Received: 1 May 2023 | Revised: 28 September 2023 | Accepted: 19 October 2023

DOI: 10.1002/jsp2.1298

SPECIAL ISSUE ARTICLE

JOR Spine OPEN ACCESS

Comparative histopathological analysis of age-associated intervertebral disc degeneration in CD-1 and C57BL/6 mice: Anatomical and sex-based differences

Jeffrey L. Hutchinson | Matthew A. Veras | Meghan E. Serjeant | Matthew R. McCann | Ashley L. Kelly | Diana Quinonez | Frank Beier | Cheryle A. Séguin

Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The Bone and Joint Institute, The University of Western Ontario, London, Ontario, Canada

Correspondence
Cheryle A. Séguin, Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, ON N6A 5C1, Canada.
Email: cheryle.seguin@schulich.uwo.ca

Funding information
Canadian Institutes of Health Research; CONNECT! NSERC CREATE Training Program; Western's Bone and Joint Institute; Arthritis Society; Ontario Graduate Scholarship

Abstract

Background: Intervertebral disc (IVD) degeneration is a major contributor to back pain and disability. The cause of IVD degeneration is multifactorial, with no disease-modifying treatments. Mouse models are commonly used to study IVD degeneration; however, the effects of anatomical location, strain, and sex on the progression of age-associated degeneration are poorly understood.

Methods: A longitudinal study was conducted to characterize age-, anatomical-, and sex-specific differences in IVD degeneration in two commonly used strains of mice, C57BL/6 and CD-1. Histopathological evaluation of the cervical, thoracic, lumbar, and caudal regions of mice at 6, 12, 20, and 24 months of age was conducted by two blinded observers at each IVD for the nucleus pulposus (NP), annulus fibrosus (AF), and the NP/AF boundary compartments, enabling analysis of scores by tissue compartment, summed scores for each IVD, or averaged scores for each anatomical region.

Results: C57BL/6 mice displayed mild IVD degeneration until 24 months of age; at this point, the lumbar spine demonstrated the most degeneration compared to other regions. Degeneration was detected earlier in the CD-1 mice (20 months of age) in both the thoracic and lumbar spine. In CD-1 mice, moderate to severe degeneration was noted in the cervical spine at all time points assessed. In both strains, age-associated IVD degeneration in the thoracic and lumbar spine was associated with increased histopathological scores in all IVD compartments. In both strains, minimal degeneration was detected in caudal IVDs out to 24 months of age. Both C57BL/6 and CD-1 mice displayed sex-specific differences in the presentation and progression of age-associated IVD degeneration.

Conclusions: These results showed that the progression and severity of age-associated degeneration in mouse models is associated with marked differences based on anatomical region, sex, and strain. This information provides a fundamental

baseline characterization for users of mouse models to enable effective and appropriate experimental design, interpretation, and comparison between studies.

KEYWORDS
aging, C57BL/6, CD-1, intervertebral disc degeneration

1 | INTRODUCTION

The *Global Burden of Disease* study reported low back pain as the leading cause of years lived with disability worldwide, with a lifetime prevalence of approximately 80%.[1] Although multifactorial, intervertebral disc (IVD) degeneration is reported to be a major contributor to low back pain, with ~40% of cases associated with IVD degeneration.[2,3] The IVD is a fibrocartilaginous joint located between the vertebral bodies of the spine made of three distinct tissues including the nucleus pulposus (NP), annulus fibrosus (AF), and cartilage endplates. During axial compression, the IVD allows flexibility along the spine axis and provides shock absorption. Given that over 20% of patients with back pain are unable to work,[4] it has enormous socioeconomic burden, estimated to be approximately $100 billion in the United States each year.[5] Current treatments for back pain are limited to pain management, surgical interventions, and physical therapy.[6–8]

Although the cause of IVD degeneration is multifactorial, at the tissue level, it is driven by breakdown of extracellular matrix, as well as changes in cellularity, biomechanics, and nutrition of the IVD.[9] In humans, it is well established that the risk of IVD degeneration and associated back pain increases with age; changes in IVD composition, including the progressive loss of aggrecan, are detected early in life, such that up to 20% of teens aged 11–16 show mild signs of IVD degeneration.[10–14] The lumbar spine in particular exhibits increased risk for age-associated IVD degeneration, associated with increased mechanical load.[4,13,15,16] Furthermore, biological sex contributes to the risk of IVD degeneration. Men have a higher prevalence of back pain and IVD degeneration than women until the fifth decade of life, after which menopause is an expected trigger for increasing risk of IVD degeneration and back pain.[17]

The prevalence and socioeconomic impact of IVD degeneration, paired with our aging population, have emphasized the need for basic research to better understand its etiology. As such, preclinical animal models have become valuable tools in IVD research. To date, however, the progression of age-associated IVD degeneration in various animal models has not been fully characterized. Current research uses various animal models, most common of which are rodent models, as well as large animal models such as dogs, sheep, goats, and pigs.[9,18] However, in large animal models, many studies use disc injury models to study IVD degeneration as a more cost-effective approach than age-associated models which are inherently associated with prolonged animal husbandry costs.[18] Murine models are an attractive and commonly used model due to the availability of transgenic techniques, relatively short lifespan, and ease of housing.[19] Importantly, they allow for the examination of age-associated IVD degeneration, which might progress through different pathways and mechanisms than injury-induced IVD degeneration. Mice share many similarities with humans in IVD geometry and the presentation of age-associated IVD degeneration, such as loss of cellularity within the NP, reduced matrix production, inflammation, and reduced range of motion.[20,21] However, unlike humans, mice retain notochordal cells within the NP throughout their lives.[22] The ability to compare findings generated in mouse models may also be limited by the specific strains of mice used to study IVD and limited knowledge of how risk factors such as age, anatomical location, and sex affect the onset and progression of IVD degeneration. Moreover, given limitations associated with the size of murine IVDs, experimental designs often pool IVDs from multiple anatomical locations or use different regions of the spine for distinct endpoint analyses. Inherent differences in the rate or extent of age-associated degeneration between anatomical regions could confound the outcome or interpretation of the resultant findings.

To address this problem, the current study was designed to directly compare age-associated IVD degeneration across anatomical regions of the spine in two common laboratory mouse strains, CD-1 and C57BL/6. In both strains, histopathological changes were assessed in both male and female mice at 6, 12, 20, and 24 months of age in the cervical, thoracic, lumbar, and caudal spine. These data were used to compare the progression of IVD degeneration between anatomical regions, and examine differences based on strain and sex over time. This information provides a fundamental baseline characterization for users of mouse models to enable effective and appropriate experimental design, interpretation, and comparison between studies.

2 | METHODS

2.1 | Mice

Male and female C57BL/6 (C57BL/6NCrl, strain code 027) and CD-1® mice (strain code 022) were obtained from Charles River (Wilmington, MA, USA). Mice were housed in conventional cages and maintained on a 12-h light/dark cycle, with rodent chow (Envigo 2018) and water available ad libitum. Mice were aged to 6, 12, 20, or 24 months of age and were euthanized by intraperitoneal injection of a lethal dose of sodium pentobarbital. All aspects of this study were conducted in accordance with the policies and guidelines set forth by the Canadian Council on Animal Care and were approved by the

Animal Use Subcommittee of the University of Western Ontario London, ON.

2.2 | Histology

Histopathological analysis was completed for spinal segments of male and female CD-1 and C57BL/6 mice at 6, 12, 20, and 24 months of age ($N = 4–7$ mice/group). Due to attrition of groups with age, 4–6 CD-1 mice were assessed for each anatomical region and time point, with the exception of cervical spines from female mice at 6 and 12 months of age ($N = 2$). Similarly, groups of 3–7 C57BL/6 mice were assessed for each anatomical region and time point, except for cervical spines from female 24-month-old mice ($N = 2$). For each, the intact spinal column was dissected and segmented into the cervical, thoracic, lumbar, and caudal regions. Tissues were fixed overnight with 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) and then decalcified using Shandon's TBD-2 (Thermo Fisher Scientific, Waltham, MA, USA) for 5–7 days at room temperature with continuous agitation. Following standard histological processing, tissues were embedded in paraffin and sectioned sagittally at a thickness of 5 µm. Midsagittal sections of the spines from each region were deparaffinized and rehydrated as previously described,[23] and stained with 0.05% Safranin-O/0.05% Fast Green. Sections were imaged on a Leica DM1000 microscope, with Leica Application Suite (Leica Microsystems: Wetzlar, DEU).

Spine sections were assessed for degenerative changes using an established mouse IVD histopathological scoring system[24] to evaluate the NP, AF, and NP/AF boundary. In this study, and as previously done by our group, IVD scores were determined by evaluating NP structure (Part AI, scores 0–4), AF structure (Part BI, scores 0–4), and the NP/AF boundary (Part C, scores 0–2).[24] For each IVD, degeneration was scored by two individuals blinded to age, strain, and sex of the sample. Scores from the NP, AF, and NP/AF boundary were summed to provide the overall degeneration score, where the maximum score of 10 reflects the highest degree of degeneration.

