Impaired Intervertebral Disc Development and Premature Disc Degeneration in Mice With Notochord-Specific Deletion of CCN2

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Objective. Currently, our ability to treat intervertebral disc (IVD) degeneration is hampered by an incomplete understanding of disc development and aging. The specific function of matricellular proteins, including CCN2, during these processes remains an enigma. The aim of this study was to determine the tissue-specific localization of CCN proteins and to characterize their role in IVD tissues during embryonic development and age-related degeneration by using a mouse model of notochord-specific CCN2 deletion.

Methods. Expression of CCN proteins was assessed in IVD tissues from wild-type mice beginning on embryonic day 15.5 to 17 months of age. Given the enrichment of CCN2 in notochord-derived tissues, we generated notochord-specific CCN2−null mice to assess the impact on the IVD structure and extracellular matrix composition. Using a combination of histologic evaluation and magnetic resonance imaging (MRI), IVD health was assessed.

Results. Loss of the CCN2 gene in notochord-derived cells disrupted the formation of IVDs in embryonic and newborn mice, resulting in decreased levels of aggrecan and type II collagen and concomitantly increased levels of type I collagen within the nucleus pulposus. CCN2-knockout mice also had altered expression of CCN1 (Cyr61) and CCN3 (Nov). Mirroring its role during early development, notochord-specific CCN2 deletion accelerated age-associated degeneration of IVDs.

Conclusion. Using a notochord-specific gene targeting strategy, this study demonstrates that CCN2 expression by nucleus pulposus cells is essential to the regulation of IVD development and age-associated tissue maintenance. The ability of CCN2 to regulate the composition of the intervertebral disc suggests that it may represent an intriguing clinical target for the treatment of disc degeneration.

Globally, the prevalence of low back pain is increasing at an alarming rate, with the most recent systematic review reporting a lifetime prevalence of 39%, which is predicted to increase substantially over the coming decades as the population ages (1). Intervertebral disc (IVD) degeneration, an underlying cause of low back pain, begins with changes in the cellular microenvironment that are initiated long before the appearance of associated symptoms, such as decreased mobility and acute or chronic pain (2). The lack of effective treatment for this widespread clinical problem is related to our limited understanding of the mechanisms that regulate the processes of IVD development, maintenance, and degeneration. In particular, there is an incomplete understanding of the relative importance of individual growth factors, secreted molecules, and matrix components that constitute the unique microenvironment of the IVD.
IVDs are specialized connective tissue structures that anchor adjacent vertebral bodies along the spine, conferring flexibility and providing mechanical stability during axial compression. Anatomically, the IVD consists of 3 structurally distinct, yet interdependent tissues: the annulus fibrosus (AF), the central nucleus pulposus (NP), and the cartilage end plates that anchor the disc to the adjacent vertebral bodies.

The mechanical properties of the IVD result from the specific organization of the extracellular matrix (ECM), which is maintained by the distinct cell populations found within the component tissues. The AF consists of concentric lamellae formed by parallel bundles of type I collagen fibers, which encapsulate the NP and provide tensile strength (3). The NP consists largely of a proteoglycan and water gel supported by an irregular network of type II collagen and elastin fibers. The major proteoglycan of the disc is aggrecan, which, due to its highly anionic glycosaminoglycan content, provides osmotic properties, enabling the NP to maintain height and turgescent against compressive loads (2,4). In contrast to the cartilage end plates and AF, in which mesenchyme-derived cell types remain relatively invariant, the NP undergoes a substantial change in its cellular and ECM composition throughout life (5). Notochord cells constitute the primordium for the NP during development; however, in humans, their numbers decrease rapidly after birth and the NP becomes gradually populated by smaller chondrocyte-like cells (6). Recent studies have demonstrated that in mice, all cells of the mature NP are derived from the embryonic notochord (7,8); however, the change in cell phenotype associated with notochord cell maturation has yet to be established.

