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RESEARCH ARTICLE

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Effect of voluntary running activity on mRNA expression of extracellular matrix genes in a mouse model of intervertebral disc degeneration

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Abstract

Introduction: Low back pain (LBP), a leading cause of global disability, is often associated with intervertebral disc degeneration (IDD). Exercise therapy is recommended for chronic LBP management and affects many tissues and organ systems. However, the ability of exercise to repair the extracellular matrix (ECM) in degenerating discs is unclear. The aims of the study were to examine mRNA expression of ECM structural components (collagen I, II, X, aggrecan) and regulators of matrix turnover (matrix metalloproteinases (MMP)-3, – 9, – 13, ADAMTS-4, – 5, TIMP1-4, CCN2) between age-matched (a) wild-type and secreted protein acidic and rich in cysteine (SPARC)-null, (b) sedentary and active, and (c) male and female mice.

Methods: At 8 months of age, male and female SPARC-null and wild-type control mice received a home cage running wheel or a control, fixed wheel for 6 months. Deletion of the *SPARC* gene results in progressive IDD beginning at 2 to 4 months of age. Increased activity was confirmed, and qPCR was performed on excised lumbar discs.

Results: Male SPARC-null mice expressed less aggrecan mRNA than wild-type controls. After 6 months of running, collagen, *MMP3*, and *MMP13* expression was increased in male and *MMP3* was increased in female SPARC-null mice. Sex differences were observed in wild-type mice and in response to IDD and long-term running.

Conclusions: Voluntary running results in changes in mRNA consistent with increased ECM turnover and disc regeneration. Improved disc ECM might contribute to the beneficial effects of exercise on LBP and may create an intradiscal environment hospitable to regenerative therapies. Sex-specific differences should be considered in the development of disc-targeting therapies.

KEYWORDS
degeneration, extracellular matrix, pain, pre-clinical models

1 | INTRODUCTION

Low back pain (LBP) is a leading cause of global disability.[1] Although intervertebral disc degeneration (IDD) can contribute to LBP, many individuals with IDD are pain free.[2] Whether there are causative or correlative relationships between IDD and LBP is currently unresolved. Existing therapies for chronic LBP, such as surgery and opioids, are either invasive, lose efficacy with time, or are associated with undesired side effects. Physical activity, including exercise and multidisciplinary rehabilitation, are therefore recommended.[3] Although physical activity is a first line therapy for chronic LBP, its effects on IDD-associated molecular dynamics are poorly understood.

Intervertebral discs (IVDs) consist of an inner jelly-like nucleus pulposus surrounded by the outer fibrous cartilage annulus fibrosus.[4] The extracellular matrix (ECM) is a noncellular network that plays critical roles in structural support and biochemical functions in all tissues and organs, including IVDs (Figure 1).[5] The major components of IVD ECM, collagen and aggrecan, are made during chondrogenesis by progenitor cells of chondrocytes.[6] Aggrecans attract and retain water while different types of collagens form net-like structures that entrap aggrecans, preventing IVD swelling by binding to collagens.[7] These constituents of ECM also affect IVD cells to regulate development, maintenance, and repair of IVDs.[8,9]

There are numerous enzymes and other components that regulate dynamic ECM turnover and maintenance. Matrix metalloproteinases (MMPs) and disintegrins and metalloproteinases with thrombospondin motifs (ADAMTSs) are proteases with diverse roles, including degradation of ECM components, such as collagens and aggrecans, and osteoclastic resorption.[10] Previous studies have suggested that certain MMPs and ADAMTSs play specific roles in IDD.[11,12] For example, one study has shown relationships between the expression of MMP9 and histologic degenerative scores and herniation grades in human patients after lumber disc herniation surgeries.[13] To ensure tight regulation of their activities, MMPs have endogenous inhibitors called tissue inhibitors of metalloproteinases (TIMPs).[11] Imbalances between proteinases and inhibitors may lead to IDD, and IDD may further alter levels of ECM components in return and interfere with ECM regulation.[14,15] Since breakdown of the ECM is a hallmark of IDD, therapies that can restore discs toward normal homeostasis with minimal side effects are needed.

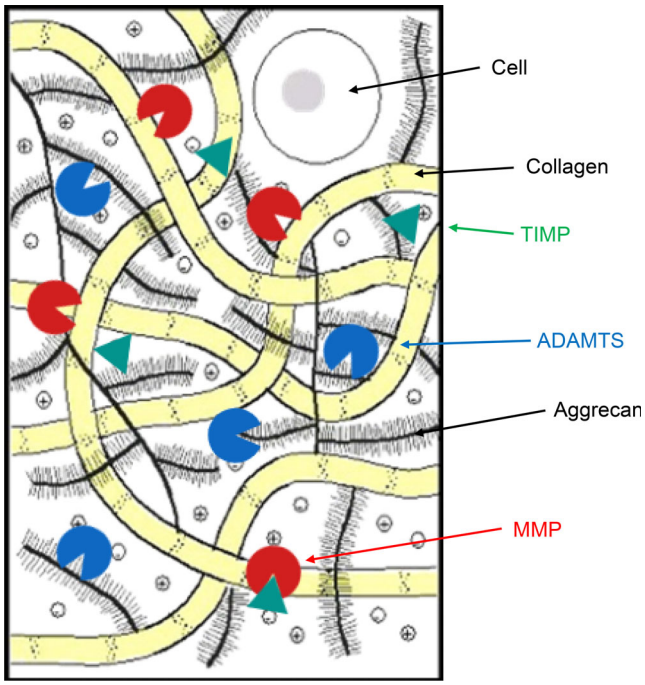

FIGURE 1 Schematic of intervertebral disc (IVD) extracellular matrix (ECM). The IVD ECM is composed primarily of collagen and aggrecan that contribute to disc structure, function, and maintenance. These components are regulated by degrading enzymes called matrix metalloproteinases (MMPs) and ADAMTSs (shown as red and blue pacmen, respectively) and their inhibitors tissue inhibitors of metalloproteinases (TIMPs) (green triangles)