2.3 | Statistical analysis

Histopathological data were analyzed by nonparametric Kruskal–Wallis H test with Dunn's multiple comparisons or Mann–Whitney U test with Tukey's multiple comparisons. $p < 0.05$ was considered significant. Statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software: San Diego, CA, USA). Cohen's weighted Kappa was used to assess inter-rater reliability for each of the scoring criteria (NP, AF, and the NP/AF boundary) from a random sampling of 141 IVDs (comprised of images from both sexes and all time points).[25] Scoring of the NP and AF displayed almost perfect strength of agreement with weighted Kappa values of 0.879 and 0.813, respectively. The NP/AF boundary displayed substantial agreement with a weighted Kappa score of 0.801.

3 | RESULTS

3.1 | Progression of age-associated disc degeneration

The progression of age-associated IVD degeneration was assessed to allow for comparison between anatomical regions, sexes, and strains in C57BL/6 and CD-1 mice at 6, 12, 20, and 24 months of age. Histopathological scores were determined at each IVD for the NP, AF, and the NP/AF boundary compartments, enabling analysis of scores by tissue compartment, summed scores for each IVD, or averaged scores for each anatomical region.[24]

To provide an overview of changes over time, the averaged histopathological scores for IVDs within each anatomical region were compared between time points up to 24 months of age. As expected, histopathological scores generally increased with age, but in an anatomical region-, strain-, and sex-specific manner. In both male and female C57BL/6 mice, changes over time in IVDs were not associated with significantly increased histopathological scores in the cervical (Figure 1A) or thoracic (Figure 1B) regions. In these regions, IVDs appeared generally healthy with only mild degenerative changes detected in the NP at 24 months of age. In contrast, IVD degeneration was observed in the cervical spine in CD-1 mice at all time points assessed (Figure 1A) with advanced degeneration noted in all IVD compartments in both male and female mice. A significant increase in histopathological scores was detected in the thoracic spine for both male and female CD-1 mice with age (Figure 1B). Histologically, these scores reflect near complete loss of the NP and AF structure as well as the loss of the boundary between regions by 24 months of age.

Age-associated changes in the lumbar IVDs were mostly associated with increased histopathological scores (Figure 1C). Lumbar spines from male C57BL/6 mice showed increased histopathological scores at 24 months of age compared to both 6 and 12 months of age (Figure 1C). While C57BL/6 female mice show no significant change in histopathological scores between 6 and 24 months, a significant increase was detected between 12 and 20 months of age. Similar trends were detected in CD-1 mice, with significant increases in histopathological scores noted in males between 12 and 20 months of age, and in females between 6 and 24 months of age. Scoring reflects histological features including almost complete loss of the NP, AF, and boundary by 24 months of age in both sexes.

Of note, histopathological features of IVD degeneration were not detected in the caudal spine (Figure 1D). In C57BL/6 mice, no significant change in histopathological scores was detected over time in male mice, while in female mice, elevated histopathological scores were noted at the 6-month time point, which decreased by 12 months of age. CD-1 mice exhibited no changes in histopathological score over time in either male or female mice.

3.2 | Differences in degeneration between spinal regions over time

Assessment of the average histopathological scores within each spinal region also enabled the comparison of degeneration between

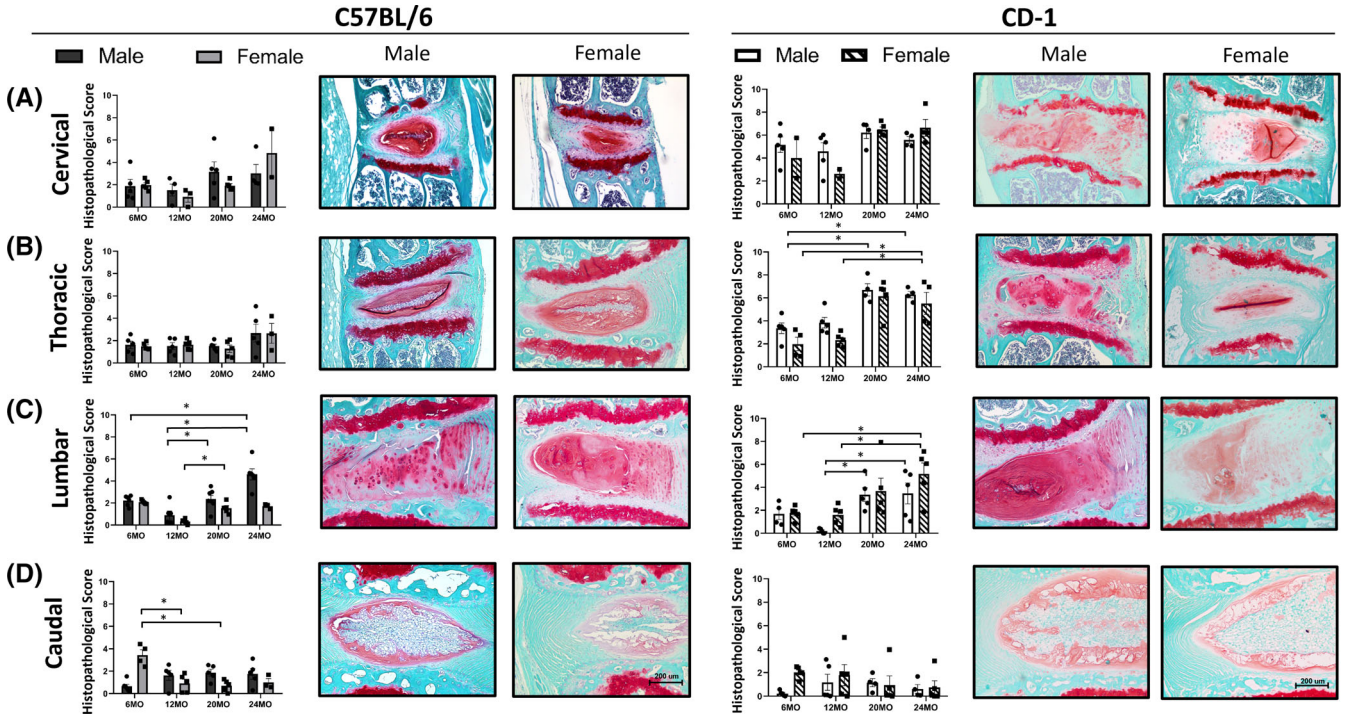

FIGURE 1 Age-associated IVD degeneration in C57BL/6 and CD-1 mice. The average histopathological score for the cervical (A), thoracic (B), lumbar (C), and caudal (D) regions was compared over time for both male and female C57BL/6 mice (left column) and CD-1 mice (right column) at 6, 12, 20, and 24 months of age. Scores presented as the average of all IVDs in each anatomical region for each mouse ($N = 3–7$ for each time point). Representative mid-sagittal sections corresponding to each anatomical region are included from both male and female mice at 24 months of age, stained with Safranin-O/Fast Green. Data presented as mean ± SEM for each group. Data analyzed by Kruskal–Wallis *H* test with Dunn's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

anatomical regions at each time point in male and female mice of each strain (Figure 2). In general, histopathological scores were similar in all anatomical regions at each time point in male C57BL/6 mice (Figure 2A), apart from the caudal spine which demonstrated decreased scores at both 6 and 24 months of age compared to the lumbar spine male mice (Figure 2A). A similar consistency in average histopathologic scores between regions at each time point was noted in female C57BL/6 mice, with a few exceptions. At 6 months of age, histopathological scores were increased in the caudal region compared to the thoracic region. At 12 months of age, histopathological scores in the thoracic region were increased compared to the lumbar region. At 20 months of age, histopathological scores in the cervical region were significantly higher than those in the caudal region.

Significant anatomical region-dependent differences in degeneration were detected in CD-1 mice over time (Figure 2B). At each time point assessed, male CD-1 mice showed increased histopathological scores in the upper spine (cervical and/or thoracic) compared to the lower spine (lumbar and/or caudal). A similar pattern was detected in female CD-1 mice at 20 and 24 months of age.

Finally, to assess strain-specific differences in the progression of degeneration, the average histopathological scores at each time point were compared between C57BL/6 and CD-1 mice for each anatomical region in both male and female mice (Figure S1). At each time point, male CD-1 mice showed increased histopathological scores in the cervical and thoracic regions compared to C57BL/6 mice. No difference was detected between male mice at any time point in the lumbar or caudal regions. In female mice, no significant differences were detected between C57BL/6 and CD-1 mice at either 6 or 12 months of age. At 20 months of age, however, a significant increase in histopathological scores was detected in CD-1 compared to C57BL/6 mice in the cervical, thoracic, and lumbar spine. While not significant, a similar trend was detected at 24 months of age in the lumbar spine.

3.3 | Assessment of IVD degeneration by level within anatomical regions over time

Given inherent differences in risk factors such as mechanical loading across the spine[4] as well as reported differences at the level of individual IVDs in humans[26] and rodent models,[27,28] we sought to generate a "map" of age-associated degeneration by assessing histopathological degeneration at the IVD level for the thoracic (Figures 3 and 4), lumbar (Figures 5 and 6), and caudal (Figures 7 and 8) spine for both male and female CD-1 and C57BL/6 mice.