Named after the first 3 members to be discovered, cysteine-rich protein 61 (Cyr61), connective tissue growth factor (CTGF), and nephroblastoma overexpressed protein (Nov), the CCN family consists of 6 secreted matricellular proteins (CCN1–CCN6) that serve as multifunctional signaling mediators that regulate the interactions of cells, growth factors, and the ECM (9). Data generated using mouse knockout models revealed that CCN proteins play key roles in angiogenesis, embryonic cartilage and bone formation, and mediate inflammation and fibrosis in adults (10,11). Of the CCN family members, CCN1 appears to possess antagonistic activity to both the CCN family members, CCN1 appears to possess antagonistic activity to CCN2 (13–15). In mice, loss of the CCN2 gene results in skeletal dysmorphisms and perinatal lethality linked with improper rib cage formation due to impaired endochondral ossification (18). In addition to its role in cartilage and bone development, several studies have suggested that CCN2 may also play a role in both the notochord and IVD. In mice, microarray-based gene expression profiling demonstrated that expression of CCN2 is enriched in the embryonic node and notochord (19). Moreover, in zebrafish, knockdown of CCN2 expression leads to notochord malformation and early embryonic lethality (20). Within the IVD, CCN2 was first identified as an anabolic factor secreted by notochord cells, which induces NP cell proliferation and aggrecan production in vitro (21–23). Recent studies localized CCN2 expression to NP cells in degenerated human IVDs and proposed that CCN2 promotes angiogenesis and accelerates IVD fibrosis and degeneration (24,25). In contrast, the association between CCN2 and IVD degeneration has led to speculation that the expression of CCN2 is associated with initiation of a reparative response that influences ECM remodeling and cell proliferation (26). Despite these correlational observations, the specific role played in vivo by CCN2 in the development, aging, and degeneration of the IVD remains unclear.

The objective of the present study was to examine selected CCN proteins that had previously been localized to IVD tissues for their expression during distinct stages of development and aging. To precisely delineate the role of CCN2 in the IVD, we used a conditional knockout strategy in mice harboring an allele that drives the expression of Cre recombinase under the control of a notochord-specific promoter (NotoCre) (8) and CCN2 alleles flanked with loxP sites (27). We found that loss of CCN2 in notochord-derived cells in vivo resulted in impaired development of IVDs and marked acceleration of age-associated IVD degeneration. Our results provide new and valuable insights into the role played by CCN2 in the IVD and suggest that modulation of CCN levels could regulate disc degeneration.

**MATERIALS AND METHODS**

**Animals.** Genetically modified mice harboring a notochord-specific deletion of Ccn2 were generated using the Cre/loxP method. Mice carrying the Ccn2 gene flanked by loxP sites (Ccn2F/F C57BL6 mice) (27) were mated with NotoCre mice (8), to generate mice bearing NotoCre and a floxed Ccn2 allele in their germline. These mice were backcrossed to homozygous floxed mice (NotoCre / Ccn2F/F × Ccn2F/F) to generate mice with inactivation of both alleles in notochord-
derived cells (genotype NotoCre/+ /Ccn2F/F). Homozygous disruption of the Noto locus is perinatal lethal (28); viable offspring have genotypes of either NotoCre/+ /Ccn2F/F (herein after referred to as CCN2-knockout) or Noto+/+ /Ccn2F/F (hereinafter referred to as wild-type). Mice were housed in standard cages and maintained on a 12-hour light/dark cycle, with rodent chow and water available ad libitum. Genotyping was performed as previously described (8,27).