Secreted protein acidic and rich in cysteine (SPARC) is a matricellular protein with functional roles in tissue turnover and/or repair.[16] Expression of SPARC decreases in IDD,[17,18] and targeted deletion of SPARC in mice (SPARC-null) results in age-associated acceleration of IDD.[19] Progressive IDD in SPARC-null is associated with behavioral signs of axial LBP and radiating leg pain beginning at a few months of age and persisting for up to 78 weeks of age[19-21]; these indices of LBP are sensitive to analgesics and anti-inflammatory treatments.[20-24] SPARC-null mice also develop defects in connective tissues such as decreased proteoglycan content[25] and altered collagen fibril diameter in IVD ECM.[17] Biomechanical changes in SPARC-null mice include increased stiffness and smaller neutral zone of IVDs.[26] To date, the effect of IDD and exercise on mRNA expression of ECM genes in intervertebral discs in SPARC-null mice have not been investigated. In addition, little is known about the impact of sex on IDD-associated changes in ECM.[27,28]

One of the most common prescriptions given by healthcare professionals to LBP patients is exercise.[29] Recent studies of Belavý et al have shown that chronic running in humans is associated with IVDs that are more hydrated with higher hydration and glycosaminoglycan content[30] and that specific types of loading may be beneficial to lumbar IVD conditions through studying IVD properties of various athletes.[31] In SPARC-null mice, the proinflammatory response to IDD in multifidus muscle was reduced in physically active mice.[32] Although it has been shown that exercise is beneficial for LBP, its effect on IDD and ECM regulation in degenerating IVDs has not been studied.

The aims of this study were to examine the mRNA expression of ECM components and dynamics between (a) healthy wild-type (WT) and SPARC-null mice, (b) sedentary and physically active mice, and (c) male and female mice.

2 | MATERIALS AND METHODS

2.1 | Animals

Two cohorts of mice were used in this study.

Male and female 8-month old SPARC (Secreted Protein, Acidic, Rich in Cysteine)-null and age-matched C57BL/6 wild-type

(WT) control mice were generated as previously described and bred in house.[20] This cohort consisted of 72 male mice (36 WT and 36 SPARC-null) and 56 female mice (29 WT and 27 SPARC-null). A subset of 28 male and 20 female mice were included in the mRNA studies. The remaining mice were processed for other endpoints.

A second cohort of male 8 to 9 month old SPARC-null (n = 31) and age-matched WT (n = 18) control mice were used in the disc thickness study.

All mice were housed in a temperature-controlled room with a 12-hour light/dark cycle in groups of 2 to 3 per pathogen-free ventilated polycarbonate cage (Allentown, Inc, Allentown, New Jersey), with corncob bedding (7097; Teklad Corncob Bedding, Envigo, United Kingdom) and cotton nesting squares for environmental enrichment that were removed upon placement of the wheels. Mice were given free access to food (2092X Global Soy Protein-Free Extruded Rodent Diet, Irradiated) and water.

All experiments were approved by the Institutional Animal Care and Use Committee of McGill University and conformed to the ethical guidelines of the Canadian Council for Animal Care and the International Association for the Study of Pain.

2.2 | Measurement of disc thickness

2.2.1 | Voluntary running

At 8 to 9 months of age, cages of male mice were randomly assigned into either running or sedentary and had free access to functional (running) or nonfunctional (sedentary) wheels in their home cages for 4 months. The wheels consisted of a spinning saucer-like wheel mounted on a dome-shaped hut for nesting (InnoDome and InnoWheel, bio-serve). Sedentary cages had wheels that were anchored to the dome so they cannot rotate.

2.2.2 | In vivo X-ray

Disc thickness was measured using dorsal X-ray images taken at ×4 using a Faxitron MX-20 (Faxitron XRay LLC, Lincolnshire, Illinois) in anesthetized mice (ketamine 100 mg/kg, xylazine 10 mg/kg, and acepromazine 3 mg/kg, ip). Disc thickness was determined by an individual blind to experimental group using ImagePro© software. Average dorsal thickness was calculated as the average of three dorsal measurements and normalized to the average length of the adjacent vertebral bones.

2.3 | Measurement of mRNA expression

2.3.1 | Voluntary running

Following the same procedure as 2.2.1, mice had free access to functional or sedentary wheels for 6 months.

2.3.2 | Counting wheels

At the end of the running period (after 6 months of intervention), each mouse was placed alone in a clean cage with a counting wheel for 60 minutes, and the number of rotations was measured and converted to distance traveled.

2.3.3 | IVD harvesting and total RNA extraction

Mice were sacrificed by decapitation under deep isoflurane anesthesia, and five IVDs (L1/L2-L5/L6) were isolated per mouse. Harvested IVDs were pooled together and immediately crushed in liquid nitrogen using a mortar and pestle. Crushed IVDs were placed in 1.5 mL Eppendorf tubes with 750 μL of QIAzol buffer (Qiagen) placed on ice. Then, a pellet pestle was used on crushed IVDs to completely homogenize them. The homogenization step was performed with the tubes embedded on ice at all times with a maximum of 30 seconds spent on each tube to avoid sample heating. After one round of homogenization, the samples went through the step two more times for a total of 1 minute and 30 seconds of homogenization. 100 μL of chloroform was added to each tube and the samples were vortexed for 10 seconds to mix. 150 μL of each sample was set aside for other uses. The remaining 550 μL of samples were centrifuged for 15 minutes at 12000xg at 4°C. The aqueous layer at the top was carefully obtained (250 μL per sample). 250 μL of 70% EtOH was added and the samples were mixed by pipetting followed by total RNA extraction using RNeasy Mini Kit (Qiagen) following the manual provided by the manufacturer, starting from step 6 of the extraction for animal tissue. The volume of elution buffer used was 40 μL. Quantity and quality of RNA were measured with Nanodrop 2000 Spectrophotometer (Thermo Fischer) and RNA Pico Chip Bioanalyzer 2100 (Agilent). The RNA integrity number (RIN) of all samples ranged from 6.8 to 9.2.