3.4 | Thoracic spine

Assessment of histopathological changes at individual disc levels demonstrated the pattern of age-associated degeneration in the thoracic

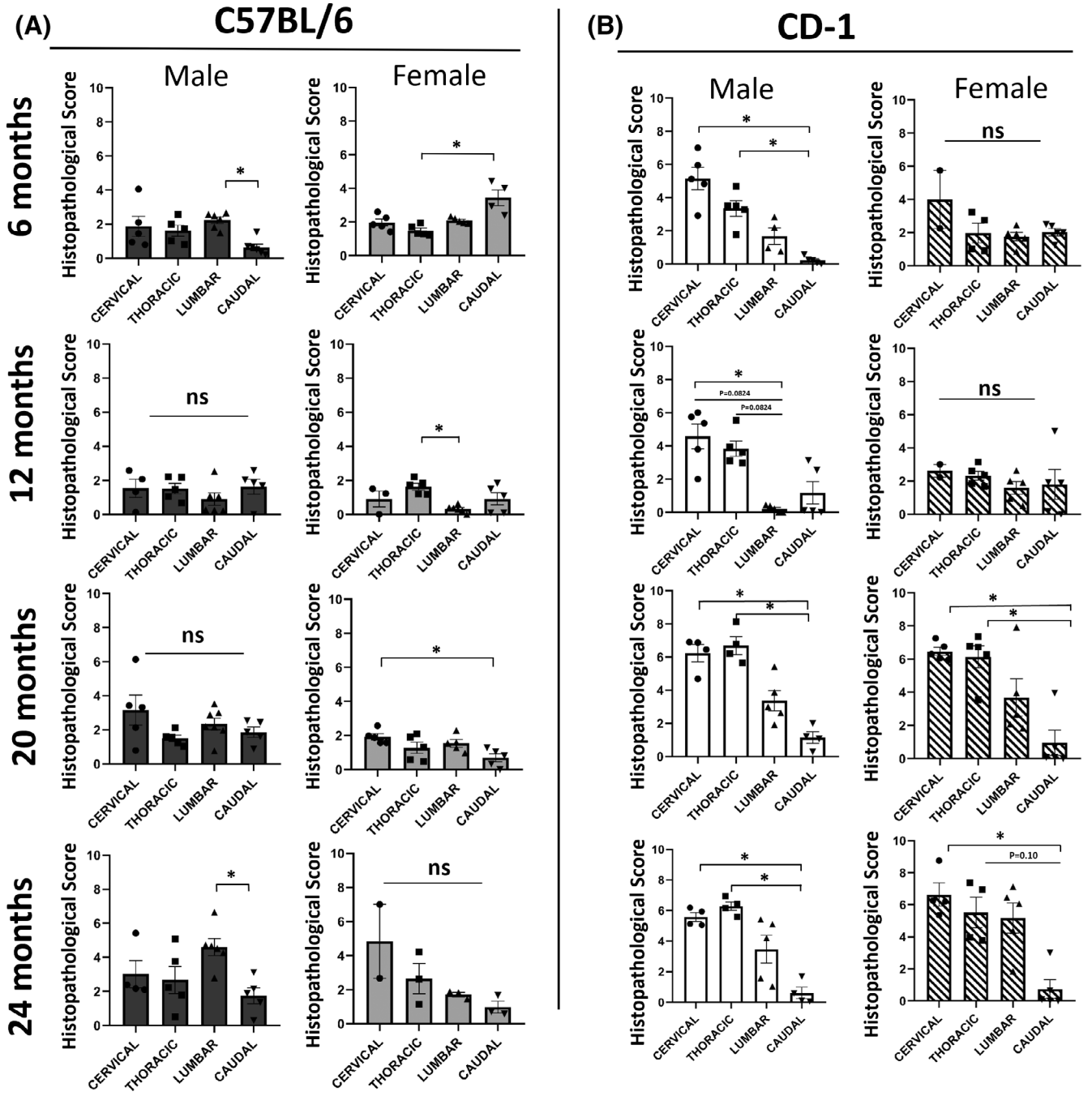

FIGURE 2 IVD degeneration by anatomical location in C57BL/6 and CD-1 mice. Average histopathological scores of IVDs from each anatomical region were compared in C57BL/6 (A) and CD-1 (B) mice. Average scores for each region in male (left column) and female (right column) were compared for each strain. Scores presented as the average of all IVDs in each anatomical region for each mouse ($N = 3–7$ for each time point, excluding cervical region). Data presented as mean ± SEM. Data analyzed by Kruskal–Wallis *H* test with Dunn's multiple comparisons, $^*p < 0.05$. IVD, intervertebral disc.

spine in C57BL/6 (Figure 3) and CD-1 (Figure 4) mice. Histological analysis showed degenerative changes in both the male and female C57BL/6 at 6 and 12 months of age, including cell cluster formation in the NP, widening of the lamellae, and detection of hypertrophic cells in the AF (Figure 3A,B). At 12 months of age, increased degeneration was noted in the upper thoracic spine, with a significant increase in histopathological scores between T2/3 and T8/9 in female mice. By 20 and 24 months of age (Figure 3C,D), increased signs of degeneration were detected, particularly in the upper thoracic spine, with high variability between mice. Histologically NP cell clusters varied in size, but typically displayed less than 50% cell loss. The AF frequently showed widened lamellae and increased prevalence of hypertrophic cells, while the boundary displayed some discontinuity and presence of hypertrophic cells. At 20 months of age, changes were associated with increased histopathological scores at T1/2 compared to T5/6, as well as at T10/11 and T11/12. To determine if

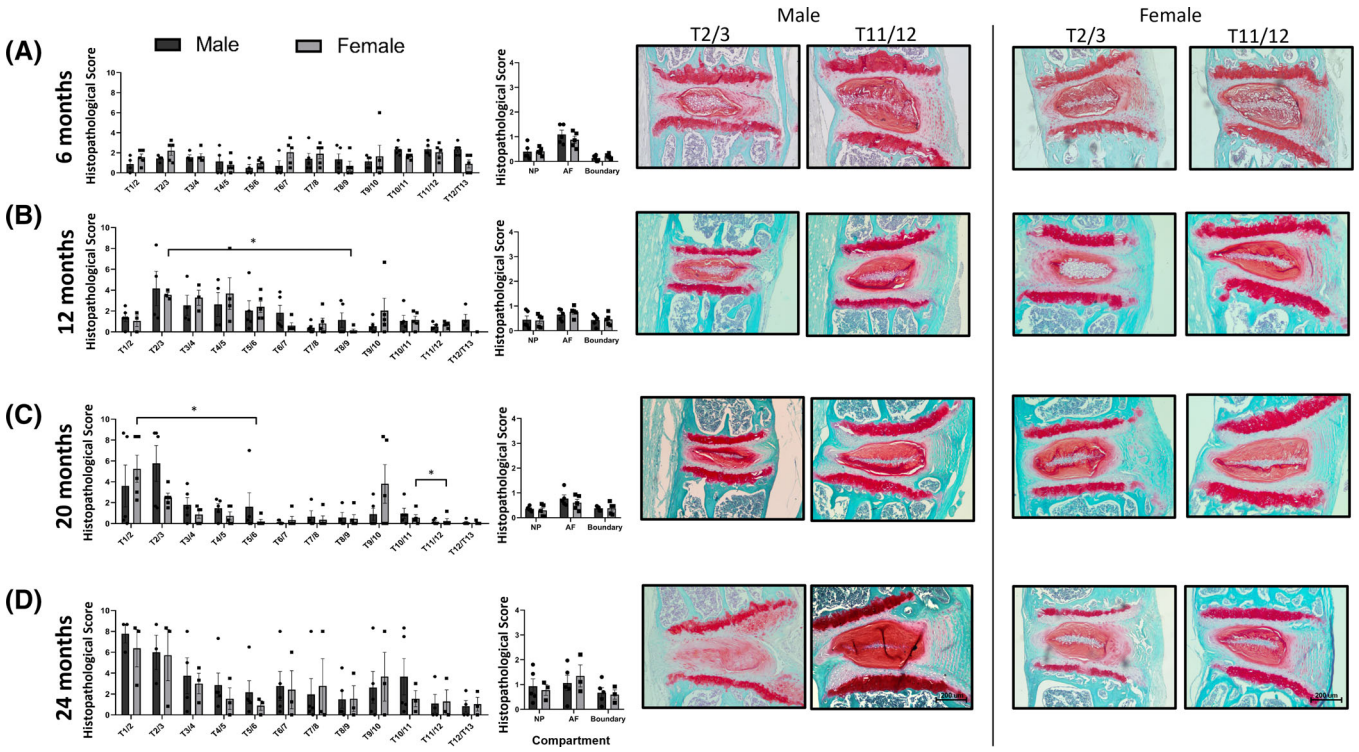

FIGURE 3 IVD degeneration by disc level in the thoracic spine of C57BL/6 mice. The average histopathological score for each IVD (NP + AF + boundary) in the thoracic spine was compared over time for both male and female C57BL/6 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all thoracic IVDs to generate the average compartment scores presented. Representative mid-sagittal sections corresponding to the upper (T2/3) and lower (T11/12) thoracic spine for both male and female mice at each time point stained with Safranin-O/Fast Green. Data presented as mean ± SEM, $N = 3–7$ mice for each time point. Data analyzed by Kruskal–Wallis H test with Dunn's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

histopathological scores were driven by changes within specific tissue compartments, the average scores for each of the NP, AF, and NP/AF boundary regions for thoracic IVDs were assessed. These scores increased slightly over time; no significant differences were detected between IVD compartments at any time point assessed.