Mice were euthanized at the following ages: embryonic day 15.5, postnatal day 1, postnatal day 28, 12 months, and 17 months. Time points were selected to reflect distinct stages of IVD development, including notochord segmentation (embryonic day 15.5) and intervertebral disc formation (postnatal day 1). To assess IVD aging, tissues were examined in fully formed IVDs prior to skeletal maturity (postnatal day 28), and in mice of advanced age prior to the onset of significant degenerative changes (12 and 17 months), which were previously reported in wild-type mice (29,30). Experimental results at each time point examined were derived from the analysis of 5–6 IVDs from each mouse, using 4 wild-type and 4 CCN2-knockout mice, with the exception of the month 17 time point where data are reported for 4 wild-type and 3 CCN2-knockout mice.

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.

**Magnetic resonance imaging (MRI).** At 12 or 17 months of age, wild-type and CCN2-knockout mice were subjected to MRI. Three-dimensional spinal IVD imaging was conducted with a 9.4T small-animal MRI scanner equipped with a 30-mm millipede coil (Agilent) and using a TrueFISP 3-dimensional pulse sequence with the following parameters: repetition time 5.4 msec, echo time 2.7 msec, field of view 51.2 mm, matrix 640 × 280 × 32 voxels, spatial resolution 80 × 80 × 500 μm, and scan time 60 minutes. The anesthetized mouse was placed supine on a tray and taped to minimize motion artifacts. The body temperature of the mouse was maintained at 37°C using a MR-compatible physiologic monitoring and gating system (SA Instruments) during the scan.

For quantification of signal intensity (an indicator of tissue hydration), a region of interest was standardized based on wild-type animals, using a box size encompassing the entire lumbar IVD. Relative signal intensities within this region were normalized to that of adjacent skeletal muscle to account for changes (12 and 17 months), which were previously reported in wild-type mice (29,30). Experimental results at each time point examined were derived from the analysis of 5–6 IVDs from each mouse, using 4 wild-type and 4 CCN2-knockout mice, with the exception of the month 17 time point where data are reported for 4 wild-type and 3 CCN2-knockout mice.

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.

**Histologic and immunofluorescence analyses.** Formalin-fixed tissue samples were decalcified for 5 days with gentle rocking, using a Shandon TBB-2 decalcifier (Thermo Scientific) at a fluid-to-tissue ratio of 10:1. Following standard histologic processing, samples were embedded in paraffin, and 5 μm–thick serial sections were cut in the coronal plane.

![Figure 1](image_url). Localization of CCN proteins in the intervertebral discs of wild-type mice during skeletal development. Representative images demonstrate the immunolocalization of CCN1, CCN2, and CCN3 in wild-type mice of the following ages: embryonic day 15.5 (E15.5) (A–C), newborn (NB) (D–F), postnatal day 28 (P28) (G–I), 12 months (J–L), and 17 months (M–O). The expression of CCN1 and CCN2, but not CCN3, is enriched in the notochord and notochord-derived nucleus pulposus (arrows). Images are oriented with rostral at the top and are representative of 4 mice per time point. Bar = 50 μm.

Sections were deparaffinized in xylene and rehydrated by successive immersion in descending concentrations of alcohol. Serial sections were processed with either hematoxylin and eosin or 0.1% Safranin O–0.02% fast green, and images were acquired using a Leica DM1000 microscope with Leica Application Suite software. For evaluation of IVD degeneration, sections of the lumbar spine from wild-type and CCN2-knockout mice were stained with Safranin O–fast green, and changes were scored according to the Thompson grading scale (32) by 2 independent observers who were blinded with regard to the experimental group.