2.3.4 | Reverse transcription cDNA synthesis and real-time PCR

First-strand cDNA synthesis from 50 ng total RNA was performed using SuperScript II Reverse Transcriptase (Thermofisher). Following the manufacturer's protocol, 50 ng of total RNA was taken from each sample and were mixed with random primers, ×5 First-strand Buffer, 0.1 M DTT, and SuperScript II RT to obtain a total of 21 μL cDNA per sample.

Real-time PCR (RT-PCR) was performed with a total volume of 10 μL per reaction containing 7 μL mastermix (5 μL of SsoAdvanced Universl SYBR Supermix, Bio-Rad and 1 μL each of forward and reverse primers of the gene of interest) plus 3 μL of diluted cDNA (1:20 dilution). Three reference genes were used in the study: GAPDH, β-actin, and β-glucuronidase. Primers were ordered from IDT using custom DNA oligos (Table 1). PCR cycles were as follows: 95°C for 3 minutes, 40 cycles at 95°C for 15 seconds, 55°C for 30 seconds, and 60°C for 20 seconds. Reactions for each sample were performed in duplicate. See Table 1 for a full list of primers used in this study.

2.3.5 | Data and statistical analysis

mRNA expression was analyzed using the $2^{-\Delta CT}$ method with the average cycle threshold (CT) of GAPDH, β-actin, and β-Glucuronidase as the reference gene value. For males, mean CT values ± SEM of the average of the housekeeping genes for the four groups (WT sedentary, WT running, SPARC-null sedentary, SPARC-null running) were 20.65 ± 0.24, 20.37 ± 0.12, 20.27 ± 0.17, and 20.82 ± 0.32, respectively. For females, the mean CT values ± SEM for the four groups were 22.57 ± 0.25, 22.90 ± 0.24, 22.00 ± 0.48, and 22.86 ± 0.24. Relative expression values do not have a unit, but rather, they are calculated from the CT values of qPCR data ($2^{-\Delta CT}$) *100 000. Fold changes comparing to a reference group were calculated by the following equation:

$$\text{Fold change} = \frac{Avg.\left(2^{-\Delta CT}{}_{Comparison\ Group}\right)}{Avg.\left(2^{-\Delta CT}{}_{Reference\ Group}\right)},$$

Graphs and statistical analysis were generated and performed using the GraphPad Prism Software. Two-way ANOVA followed by Tukey's multiple comparisons test (Figures 2,3,5-7 and Table 2) or Mann-Whitney *U* test (Figure 4). All data are shown as mean +/− SEM, and *P* value <.05 is considered statistically significant.

TABLE 1 Primer Sequences for RT-PCR

Gene	Forward primer	Reverse primer
GAPDH	GTGAAGGTCGGTGTGAAC	AATCTCCACTTTGCCACTG
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATG
β-glucuronidase	CCGACCTCTCGAACAACCG	GCTTCCCGTTCATACCACACC
SPARC	AGCCCCTCAGCAGACTGAA	CCTCTGCACCGTCCTCAAAT
Col1a1	CTGGCGGTTCAGGTCCAA	TCCAGGCAATCCAGGAGC
Col2a1	GCACATCTGGTTTGGAGAGACC	TAGCGGTGTTGGGAGCCA
Col10a1	GGGACCCCAAGGACCTAAAG	GCCCAACTAGACCTATCTCACCT
ACAN	CTGGGATCTACCGCTGTGAAG	GTGTGGAAATAGCTCTGTAGTGGAA
MMP3	AGGTGGACCTAGAAGGAGGC	CTGTCATCTCCAACCCGAGG
MMP9	CAGCCGACTTTTGTGGTCTTC	GTACAAGTATGCCTCTGCCA
MMP13	CTTCTTCTTGTTGAGCTGGAACTC	CTCTGTGGACCTCACTGTAGACT
ADAMTS4	GAGGAGGAGATCGTGTTTCCA	CAAACCCTCTACCTGCACCC
ADAMTS5	GGAGCGAGGCCATTTACAAC	GCGTAGACAAGGTAGCCCACTTT
TIMP1	CACACCAGAGCAGATACCAT	CCCTTATGACCAGGTCCGAG
TIMP2	CAGCCTCTCCCGTCTTTTGT	GTGGCTAGAAACCCCAGCAT
TIMP3	TGAGGAGGAGAGAACCCGAG	GGGTTTTCTCTGGCTGGTGT
TIMP4	TGGCTGCCAATGCCATGTA	AGGGCTGGATGATGTCAACG
CCN2	ACCCAACTATGATGCGAGCC	GGTAACTCGGGTGGAGATGC