Age-associated degeneration of thoracic IVDs was more striking in CD-1 mice (Figure 4). At each time point, histopathological scores were higher in CD-1 mice than in C57BL/6 mice, with no apparent trend based on location or degenerative changes within specific IVD compartments. At 12 months of age, a significant increase in histopathological scores was detected at T3/4 compared to T12/13 in female mice (Figure 4B). By 20 and 24 months of age (panels C and D), severe degeneration was detected across the thoracic spine, with decreased cellularity and near complete loss of structure in all IVD compartments. Composite figures with representative histological images of thoracic IVDs at each level are presented to highlight trends observed over time in C57BL/6 (Figure S2) and CD-1 (Figure S3) mice.

3.5 | Lumbar spine

A consistent gradient of degeneration was noted for C57BL/6 mice with increased histopathological scores in the lower lumbar IVDs in both male and female mice at 6, 20, and 24 months of age (Figure 5). At 6 months of age, a significant increase in degeneration was detected at the L5/6 and L6/S1 IVDs in both male and female mice compared to IVDs in the upper lumbar spine (Figure 5A). Histopathological scores generally increased with age, particularly in the lower lumbar spine. The pattern of increased degenerative scores at the L5/6 and L6/S1 IVDs compared to the upper lumbar spine was likewise detected at 20 months of age, associated with similar scores in the NP, AF, and boundary compartments (Figure 5C). At 24 months of age, female mice displayed increasing degenerative scores down the lumbar spine (Figure 5D). In contrast to earlier time points, increased degeneration was detected consistently across the lower lumbar spine in male mice, associated with complete loss of NP cellular organization and AF structure.

A similar pattern of lumbar spine degeneration was detected in CD-1 mice (Figure 6). At 6 months of age, lumbar IVDs showed only early degenerative changes such as cell cluster formation in the NP and widened AF lamellae, with no significant differences between histopathological scores for disc levels in either male or female mice (Figure 6A). At 12 months of age, increased degeneration was noted in lower lumbar IVDs, with a significant increase in degeneration score at L6/S1 for males, and L5/6 for females compared to IVDs in the upper lumbar spine (Figure 6B). At 20 months of age, increased histopathological scores were detected at L6/S1 in male mice compared to the L2/3 and L4/5 IVDs; however, no differences in degeneration

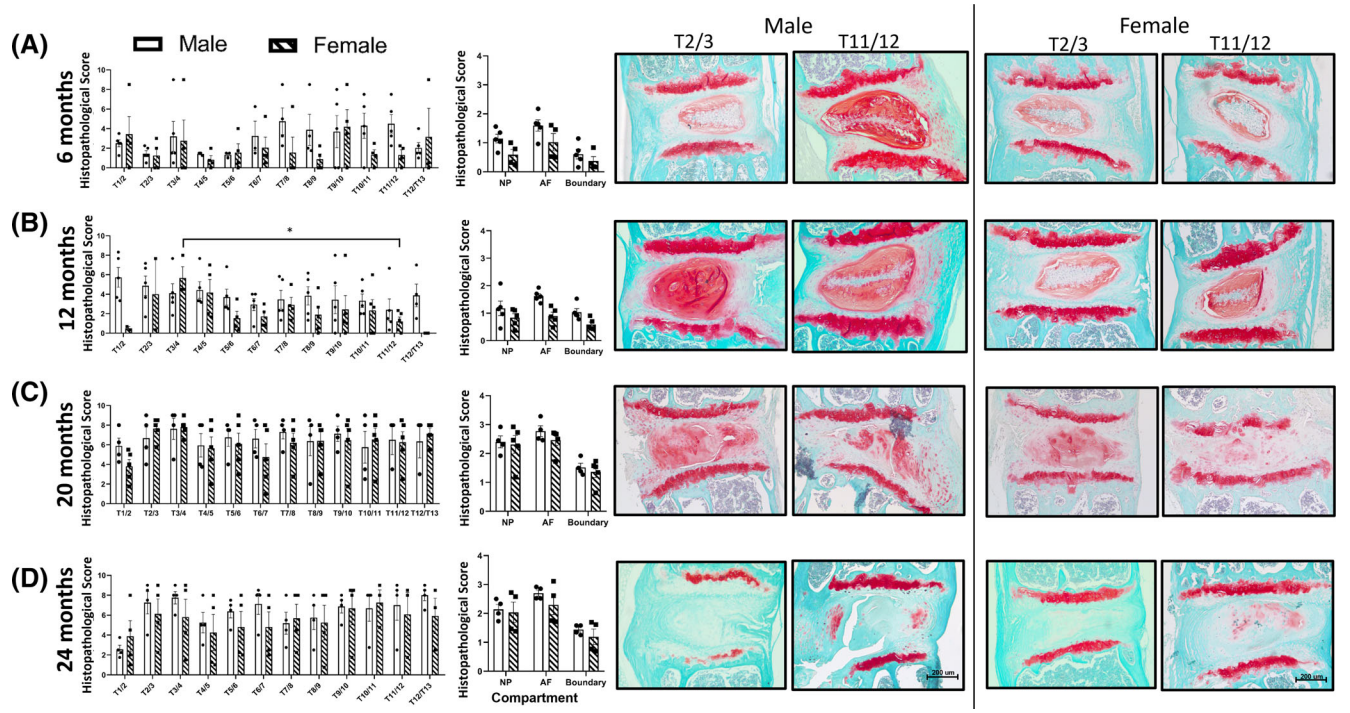

FIGURE 4 IVD degeneration by disc level in the thoracic spine of CD-1 mice. The average histopathological score for each IVD (NP + AF + boundary) in the thoracic spine was compared over time for both male and female CD-6 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all thoracic IVDs to generate the average compartment scores presented. Representative mid-sagittal sections corresponding to the upper (T2/3) and lower (T11/12) thoracic spine for both male and female mice at each time point stained with Safranin-O/Fast Green. Data presented as mean ± SEM, $N = 3–7$ mice for each time point. Data analyzed by Kruskal–Wallis H test with Dunn's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

scores were detected based on IVD level in female mice at this age (Figure 6C). By 24 months of age, increased histopathological scores were detected at all IVDs in the lumbar spine, associated with complete loss of NP cellular organization, the presence of hypertrophic cells within the AF, and loss of AF lamellar organization. Age-associated degeneration in the lumbar spine involved changes to all IVD compartments; no significant differences were detected between IVD compartments at any time point assessed. Composite figures with representative histological images of lumbar IVDs at each level are presented to highlight trends observed over time in C57BL/6 (Figure S4) and CD-1 (Figure S5) mice.

3.6 | Caudal spine

Level-by-level analysis of the caudal spine revealed a consistent pattern distinguishing the upper from lower caudal IVDs; as such, we pooled IVDs within each region to compare histopathological scores of upper (C5/6–C7/8) and lower caudal (C8/9–C11/12) discs (Figures 7 and 8). Evidence of histopathological degeneration was largely limited to the upper caudal spine in both C57BL/6 and CD-1 mice. At 6 months of age, female C57BL/6 mice showed significantly higher histopathological scores in IVDs of the upper caudal region than age-matched males (Figure 7A). This difference was associated with a significant increase in degeneration scores in all IVD compartments driven by features such as changes in NP cell organization, increased presence of hypertrophic cells in the AF, and loss of the NP/AF boundary. From 12 to 24 months of age, evidence of mild to moderate degeneration was detected in the upper caudal spine of both male and female mice, while few degenerative changes were detected in the lower caudal spine at any time point (panels B–D).

These findings were consistent in CD-1 mice (Figure 8). At 6 months of age, significantly higher histopathological scores were detected in IVDs of the upper caudal spine in females compared to age-matched males (Figure 8A). This difference was associated with increased degeneration scores in both the NP and AF, driven by features such as cell cluster formation in the NP and the presence of serpentine and/or widened lamellae in the AF. The boundary between the NP and AF remained well-defined in this group. In both strains, only mild to moderate degeneration was detected in the upper caudal spine of male and female mice up to 24 months of age, with few degenerative changes detected in the lower caudal spine at any time point (panels B–D).

