviewed used young rodents and focused on cartilage changes. Gillmer *et al.* showed that an ovarian toxin to induce a gradual transition to menopause in middle-aged mice increased OA, which was blunted by hormone replacement[30], and D’Amico *et al.* used this approach to generate tissues from menopausal and non-menopausal females for in vitro studies[15]. Future studies using this or similar human-mimicking hormonal perturbations in a wide range of strains with different propensity for OA would help determine whether higher risk with menopause is true across different animal models of age-related OA.

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