For immunohistochemistry, samples were processed as described above and antigen retrieval was performed with 10 mM sodium citrate (pH 6.0) for 12 minutes at 95°C. Slides were blocked by incubation for 1 hour with the appropriate species-specific serum albumin (5%) in phosphate buffered saline plus 0.1% Triton X-100 (Sigma). Primary antibodies were added and incubated overnight at 4°C in a humidified chamber, followed by incubation with secondary antibodies for 1 hour at room temperature. Samples were mounted using Vectashield mounting medium with DAPI (Vector). The following antibodies and dilutions were used: anti-COL1A2 at a 1:200 dilution (catalog no. sc-28654; Santa Cruz Biotechnology),
anti-COL2A1 at a 1:200 dilution (catalog no. 70R-CR008; Fitzgerald), antiaggrecan at a 1:50 dilution (catalog no. sc-25674; Santa Cruz Biotechnology), anti-CCN1/Cyr61 1:100 (catalog no. sc-13100; Santa Cruz Biotechnology), anti-CCN2/CTGF 1:200 (clone L-20; Santa Cruz Biotechnology), anti-CCN3/Nov 1:1,000 (catalog no. AF-1976; R&D Systems), DyLight 488–conjugated anti-goat, anti-rabbit, and anti-mouse IgG at a 1:500 dilution (Jackson ImmunoResearch), and DyLight 594–conjugated anti-goat and anti-rabbit IgG at a 1:500 dilution (Jackson ImmunoResearch). Species-specific isotype IgG controls (Invitrogen) were used to assess antibody specificity (see Supplementary Figure 2, available on the Arthritis & Rheumatism web site at http://onlinelibrary.wiley.com/doi/10.1002/art.38075/abstract). Images were captured with a Zeiss Axio Imager.M1 fluorescence microscope and processed with Northern Eclipse software.

RESULTS

Tissue-specific expression patterns of CCN proteins in murine IVDs. To determine the distribution of CCN proteins within both notochord- and mesenchyme-derived tissues of the IVD, immunolocalization studies were conducted on spinal segments from wild-type mice ranging in age from embryonic day 15.5 to 17 months of age (Figure 1). Following notochord segmentation and the initiation of disc formation on embryonic day 15.5, CCN1 expression was not detected (Figure 1A), CCN2 expression was specific for the notochord-derived NP (Figure 1B), and CCN3 expression was detected in the NP of CCN2-knockout mice as compared to wild-type littermate controls. Arrows indicate nucleus pulposus (NP) tissue. B, Representative images of the ECM composition show decreased levels of type II collagen and aggrecan proteins and increased levels of type I collagen within the NP of CCN2-knockout mice as compared to wild-type littermate controls. Images are oriented with rostral at the top and are representative of 4 mice per genotype. Bars = 50 μm. H&E = hematoxylin and eosin.
enriched in the NP (Figure 1), suggesting that these proteins may play a role in NP function.

**Disruption of embryonic IVD formation in notochord-specific CCN2-knockout mice.** To investigate the role of CCN2 in NP function, notochord-specific CCN2–knockout mice were generated. Notochord segmentation and IVD patterning did not appear to be disrupted in CCN2-knockout mice as compared to their wild-type littermate controls (Figure 2). On embryonic day 15.5, loss of CCN2 resulted in increased expression of CCN3 within the putative NP (Figure 2A). Compared to wild-type littermate controls, CCN2-knockout mice demonstrated decreased aggrecan and type II collagen protein expression within the putative NP, concomitant with increased type I collagen protein expression (Figure 2B). By postnatal day 1, when IVD development is completed, perturbation of the ECM composition of the IVD in CCN2-knockout mice was more pronounced (Figure 3). Notably, deletion of CCN2 in notochord-derived cells resulted in decreased CCN1 expression and increased CCN3 expression within the NP (Figure 3A). Moreover, compared to wild-type littermate controls, CCN2-knockout mice displayed decreased levels of aggrecan in the NP, as well as decreased levels of type II collagen and increased levels of type I collagen throughout the IVD (Figure 3B).