4 | DISCUSSION

There are multiple risk factors associated with the development of IVD degeneration including age,[28] sex,[17] genetics,[29] and anatomical location.[9,19,28] Despite the now widespread use of mouse models to

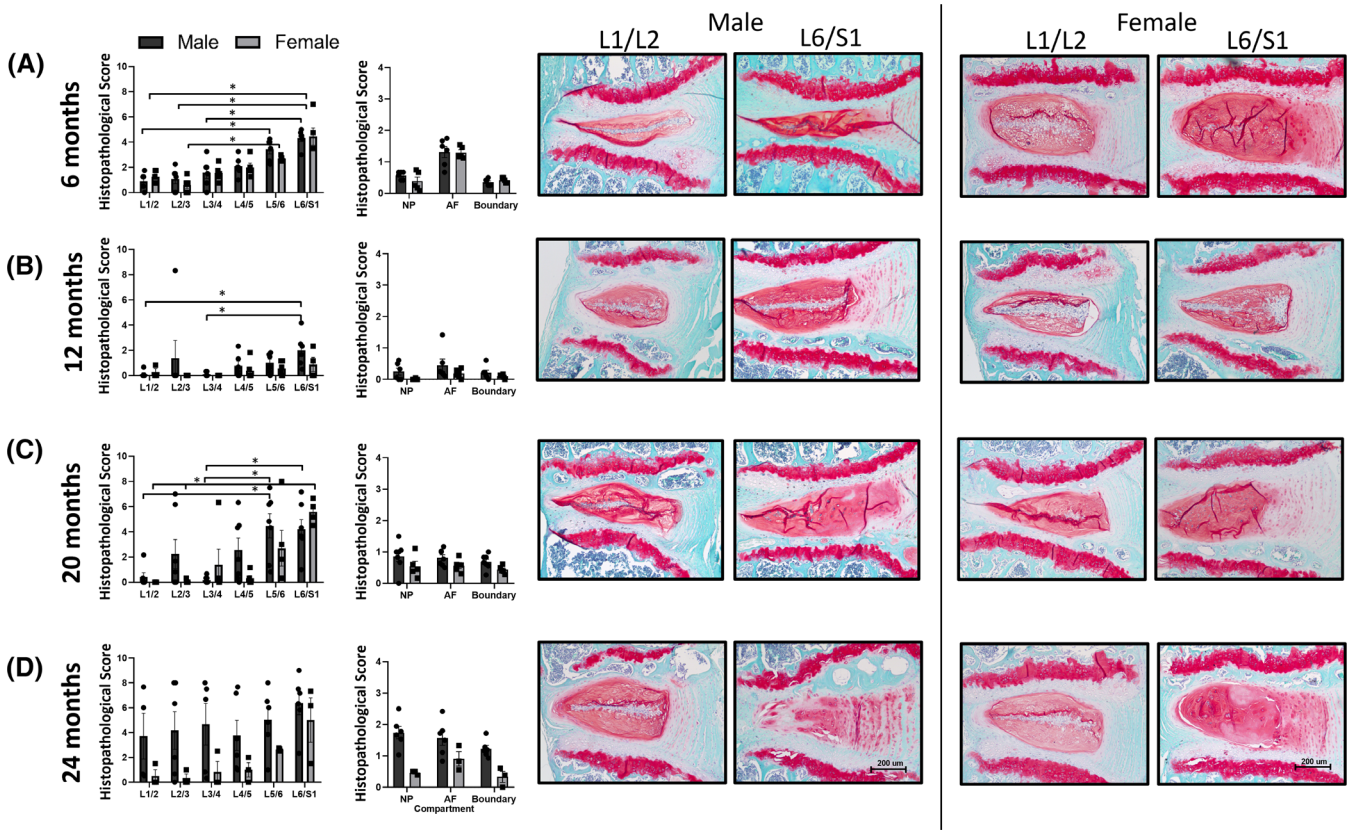

FIGURE 5 IVD degeneration by disc level in the lumbar spine of C57BL/6 mice. Average histopathological scores for each IVD (NP + AF + boundary) in the lumbar spine were compared for both male and female C57BL/6 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all lumbar IVDs to generate the average compartment scores presented. Representative mid-sagittal sections correspond to upper (L1/2) and lower (L6/S1) lumbar IVDs stained with Safranin-O/Fast Green. Data presented as mean ± SEM for each group, $N = 3–7$ mice for each time point. Data analyzed by Kruskal–Wallis H statistical test with Dunn's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

study IVD biology and disc degeneration, there have been limited studies characterizing the progression of age-associated degeneration to understand the effects of anatomical location, genetic strain, and sex.[28] Here, we report a detailed histopathological characterization of age-associated IVD degeneration in both male and female C57BL/6 and CD-1 mice. These mouse strains were specifically selected as they are commonly used for the generation of transgenic strains and/or in studies of IVD development, injury, and degeneration.[23,28,30–34] In keeping with previous studies,[28] C57BL/6 mice displayed only mild IVD degeneration until 24 months of age; at this point, the lumbar spine demonstrated the most degeneration compared to other regions. In contrast, degeneration was detected earlier in the CD-1 mice (20 months of age) in both the thoracic and lumbar spine; moreover, moderate to severe degeneration was noted in the cervical spine at all time points assessed. In both strains, age-associated IVD degeneration in the thoracic and lumbar spine was associated with increased histopathological scores in all IVD compartments and not driven by a single compartment. The differences observed in this study should serve to guide future studies aimed at investigating the etiology or potential disease-modifying targets for IVD degeneration using mouse models.

Of note, our study highlighted a remarkable lack of age-associated degeneration in caudal IVDs from male and female mice up to 24 months of age in both C57BL/6 and CD-1 strains. Even with advanced age, caudal IVDs maintained cellularity and matrix structure in all regions of the IVD throughout the caudal spine. This represents a notable difference to the LG/J and SM/J mouse models which show age-associated degeneration in caudal IVDs.[28] Mouse caudal IVDs have been previously reported to differ from lumbar IVDs in mechanical properties[35,36] and are subjected to different mechanical loads.[37] These findings suggest that caudal IVDs in C57BL/6 and CD-1 mice may be resistant to age-associated degeneration. Many studies have been conducted using caudal IVDs to study the effects of mechanical loading,[38,39] to implement models of injury-induced degeneration,[40–42] and as a tissue source for cell culture experiments[43,44] in small and large animal models given their ease of access. Our findings are consistent with previous reports[37] and highlight significant inherent differences in caudal IVDs relative to those in all other anatomical regions that may influence cell phenotype and function in vivo and in vitro. We postulate that these differences may influence biological readouts such as phenotypic changes in genetically modified mouse strains, the effects of pharmacological modulation of IVD cells, and as such, the

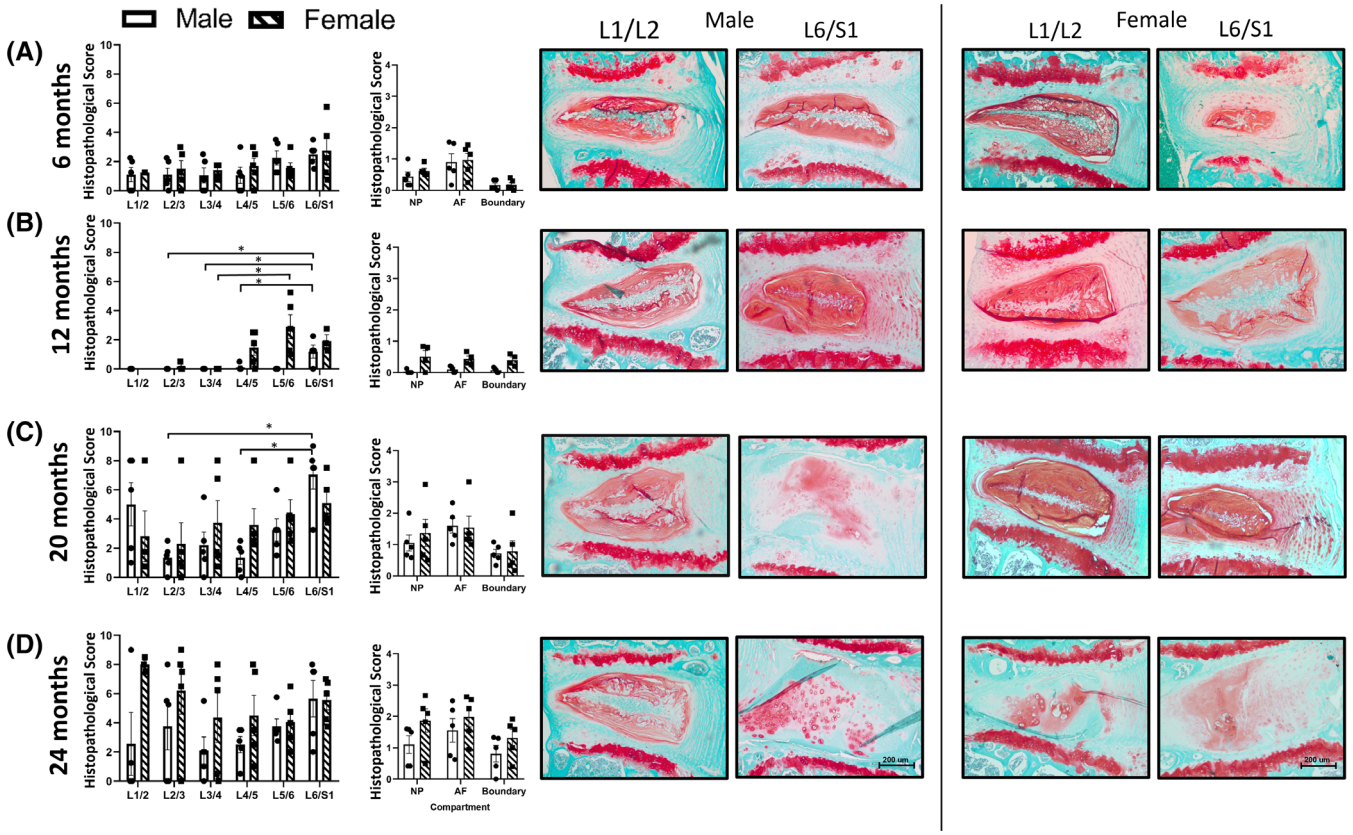

FIGURE 6 IVD degeneration by disc level in the lumbar spine of CD-1 mice. Average histopathological scores for each IVD (NP + AF + boundary) in the lumbar spine were compared for both male and female CD-1 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all lumbar IVDs to generate the average compartment scores presented. Representative mid-sagittal sections correspond to upper (L1/2) and lower (L6/S1) lumbar IVDs stained with Safranin-O/Fast Green. Images are representative of 3–7 mice per time point and 5–6 IVDs per mouse. Data presented as mean ± SEM for each group, $N = 3–7$ mice for each time point. Data analyzed by Kruskal–Wallis H statistical test with Dunn's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

extrapolation or translation of findings based on studies conducted exclusively using caudal IVDs.