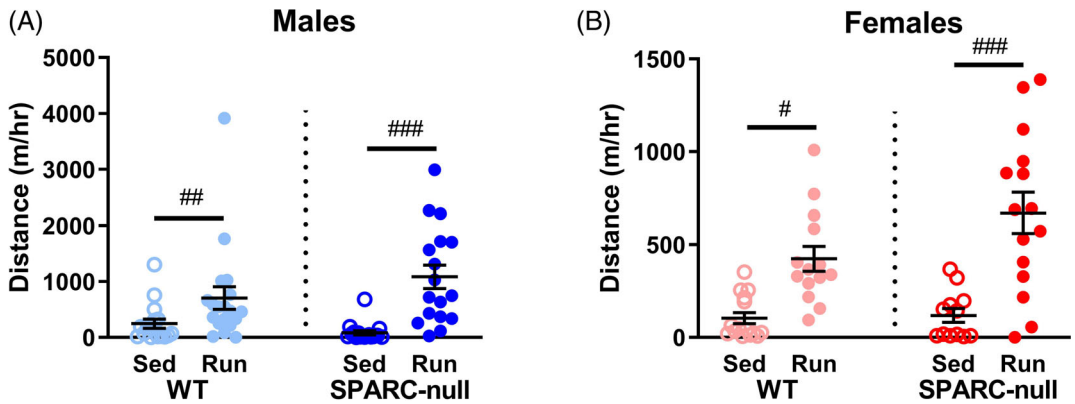

FIGURE 2 Increased voluntary activity in running vs sedentary wild-type (WT) and secreted protein acidic and rich in cysteine (SPARC)-null mice. The number of wheel rotations was measured for 60 minutes to confirm increased voluntary running and converted to converted to total distance ran in meters/hour. Sed = sedentary, Run = runners. Results are represented as mean ± SEM. Two-way ANOVA followed by Tukey multiple comparisons test. #*P* < .05, ##*P* < .01, ###*P* < .001. n = 27 to 36

3 | RESULTS

3.1 | Confirmation of voluntary running

Counting wheels were used to confirm the effectiveness of the running intervention. WT and SPARC-null animals of both sexes with functional home cage wheels ran significantly more than the sedentary groups (Figure 2A,B).

3.2 | Lumbar disc thickness

Changes in lumbar IVD thickness was examined in 12 to 13-month-old male mice by in vivo X-ray (Figure 3).These mice had functional or sedentary wheels in their home cages for 4 months prior to analysis. In both sedentary and running groups, SPARC-null mice had significantly thinner IVDs compared to WT mice. Although not statistically significant, a trend toward reduced thickness was observed in SPARC-null runners compared to sedentary mice (P = .1).

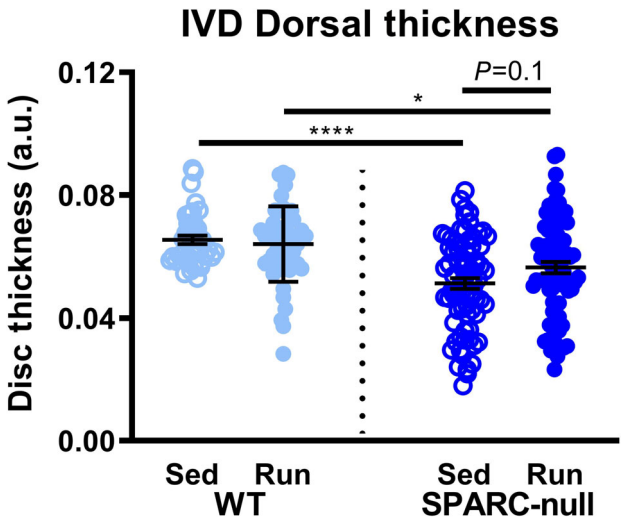

FIGURE 3 Disc thickness in running vs sedentary wild-type (WT) and secreted protein acidic and rich in cysteine (SPARC)-null mice. Dorsal thickness of intervertebral discs (IVDs) in WT and SPARC-null sedentary (Sed) and running (Run) male mice was determined using X-ray image analysis for each of the five lumbar discs normalized to the length of the adjacent vertebral bodies. Results are represented as mean ± SEM in arbitrary units (a.u.). Two-way ANOVA followed by Tukey multiple comparisons test. *P < .05, ****P < .0001, WT vs SPARC-null. n = 8 to 16

3.3 | mRNA expression of SPARC and ECM structural components (collagens and aggrecan)

The deletion of *SPARC* was confirmed by measuring the mRNA expression using RT-PCR in 14 month old mice (Figure 4). There were significant differences in the expression levels between WT and SPARC-null mice in both sedentary and running groups, confirming negligible expression levels in SPARC-null mice.

mRNA expression of ECM structural components (*Col1a1*, *Col2a1*, *Col10a1*, *and ACAN)* were measured in lumbar IVDs of the same mice (Figure 5). In males, there were no significant differences in collagen mRNA expression between sedentary WT and SPARC-null mice (Figure 5A,B,C), but there was a decrease in aggrecan mRNA in SPARC-null mice compared to WT (Figure 5D). Exercise did not alter expression of these collagens or aggrecan in WT mice. In contrast, there were significant increases in *Col1a1*, *Col2a1*, *and Col10a1* after exercise in SPARC-null mice compared to matched sedentary controls.

In females, there were no significant differences in mRNA expression of *Col1a1*, *Col2a1*, *Col10a1*, or *ACAN* between age-matched WT and SPARC-null mice in both sedentary and running groups, (Figure 5E-H) and no effects of exercise were observed.

In summary, running resulted in increased mRNA expression of *Col1a1*, *Col2a1*, and *Col10a1* in SPARC-null male mice.