Based on these observations, we anticipated that the alterations in the IVD matrix composition detected during development in notochord-specific CCN2-knockout mice would disrupt disc function in the mature IVD. However, on postnatal day 28, neither the tissue architecture nor the ECM content was appreciably altered in CCN2-knockout mice as compared to their littermate controls (Figures 4A and B). Intriguingly, although the CCN2 gene was deleted in notochord-derived cells of the NP, CCN2 protein was detected

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*Figure 3.* Histologic appearance and localization of ECM proteins in IVDs obtained from 1-day-old wild-type mice and CCN2-knockout mice. **A**, Representative images of IVD tissues on postnatal day 1 demonstrate the histologic appearance and localization of CCN proteins. Similar to the findings at embryonic time points, CCN1 expression is reduced and CCN3 expression is enhanced in CCN2-knockout mice as compared to wild-type littermate controls. **Arrows** indicate NP tissue. **B**, Representative images examining the ECM composition show decreased levels of type II collagen throughout the IVD, decreased levels of aggrecan within the NP, and increased levels of type I collagen within the NP of CCN2-knockout mice as compared to wild-type littermate controls. Images are oriented with rostral at the top and are representative of 4 mice per genotype. Bars = 50 μm. See Figure 2 for definitions.
throughout the IVD at this time point (Figure 4A). These data suggest that diffusion of CCN2 into the NP from adjacent mesenchymal tissues (i.e., hypertrophic chondrocytes [Figure 1H]) is likely occurring and, hence, rescues the effects of notochord-specific CCN2 knock-out at this time point.

**Figure 4.** Histologic appearance and localization of ECM proteins in IVDs obtained from 28-day-old wild-type and CCN2-knockout mice. A, Representative images of IVD tissues in fully formed IVDs demonstrate the histologic appearance and localization of CCN proteins. Arrows indicate NP tissue. B, Representative images examining the ECM composition show no remarkable changes in CCN2-knockout mice as compared to wild-type littermate controls. Images are oriented with rostral at the top and are representative of 4 mice per genotype. Bars = 50 μm. See Figure 2 for definitions.

**Acceleration of age-associated degeneration of lumbar IVDs by notochord-specific CCN2 deletion.** Reports of the localization of CCN2 to degenerated human IVDs have led to speculation that CCN2 plays a role in the initiation of tissue repair (24–26). To begin to test this hypothesis, we compared the structure and tissue composition of IVDs from 12-month-old CCN2-knockout and wild-type mice (n = 4 per genotype). Histologic examination revealed that CCN2-knockout mice possessed gross changes in tissue architecture, with the loss of distinct AF–NP boundaries, a decrease in NP cellularity, and disorganization of IVD structure, associated with a significant increase in the degeneration score, in both the NP and the AF (Figures 5A and C). No changes in the expression of CCN1 or CCN3 were detected (see Supplementary Figure 3A, available on the Arthritis & Rheumatism web site at http://online library.wiley.com/doi/10.1002/art.38075/abstract). However, CCN2-knockout mice had reduced aggrecan levels within the NP (Figure 5A). Degenerative changes (Thompson score >2) were observed in 30% of lumbar IVDs from CCN2-knockout mice at 12 months of age, while in the wild-type littermates, degenerative changes were detected in <5% of lumbar IVDs (Figure 5C).

To assess the impact of notochord-specific CCN2 deletion on the overall health of the IVD tissue, T2/T1-weighted MRI was used to quantify the hydration-dependent signal intensity. The signal intensity of lumbar IVDs from CCN2-knockout mice was significantly reduced (P < 0.001) compared to that of lumbar IVDs from wild-type control mice at 12 months of age (Figures 5B and D). The pixel standard deviation within the IVD,
a measure of disc degeneration that quantifies the loss of a distinct AF–NP boundary (31), was also significantly reduced ($P = 0.0001$) in CCN2-knockout mice as compared to wild-type controls (see Supplementary Figure 4A, available on the *Arthritis & Rheumatism* web site at...
compared to wild-type mice. Based on these findings, we propose that the expression of CCN2 by the notochord and notochord-derived cells of the NP is an essential component of the IVD microenvironment that contributes to the maintenance of tissue homeostasis.