The level-by-level assessment of IVD degeneration conducted enabled the identification of spinal regions more prone to degeneration within the thoracic and lumbar regions. Both strains and sexes displayed high variability between individual mice and between IVDs at each level, particularly in the thoracic spine. In both male and female C57BL/6 mice, increased degeneration was detected in the upper thoracic spine (T1/2–T3/4) compared to the mid and lower regions from 12 to 24 months of age. While a similar pattern was detected in CD-1 mice at 12 months of age, both male and female CD-1 mice displayed features of severe histopathological degeneration across the entire thoracic spine by 20 months of age in both male and female mice. In fact, the average degeneration scores in the thoracic spine were greater than those in the lumbar spine in CD-1 mice. While the current study did not explore the mechanisms underlying the differences in susceptibility to degeneration between anatomical regions, it would be intriguing to explore the relative contribution of factors including cellular composition, ECM levels, or mechanical loading in future studies. The striking strain-specific difference in age-associated degeneration in the thoracic spine should however be considered as an important factor in the design of experiments including these tissues.

Consistent trends in the pattern of age-associated disc degeneration across the lumbar spine were detected in both C57BL/6 and CD-1 mice. Similar to humans,[17] both mouse strains demonstrated a gradient of increased IVD degeneration towards the lower lumbar spine, with the highest histopathological scores detected at L5/6 and L6/S1 in male and female mice. Interestingly, by 24 months of age both strains showed high levels of degeneration in nearly all lumbar IVDs. The gradient of age-associated degeneration detected across the lumbar spine may be important to recognize for future characterization of genetically modified mouse strains where phenotypic changes could be masked by evaluations based on average histopathological scores from the lumbar spine.

The current study was designed to allow for a baseline characterization of sex-specific differences in age-associated disc degeneration in wild-type mice. In humans, IVD degeneration is more prevalent in women than men overall, but shows a temporal relationship in presentation.[17] Males display higher incidence and severity of IVD

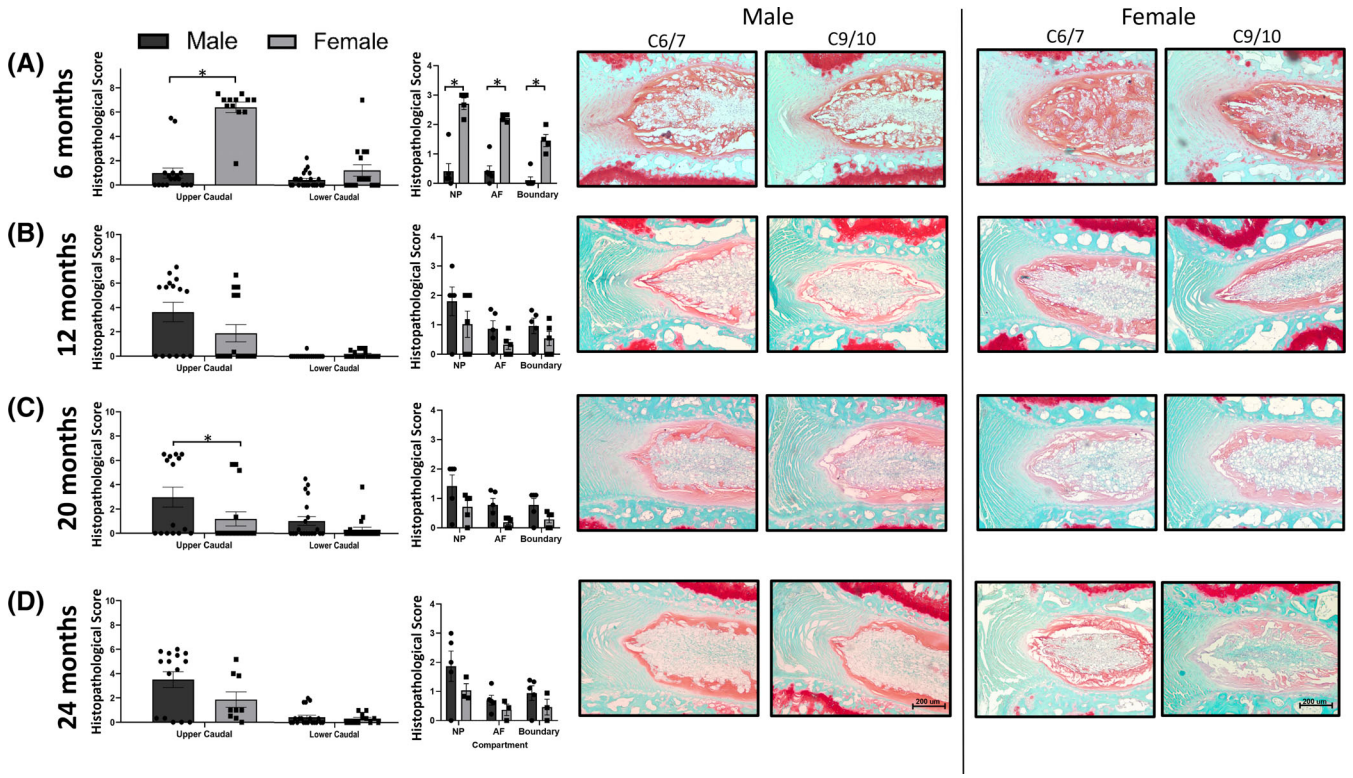

FIGURE 7 IVD degeneration in the upper and lower caudal spine of C57BL/6 mice. Histopathological scores for individual IVDs were averaged for the upper (C5–8) and lower caudal (C9–12) spine for both male and female C57BL/6 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all caudal IVDs to generate the average compartment scores presented. Representative mid-sagittal sections correspond to upper (C6/7) and lower (C9/10) caudal IVDs stained with Safranin-O/Fast Green. Images are representative of 3–7 mice per time point and 4–7 IVDs per mouse. Data presented as mean ± SEM for each group, $N = 3–7$ mice for each time point. Data analyzed by Mann–Whitney *U* statistical test with Tukey's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. c.

degeneration than women reported as early as the second decade of life[13] and this is speculated to be due to increased rates of physical injury and increased mechanical loading.[17] However, postmenopausal women (after 50 years of age) have an increased prevalence and severity of IVD degeneration compared to age-matched males.[45,46] Of note, analysis of human IVDs by MRI and Thompson scoring reported minimal differences in degeneration grades between males and females, concluding that sex had no significant effect on IVD degeneration.[47] Here, however, we show that both C57BL/6 and CD-1 mice display sex-specific differences in the temporal presentation of age-associated IVD degeneration in select anatomical regions of the spine. In C57BL/6 mice, consistent patterns of age-associated degeneration were detected in male and female mice in the cervical and thoracic spine. However, male mice showed accelerated degeneration across the lumbar spine compared to female mice at 24 months of age. Similar to C57BL/6 mice, CD-1 mice demonstrated similar rates and extent of IVD degeneration in the cervical and thoracic spine between male and female mice. When the average histopathological scores were compared between regions in CD-1 mice, male mice displayed significant differences with higher degeneration scores in the upper regions (cervical, thoracic) compared to the lower regions (lumbar caudal) at all time points assessed. This pattern was not detected in female mice until 20 months of age, suggesting a delay in the onset of degeneration. These differences in the onset of region-specific degeneration between male and female CD-1 mice may be consistent with the accelerated lumbar IVD degeneration detected in male C57BL/6 mice compared to females with advanced age. At 24 months of age, female CD-1 mice showed severe degeneration across the lumbar spine, while male mice demonstrated the gradient of increased degeneration toward the lower lumbar region. We postulate that strain-specific differences in age-associated degeneration confound our ability to directly compare sex-related differences between C57BL/6 and CD-1 mice at the time points examined. Sex-related differences have likewise been reported in rat models of IVD degeneration, demonstrating increased AF degeneration in females compared to males following annular puncture,[48] as well as sex-based differences in the relationship between degeneration and pain.[49] Interestingly, recent studies in humans have begun to explore the associations between risk factors for IVD degeneration, highlighting the interaction between sex and factors including genetic polymorphisms and obesity and the role of sex hormones.[50,51] Taken together, these findings further underscore the importance of evaluating sex as a biological factor for the development of age-associated IVD degeneration in the identification of disease-associated factors or the assessment of potential therapeutic interventions in murine models.