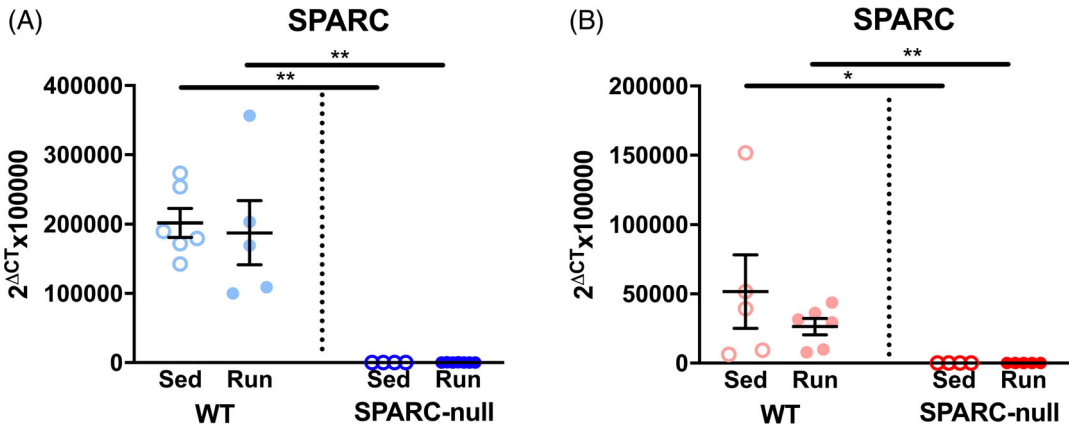

FIGURE 4 Confirmation of secreted protein acidic and rich in cysteine (*SPARC*) deletion. mRNA expression of *SPARC* in wild-type (WT) and SPARC-null sedentary (Sed) and running (Run) mice was assessed with qPCR. Results are represented as mean ± SEM. Mann-Whitney *U* test. *P < .05, **P < .01, WT vs SPARC-null. n = 4 to 7

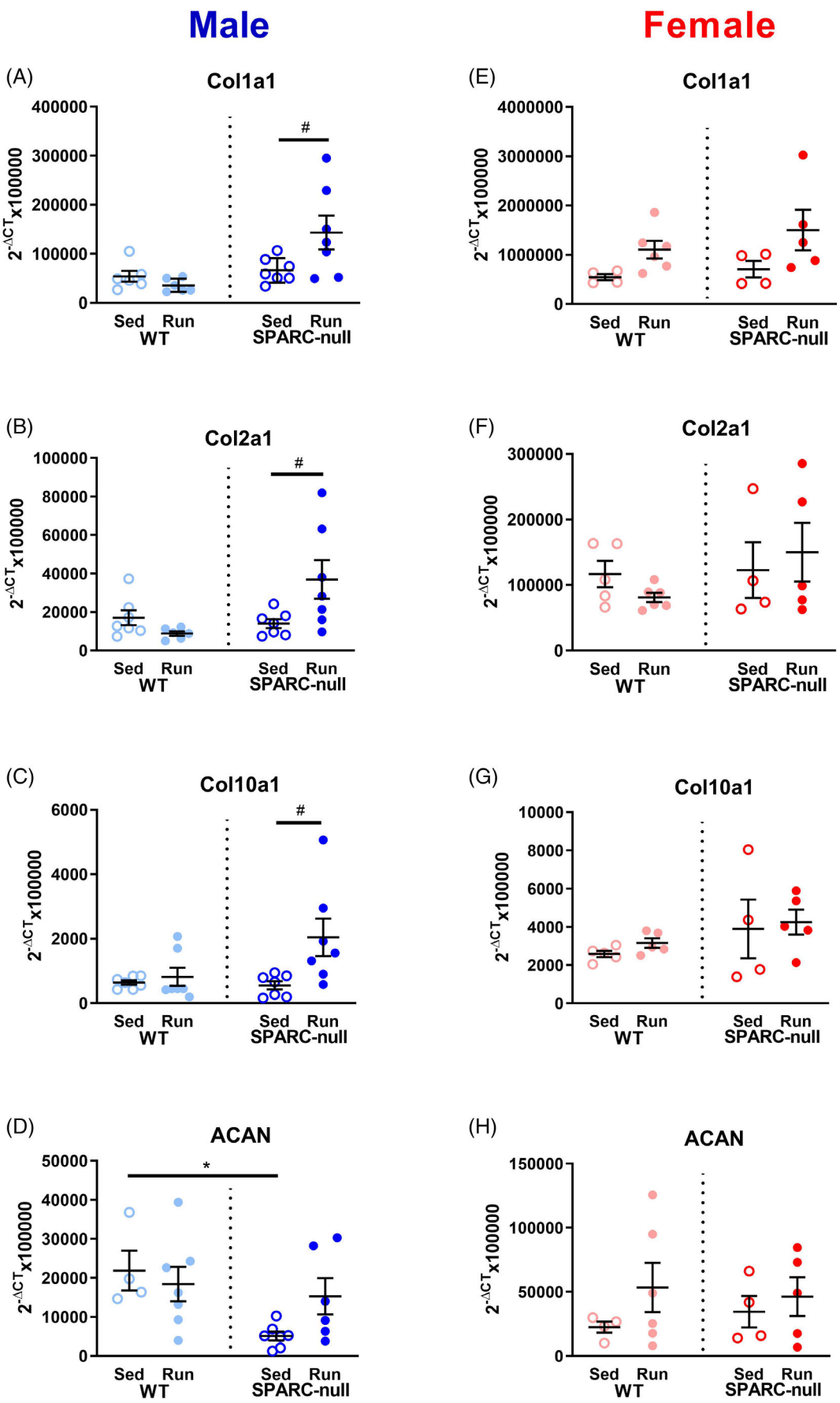

FIGURE 5 Legend on next page.

3.4 | Expression of ECM proteases (MMPs and ADAMTSs)

In males, no differences in mRNA expression were observed for MMPs (*MMP3*, *MMP9*, and *MMP13)* between sedentary WT and SPARC-null mice (Figure 6A,B,C). However, there was a nonsignificant trend toward decreased expression of *ADAMTS5* ($P = .053$) in SPARC-null compared to WT mice (Figure 6E). After exercise, significant increases in *MMP3 and MMP13* were observed in male SPARC-null mice (Figure 6A,C) and a trend toward increased expression was observed in *ADAMTS4* ($P = .08$, Figure 6D).