We first examined the expression and localization of specific CCN family members in IVD tissues from mice at distinct stages of skeletal development, ranging from embryonic day 15.5 to 17 months of age. The expression of CCN1 is known to regulate mesenchymal differentiation toward both the osteogenic (33) and chondrogenic (34) lineages. In the spine, previous studies have localized CCN1 expression to areas of cartilage formation in the developing prevertebrae (35); however, our study is the first to demonstrate enhanced expression of CCN1 in NP cells following IVD formation. Consistent with the findings of a previous global microarray analysis (19), our findings indicate that CCN2 expression was enriched in the embryonic notochord and notochord-derived cells of the NP. Similar to previous reports (26), we showed that CCN2 was localized to the cytosol and ECM of the NP, AF, and cartilage end plates in newborn mice. However, in contrast to previous studies reporting that CCN2 levels were higher in the NP of mature rat discs compared to neonatal tissues, we showed that NP-specific CCN2 was elevated at embryonic time points and remained enriched in NP tissues at all time points examined.

Our findings of elevated CCN3 expression in the notochord on embryonic day 15.5 are similar to previous reports of notochord-specific CCN3 expression in both chick and mouse embryos (13,36). Although Tran et al (13) reported robust CCN3 expression in the NP of skeletally mature rat IVDs, the current study demonstrated that CCN3 protein expression is not enriched in the NP at postnatal time points. It remains to be established whether there are species-specific differences in the levels of CCN proteins within the IVD. The reciprocal expression patterns of CCN1/CCN2 and CCN3 demonstrated in the intervertebral disc is similar to those reported in other musculoskeletal cell types, and are consistent with the hypothesis that CCN3 appears to have opposing actions to CCN1/CCN2 (14,15). It is tempting to speculate that the expression of CCN3 in the AF contributes to the regulation of CCN2 expression within the IVD and its enrichment in notochord-derived cells, similar to the complex molecular network regulated by interaction between CCN family members in other tissues (37).

It is important to note that in contrast to our current findings in mice, previous reports of CCN2 localization in human IVDs demonstrated no detectable...
expression of CCN2 within the NP and ECM of non-degenerated IVDs (24,25). In those studies, CCN2 expression was restricted to the NP and inner AF of degenerated IVDs and was found to be associated with discogenic low back pain. The discrepancy between the CCN2 expression patterns in mice and humans may be related to differences in the cellular composition of the IVD. In humans, the numbers of notochord cells decrease rapidly after birth and are undetectable in the NP past early adolescence (38). Our previous studies have shown that in mice, however, a subset of cells expressing notochord markers is present within the NP up to 9 months of age (8). We therefore suggest that the persistence of undifferentiated notochord cells in the mouse NP leads to continued CCN2 expression, which contributes to the maintenance of tissue homeostasis. Given the reported ability of notochord-derived factors to stimulate NP cell proliferation and ECM synthesis and to prevent cytokine-mediated cell apoptosis in vitro (22,23,39), further studies are required to delineate which of these functions are directly regulated by CCN proteins in vivo.

The findings of the current study demonstrate that the production of CCN2 by NP cells contributes to the maintenance of IVD tissue homeostasis by promoting proper ECM synthesis. Our findings are consistent with previous reports that identified CCN2 as an anabolic factor capable of promoting aggrecan gene expression in NP cells (23), as well as with recent reports demonstrating decreased aggrecan immunoreactivity in NP tissue from the whole-body CCN2–knockout mouse on embryonic day 18.5 (40). The role of CCN2 in promoting type II collagen expression in the NP is consistent with previous reports (13,40). In contrast, the detection of increased type I collagen within the NP of CCN2-knockout mice is unexpected, given previous studies that demonstrated a positive correlation between CCN2 and type I collagen gene expression in fibroblasts (41,42), chondrocytes (43), and NP cells (13,40). Further studies are required to determine if CCN2-dependent regulation of type I collagen varies based on the stage notochord cell differentiation, the concentration of extracellular CCN2, the 3-dimensional cellular microenvironment, or a combination of multiple factors.