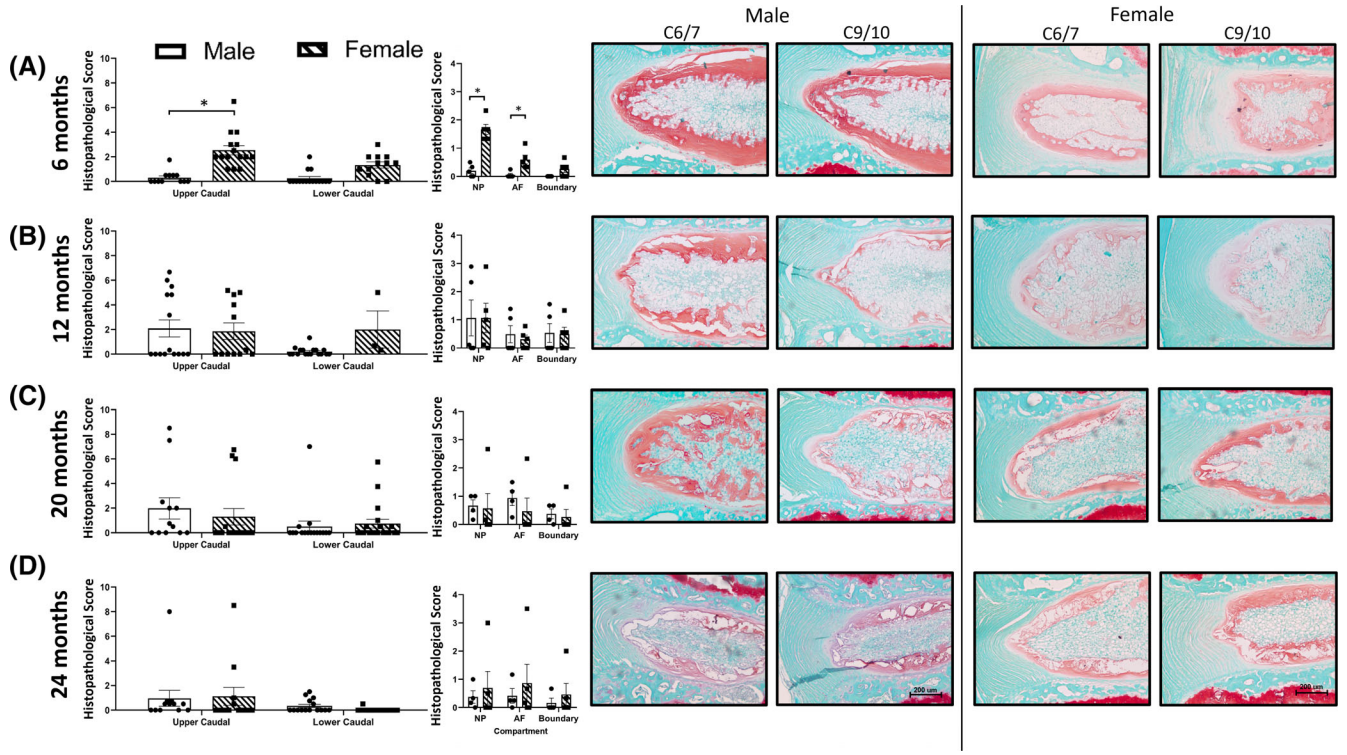

FIGURE 8 (A) IVD degeneration in the upper and lower caudal spine of CD-1 mice. Histopathological scores for individual IVDs were averaged for the upper (C5–8) and lower caudal (C9–12) spine for both male and female CD-1 mice at 6 (A), 12 (B), 20 (C), and 24 (D) months of age. The individual scores for each of the NP, AF, and boundary were averaged across all caudal IVDs to generate the average compartment scores presented. Representative mid-sagittal sections correspond to upper (C6/7) and lower (C9/10) caudal IVDs stained with Safranin-O/Fast Green. Data presented as mean ± SEM for each group, $N = 3–7$ mice for each time point. Data analyzed by Mann–Whitney *U* statistical test with Tukey's multiple comparisons, $^*p < 0.05$. Scale bar = 200 μm. IVD, intervertebral disc.

Despite the design of a longitudinal study that evaluated four groups of mice at four time points each from a total of over 80 mice and approximately 2400 IVDs, our study was associated with a number of limitations. First, our analysis was limited to that of two mouse strains commonly used for the generation of gene-modified mice but did not include additional strains that have been previously investigated in the context of IVD degeneration such as the SM/J or LG/J mice which demonstrate differences in regenerative capacities.[52,53] Second, our strategy of spine region segmentation at dissection was adopted to ensure consistency of tissue processing and histological analysis; however, we encountered difficulties in obtaining mid-sagittal sections of sequential cervical IVDs limiting our analysis of this region. Assessment of IVD degeneration was limited to changes in histopathology, whereas human IVD degeneration is largely attributed to loss of MRI signal and disc height. In addition, calcification of the IVD characteristic of human degeneration is not well modeled in most mouse strains including CD-1 and C57BL/6,[28] and as such this feature was not assessed in this study. Similarly, our analysis focused on degenerative changes in the NP, AF, and boundary between these regions using an established histopathological scoring system.[24] Our analysis did not include the assessment of degenerative features in the cartilage endplates of the IVD, an analysis that would be well suited for future studies given the importance of the endplate to disc degeneration and the recent validation of histopathological scoring systems that would enable its analysis.[54]

5 | CONCLUSION

This study highlights how anatomical region, strain, and sex contribute to the presentation and progression of age-associated IVD degeneration in mouse models. In general, age-associated degeneration was accelerated in CD-1 mice compared to C57BL/6 mice. In both strains, while histopathological features of age-associated degeneration were characterized in the thoracic and lumbar spine, IVDs within the caudal spine did not show degenerative changes with age. Both C57BL/6 and CD-1 mice displayed sex-specific differences in the presentation of age-associated IVD degeneration. This study aimed to provide a baseline characterization to guide future studies using mouse models to study IVD degeneration, and demonstrates that consideration of strain, sex, and anatomical region is key to the interpretation and the translatability of findings.

ACKNOWLEDGMENTS

This work was funded by the Canadian Institutes of Health Research and the Arthritis Society (grants to CAS). JH was supported by awards

from the CONNECT! NSERC CREATE Training Program, a Transdisciplinary Training Award from Western's Bone and Joint Institute, the Arthritis Society, and the Ontario Graduate Scholarship (OGS). MAV was supported by a Transdisciplinary Training Award from Western's Bone and Joint Institute, an Ontario Graduate Scholarship, and the Arthritis Society. MES was supported by awards from Western's Bone and Joint Institute and the Canadian Institute of Health Research. FB holds the Canada Research Chair in Musculoskeletal Research. CAS is supported by a Career Development award from the Arthritis Society.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Jeffrey L. Hutchinson https://orcid.org/0000-0003-2011-389X
Matthew A. Veras https://orcid.org/0000-0002-4504-5187
Matthew R. McCann https://orcid.org/0000-0003-2953-6258
Frank Beier https://orcid.org/0000-0002-8505-7537
Cheryle A. Séguin https://orcid.org/0000-0001-6503-9107