In females, no differences were found in mRNA levels of *MMP3*, *MMP9*, *MMP13*, *ADAMTS4*, *or ADAMTS5* between sedentary WT and SPARC-null mice (Figure 6F-J). After exercise, *MMP3* expression was increased in female SPARC-null mice (Figure 6F).

In summary, expressions of one or more *MMP* mRNAs are increased in SPARC-null mice of both sexes upon running. Nonsignificant trends toward decreased *ADAMTS5* expression in SPARC-null male mice and toward increased *ADAMTS4* expression in male SPARC-null runners and female WT runners were also observed (Figure 6D,E,I).

3.5 | Expression of protease inhibitors (TIMPs) and growth factor (CCN2)

In males, there were no statistically significant changes in expressions of *TIMP1*, *TIMP2*, *TIMP3*, *TIMP4*, or *CCN2* between sedentary WT and SPARC-null mice (Figure 7A-E), although a trend toward down-regulation was observed in *CCN2* (Figure 7E). No significant changes were observed in WT or SPARC-null mice after running, although there is a general trend toward increased expression in SPARC-null runners compared to sedentary mice (Figure 7A-E).

In females, SPARC-null mice showed a trend toward decreased expression of *TIMP4* mRNA compared to WT mice ($P = .1$, Figure 7I). No significant effects of exercise were observed in either SPARC-null or WT female mice (Figure 7F-J).

3.6 | Sex differences in ECM mRNA expression levels

Expression values for each gene and differences (expressed as fold change) in expression between sedentary males and sedentary females are shown in Table 2. In 14-month-old WT sedentary mice, females had higher (red text) mRNA expression of*Col1a1*, *MMP13*, *ADAMTS4*, and *ADAMTS5* compared to males of 10-, 40-, 16-, and 3-fold, respectively. No sex-specific differences were observed in WT mice for *Col2a1*, *Col10a1*, *ACAN*, *MMP3*, *MMP9*, *TIMP1*, *TIMP2*, *TIMP3*, *TIMP4*, or *CCN2*.

In sedentary SPARC-null mice, *Col1a1*, *Col2a1*, *Col10a1*, *ACAN*, *MMP13*, *ADAMTS4*, and *ADAMTS5* were significantly increased (red text) in female compared to male mice, with increases of 11-, 9-, 7-, 6-, 28-, 47-, and 4-fold, respectively. In contrast, expression of *TIMP1* was significantly reduced (blue text) in female vs male mice by 3-fold (Table 2).

4 | DISCUSSION

Persistent LBP is associated with enormous costs to individuals and societies. Degeneration of the IVDs in the spine is associated with chronic LBP in some individuals. Although it is well established that exercise is beneficial for LBP,[3,30] it is currently unknown if physical activity can attenuate pathological IDD. Here we demonstrate physical activity-related increases in mRNA expression of multiple ECM components important in IVD structure (i.e., collagens, aggrecan) and in regulating matrix turnover (i.e., MMPs, TIMPs), primarily in male IVDs. Using the SPARC-null model of pathological IDD associated with LBP, we observed that the potentially restorative effects on mRNA expression following long-term exposure to activity wheels were specific to mice with IDD. Finally, we report significant sex differences in the relative mRNA expression of ECM components in WT and SPARC-null mice and in the response to physical activity. Overall, these results suggest that the improved clinical outcomes associated with physical activity may, in part, be mediated at the level of the IVD and encourage exploration of multimodal therapies that combine exercise with analgesics or tissue regenerative strategies.

4.1 | Effect of *SPARC* deletion and running activity on disc thickness

We have previously reported severe IDD in SPARC-null compared to WT mice by histology, X-ray, and MRI in animals 6 weeks or older. Around the age of 12 to 14 month, which is the age of mice used in the current study, behavioral and histological changes seen in SPARC-null mice include increased cold sensitivity, axial discomfort, increased frequency and severity of lumbar IDD and decreased IVD height.[19,20] In the current study, we confirm reduced disc thickness in SPARC-null mice compared to age-matched WT (Figure 3). SPARC-null mice with functional home cage running wheels showed a trend towards thicker IVDs compared to sedentary controls. This is consistent with a study in humans showing that IVD height of long-distance runners was greater than the control group or short-distance runners, and that of

FIGURE 5 mRNA expression of extracellular matrix (ECM) structural components. mRNA expression of ECM structural genes in wild-type (WT) and secreted protein acidic and rich in cysteine (SPARC)-null sedentary (Sed) and running (Run) mice was assessed with qPCR. Results are represented as mean ± SEM. Two-way ANOVA followed by Tukey multiple comparisons test. *$P < .05$, WT vs SPARC-null, #$P < .05$, Sed vs Run. n = 4 to 7