CCN2-dependent regulation of ECM synthesis appears to protect the IVD from age-associated degenerative changes, since CCN2-knockout mice demonstrated significantly accelerated degeneration of lumbar IVDs. These findings are consistent with the proposed chondroprotective role of CCN2 demonstrated in vivo by the ability of recombinant CCN2 to stimulate the repair of cartilage tissues following focal damage and experimentally induced osteoarthritis (44). Intriguingly, the effects of notochord-specific CCN2 knockout during both development and aging were not limited to notochord-derived NP tissues, but affected adjacent IVD structures as well. CCN2-knockout mice show altered collagen content in the AF and cartilage end plates, and AF tissues displayed increased degenerative changes at 12 months and 17 months of age. This widespread effect of CCN2 deletion is likely due to both the diffusible nature of the CCN2 protein within connective tissues (45) and the ability of CCN2 to control the activity of numerous secreted growth factors and cytokines that regulate ECM production in connective tissues (46). Taken together, these potential functions suggest an important role for CCN2 in regulating cell–cell interactions between distinct IVD cell types and biologic activity within the IVD microenvironment.

Consistent with the ability of CCN2 to regulate multiple tissue types within the IVD, our data demonstrated that in contrast to earlier time points, CCN2-null IVDs did not show alterations in ECM composition as compared to wild-type IVDs on postnatal day 28. Interestingly, this period coincides with the induction of CCN2 expression within both the AF (postnatal day 1) and hypertrophic chondrocytes of the adjacent vertebral bone (postnatal day 28). In fact, we detect CCN2 immunolocalization at the periphery of the CCN2-knockout NP on postnatal days 1 and 28. We speculate that during this period, the expression of CCN2 by adjacent tissues serves to replenish CCN2 within the IVD microenvironment, possibly via transcytosis, which has recently been demonstrated to regulate the distribution of CCN2 in cartilage (45). In the context of age-associated degeneration, acceleration of degenerative changes in adjacent IVD tissues may also be associated with changes in the mechanical properties of the NP in CCN2-knockout mice, where the decreased tissue hydration observed may alter the ability of the IVD to withstand compressive loading, transferring increased strain onto the AF and thereby accelerating overall IVD degeneration.

To quantify changes in IVD health following the loss of CCN2, we used MRI, a technique used extensively in the clinical detection of early disc degeneration and as a diagnostic tool for assessing the morphology and biochemical composition of human IVDs (47). Recent clinical studies have demonstrated that progressive changes in IVD structure and composition associated with the gradual substitution of type II collagen fibers by type I collagen fibers (2) leads to NP
dehydration and the progressive loss of AF–NP distinction. This transition can be captured by MRI using the decrease in inhomogeneity descriptors, such as pixel standard deviation, that measure gray-level variation within an image. In fact, the correlational strength with patient age and clinical diagnosis of degeneration severity is higher for inhomogeneity descriptors such as standard deviation than the corresponding adjusted mean signal intensity (31). The current study establishes that similar to studies in humans, advanced MRI analysis using inhomogeneity descriptors accurately detects degenerative changes that correspond to histologic grade of tissue degeneration in mice.

The fundamental mechanisms that underlie disc degeneration are largely unknown; however, it has been suggested that the local microenvironment of IVD cells plays a substantial role in mediating turnover of the ECM (48). Matricellular proteins are secreted into the microenvironment during tissue formation and repair, and they mediate the interactions between cells and the surrounding ECM (49). In this regard, it is informative that in this study, we identified the matricellular protein CCN2 as a key player in the development and maintenance of the IVD. Endogenous CCN2 was required for ECM synthesis and tissue composition of the IVD; loss of CCN2 from the NP resulted in pronounced age-related disc degeneration. These data suggest that CCN2 may represent an intriguing clinical target for promoting the repair of degenerated discs.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Séguin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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