REFERENCES

1. Vos T, Lim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;*396*:1204-1222.
2. DePalma MJ, Ketchum JM, Saullo T. What is the source of chronic low back pain and does age play a role? *Pain Med*. 2011;*12*:224-233.
3. Hoy D, Brooks P, Blyth F, Buchbinder R. The epidemiology of low back pain. *Best Pract Res Clin Rheumatol*. 2010;*24*:769-781.
4. Vergroesen P-PA, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. *Osteoarthr Cartil*. 2015;*23*:1057-1070.
5. Katz JN. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. *J Bone Joint Surg Am*. 2006;*88*(Suppl 2):S21-S24.
6. Roelofs PDDM, Deyo RA, Koes BW, Scholten RJPM, van Tulder MW. Nonsteroidal anti-inflammatory drugs for low back pain: an updated Cochrane review. *Spine*. 2008;*33*:1766-1774.
7. Zhao L, Manchikanti L, Kaye AD, Abd-Elsayed A. Treatment of discogenic low back pain: current treatment strategies and future options—a literature review. *Curr Pain Headache Rep*. 2019;*23*:86.
8. Oichi T, Taniguchi Y, Oshima Y, Tanaka S, Saito T. Pathomechanism of intervertebral disc degeneration. *JOR Spine*. 2020;*3*:e1076.
9. Daly C, Ghosh P, Jenkin G, Oehme D, Goldschlager T. A review of animal models of intervertebral disc degeneration: pathophysiology, regeneration, and translation to the clinic. *Biomed Res Int*. 2016;*2016*:5952165.
10. Wong CK, Mak RYW, Kwok TSY, et al. Prevalence, incidence, and factors associated with non-specific chronic low back pain in community-dwelling older adults aged 60 years and older: a systematic review and meta-analysis. *J Pain*. 2022;*23*:509-534.
11. Sivan SS, Wachtel E, Roughley P. Structure, function, aging and turnover of aggrecan in the intervertebral disc. *Biochim Biophys Acta*. 2014;*1840*:3181-3189.
12. Kirnaz S, Capadona C, Wong T, et al. Fundamentals of intervertebral disc degeneration. *World Neurosurg*. 2022;*157*:264-273.
13. Miller JA, Schmatz C, Schultz AB. Lumbar disc degeneration: correlation with age, sex, and spine level in 600 autopsy specimens. *Spine*. 1988;*13*:173-178.
14. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*. 2002;*27*:2631-2644.
15. Hangai M, Kaneoka K, Hinotsu S, et al. Lumbar intervertebral disk degeneration in athletes. *Am J Sports Med*. 2009;*37*:149-155.
16. Abdalkader M, Guermazi A, Engebretsen L, et al. MRI-detected spinal disc degenerative changes in athletes participating in the Rio de Janeiro 2016 Summer Olympics games. *BMC Musculoskelet Disord*. 2020;*21*:45.
17. Wáng YXJ, Wáng J-Q, Káplár Z. Increased low back pain prevalence in females than in males after menopause age: evidences based on synthetic literature review. *Quant Imaging Med Surg*. 2016;*6*:199-206.
18. Lee NN, Salzer E, Bach FC, et al. A comprehensive tool box for large animal studies of intervertebral disc degeneration. *JOR Spine*. 2021;*4*:e1162.
19. Jin L, Balian G, Li XJ. Animal models for disc degeneration—an update. *Histol Histopathol*. 2018;*33*:543-554.
20. Tang SN, Walter BA, Heimann MK, et al. In vivo mouse intervertebral disc degeneration models and their utility as translational models of clinical discogenic back pain: a comparative review. *Front Pain Res*. 2022;*3*:894651.
21. O'Connell GD, Vresilovic EJ, Elliott DM. Comparison of animals used in disc research to human lumbar disc geometry. *Spine*. 2007;*32*:328-333.
22. McCann MR, Séguin CA. Notochord cells in intervertebral disc development and degeneration. *J Dev Biol*. 2016;*4*:3.
23. McCann MR, Patel P, Pest MA, et al. Repeated exposure to high-frequency low-amplitude vibration induces degeneration of murine intervertebral discs and knee joints. *Arthritis Rheumatol*. 2015;*67*:2164-2175.
24. Tam V, Chan WCW, Leung VYL, et al. Histological and reference system for the analysis of mouse intervertebral disc. *J Orthop Res*. 2018;*36*:233-243.
25. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;*33*:159-174.
26. Adams MA, Dolan P. Intervertebral disc degeneration: evidence for two distinct phenotypes. *J Anat*. 2012;*221*:497-506.
27. Bailey JF, Hargens AR, Cheng KK, Lotz JC. Effect of microgravity on the biomechanical properties of lumbar and caudal intervertebral discs in mice. *J Biomech*. 2014;*47*:2983-2988.
28. Novais EJ, Tran VA, Miao J, et al. Comparison of inbred mouse strains shows diverse phenotypic outcomes of intervertebral disc aging. *Aging Cell*. 2020;*19*:e13148.
29. Feng Y, Egan B, Wang J. Genetic factors in intervertebral disc degeneration. *Genes Dis*. 2016;*3*:178-185.
30. Kerr GJ, To B, White I, et al. Diet-induced obesity leads to behavioral indicators of pain preceding structural joint damage in wild-type mice. *Arthritis Res Ther*. 2021;*23*:93.
31. Ohnishi T, Sudo H, Tsujimoto T, Iwasaki N. Age-related spontaneous lumbar intervertebral disc degeneration in a mouse model. *J Orthop Res*. 2018;*36*:224-232.
32. Alvarez-Garcia O, Matsuzaki T, Olmer M, Masuda K, Lotz MK. Age-related reduction in the expression of FOXO transcription factors and correlations with intervertebral disc degeneration. *J Orthop Res*. 2017;*35*:2682-2691.
33. Gorth DJ, Shapiro IM, Risbud MV. A new understanding of the role of IL-1 in age-related intervertebral disc degeneration in a murine model. *J Bone Miner Res*. 2019;*34*:1531-1542.
34. Vincent K, Mohanty S, Pinelli R, et al. Aging of mouse intervertebral disc and association with back pain. *Bone*. 2019;*123*:246-259.
35. Holguin N, Aguilar R, Harland RA, Bomar BA, Silva MJ. The aging mouse partially models the aging human spine: lumbar and coccygeal disc height, composition, mechanical properties, and Wnt

signaling in young and old mice. *J Appl Physiol*. 2014;*1985*(116):1551-1560.
36. Elliott DM, Sarver JJ. Young investigator award winner: validation of the mouse and rat disc as mechanical models of the human lumbar disc. *Spine*. 2004;*29*:713-722.
37. Brendler J, Winter K, Lochhead P, Schulz A, Ricken AM. Histological differences between lumbar and tail intervertebral discs in mice. *J Anat*. 2022;*240*:84-93.
38. Walter BA, Korecki CL, Purmessur D, Roughley PJ, Michalek AJ, Iatridis JC. Complex loading affects intervertebral disc mechanics and biology. *Osteoarthritis Cartilage*. 2011;*19*:1011-1018.
39. MacLean JJ, Lee CR, Grad S, Ito K, Alini M, Iatridis JC. Effects of immobilization and dynamic compression on intervertebral disc cell gene expression in vivo. *Spine*. 2003;*28*:973-981.
40. Tian Z, Ma X, Yasen M, et al. Intervertebral disc degeneration in a percutaneous mouse tail injury model. *Am J Phys Med Rehabil*. 2018;*97*:170-177.
41. Serjeant M, Moon PM, Quinonez D, Penuela S, Beier F, Séguin CA. The role of Panx3 in age-associated and injury-induced intervertebral disc degeneration. *Int J Mol Sci*. 2021;*22*:1080.
42. Korecki CL, Costi JJ, Iatridis JC. Needle puncture injury affects intervertebral disc mechanics and biology in an organ culture model. *Spine*. 2008;*33*:235-241.
43. Panebianco CJ, Dave A, Charytonowicz D, Sebra R, Iatridis JC. Single-cell RNA-sequencing atlas of bovine caudal intervertebral discs: discovery of heterogeneous cell populations with distinct roles in homeostasis. *FASEB J*. 2021;*35*:e21919.
44. Bezci SE, Werbner B, Zhou M, et al. Radial variation in biochemical composition of the bovine caudal intervertebral disc. *JOR Spine*. 2019;*2*:e1065.
45. Wang Y-XJ, Griffith JF. Effect of menopause on lumbar disk degeneration: potential etiology. *Radiology*. 2010;*257*:318-320.
46. Wang YXJ. Postmenopausal Chinese women show accelerated lumbar disc degeneration compared with Chinese men. *J Orthop Translat*. 2015;*3*:205-211.
47. Siemionow K, An H, Masuda K, Andersson G, Cs-Szabo G. The effects of age, gender, ethnicity, and spinal level on the rate of intervertebral disc degeneration. A review of 1712 intervertebral discs. *Spine*. 2011;*36*:1333-1339.
48. Mosley GE, Hoy RC, Nasser P, et al. Sex differences in rat intervertebral disc structure and function following annular puncture injury. *Spine*. 2019;*44*:1257-1269.
49. Mosley GE, Wang M, Nasser P, et al. Males and females exhibit distinct relationships between intervertebral disc degeneration and pain in a rat model. *Sci Rep*. 2020;*10*:15120.
50. Shelby T, Mills ES, Ton A, et al. The role of sex hormones in degenerative disc disease. *Glob Spine J*. 2023;*13*:2096-2099. doi:10.1177/21925682231152826
51. Chen H-H, Hsu H-T, Liao M-H, Teng M-S. Effects of sex and obesity on LEP variant and leptin level associations in intervertebral disc degeneration. *Int J Mol Sci*. 2022;*23*:12275.
52. Choi H, Tessier S, Silagi ES, et al. A novel mouse model of intervertebral disc degeneration shows altered cell fate and matrix homeostasis. *Matrix Biol*. 2018;*70*:102-122.
53. Zhang Y, Xiong C, Kudelko M, et al. Early onset of disc degeneration in SM/J mice is associated with changes in ion transport systems and fibrotic events. *Matrix Biol*. 2018;*70*:123-139.
54. Melgoza IP, Chenna SS, Tessier S, et al. Development of a standardized histopathology scoring system using machine learning algorithms for intervertebral disc degeneration in the mouse model-An ORS spine section initiative. *JOR Spine*. 2021;*4*:e1164.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hutchinson, J. L., Veras, M. A., Serjeant, M. E., McCann, M. R., Kelly, A. L., Quinonez, D., Beier, F., & Séguin, C. A. (2023). Comparative histopathological analysis of age-associated intervertebral disc degeneration in CD-1 and C57BL/6 mice: Anatomical and sex-based differences. *JOR Spine*, *6*(4), e1298. https://doi.org/10.1002/jsp2.1298