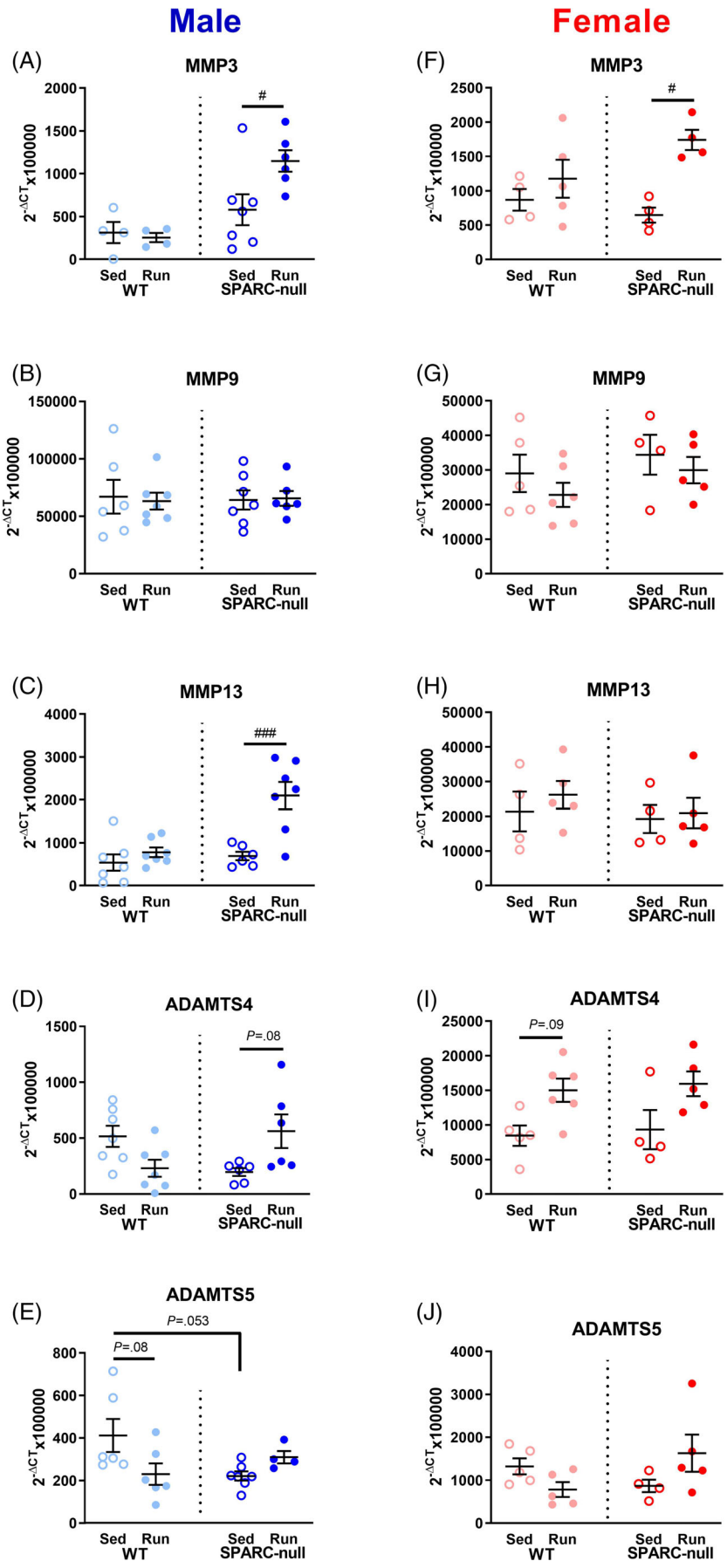

FIGURE 6 mRNA expression of extracellular matrix (ECM) degrading enzymes. mRNA expression of ECM degrading enzymes in wild-type (WT) and secreted protein acidic and rich in cysteine (SPARC)-null sedentary (Sed) and running (Run) mice was assessed with qPCR. Results are represented as mean ± SEM. Two-way ANOVA followed by Tukey multiple comparisons test. #*P* < .05, ###*P* < .001, Sed vs Run. n = 4 to 7

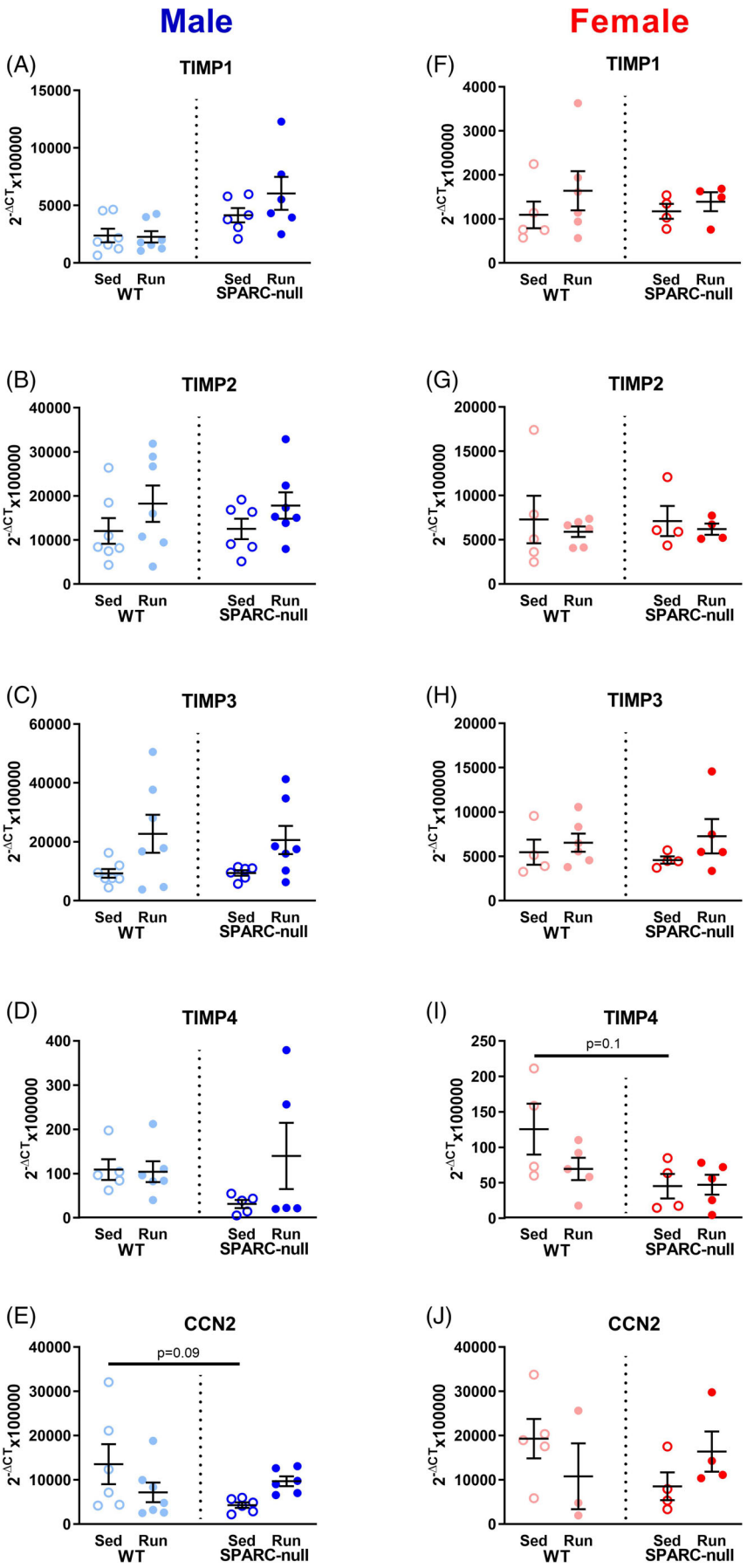

FIGURE 7 mRNA expression of matrix metalloproteinase (MMP) inhibitors and the growth factor CCN2. mRNA expression of tissue inhibitors of metalloproteinases (TIMPs) (MMP inhibitors) and connective tissue growth factor (CCN2) in wild-type (WT) and secreted protein acidic and rich in cysteine (SPARC)-null sedentary (Sed) and running (Run) mice was assessed with qPCR. Results are represented as mean ± SEM. Two-way ANOVA followed by Tukey multiple comparisons test. n = 4 to 7

TABLE 2 Comparison of mRNA expression of ECM components between sedentary male and female WT and SPARC-null mice

	WT			SPARC-null		
	M	F	Fold change	M	F	Fold change
Col1a1	54 114 ± 11 060	545 609 ± 63 213	10.08 ***	66 312 ± 9480	707 576 ± 166 908	10.67****
Col2a1	16 956 ± 3860	116 914 ± 20 039	6.90	13 981 ± 2309	122 713 ± 42 474	8.78**
Col10a1	639 ± 68	2581 ± 167	4.04	553 ± 127	3896 ± 1534	7.05 **
ACAN	21 883 ± 5076	22 434 ± 4325	1.03	5113 ± 1131	34 498 ± 12 311	6.75 *
MMP3	312 ± 123	867 ± 157	2.78	579 ± 181	646 ± 109	1.12
MMP9	67 034 ± 14 698	29 027 ± 5405	2.31	64 216 ± 8365	34 411 ± 5781	1.87
MMP13	538 ± 190	21 374 ± 5728	39.73 ***	691 ± 99	19 211 ± 4040	27.80 ***
ADAMTS4	516 ± 94	8456 ± 1467	16.39 ***	198 ± 35	9324 ± 2843	47.09 ***
ADAMTS5	412 ± 78	1321 ± 187	3.21 ****	221 ± 21	867 ± 146	3.92 **
TIMP1	2381 ± 598	1093 ± 303	2.18	4143 ± 618	1172 ± 170	3.53 **
TIMP2	12 040 ± 2914	7293 ± 2685	1.65	12 509 ± 2314	7110 ± 1704	1.76
TIMP3	9307 ± 1455	5473 ± 1421	1.70	9423 ± 917	4590 ± 409	2.05
TIMP4	109 ± 23	126 ± 36	1.16	31 ± 9	45 ± 17	1.45
CCN2	13 552 ± 4527	19 293 ± 4440	1.42	4283 ± 615	8542 ± 3141	1.99

Note: mRNA expression levels of ECM genes in WT and SPARC-null sedentary mice were compared between male and female after normalization to house-keeping genes. Red values represent higher expression in females and blue values represent higher expression in males. Results are expressed as mean ± SEM. Two-way ANOVA followed by Tukey multiple comparisons test, *P < .05, **P < .01, ***P < .005,****P < .001. n = 4 to 7.

short-distance runners was greater than the control group.[30] It is possible to infer that the running activity protected against loss of IVD thickness that is normally observed in SPARC-null mice.

4.2 | Effect of *SPARC* deletion and long-term running activity on ECM structural components

We hypothesized that SPARC-null mouse IVDs would have decreased mRNA expression for collagen I and II and aggrecan, and increased expression of collagen X compared to WT controls; in fact, no strain differences in collagen mRNA were observed. However, a statistically significant decrease in aggrecan (*ACAN*) was observed in male SPARC-null mice compared to WT (Figure 5D). As aggrecan is an important player in regulating water content of IVDs, the decrease in aggrecan expression in SPARC-null mice is consistent with the dehydration, that is, a hallmark of degenerating discs.[33,34] The absence of change in collagen expression may indicate that these collagens are not involved in the SPARC-null phenotype. An alternative explanation is that differences are masked by age-related degeneration in the WT mice, which were 14 months old in this study. We have previously shown that 15-month-old WT mice present with behavioral changes reminiscent of SPARC-null mice.[18]

Long-term running activity had a greater impact of IVD expression of ECM in SPARC-null mice than in WT mice. For example, in male SPARC-null mice, mRNA expression of all three types of collagen were significantly higher in IVDs of the running vs the sedentary groups. In contrast, no changes were observed between male WT runners vs sedentary mice. Our WT results are in contrast with reports of exercise-related increases in ECM in healthy rat discs after 3 weeks of treadmill training.[35] While the source of these differences is unclear, the current work suggests that running may have a greater impact on degenerating discs compared to healthy discs.

4.3 | Effect of *SPARC* deletion and long-term running activity on MMPs and ADAMTSs

Changes in ECM components arise from disrupted equilibrium between synthesis and degradation of ECM. Three types of matrix metalloproteinases (MMPs), collagenases, gelatinases, and stromelysin play major roles in ECM degradation in IVDs. Aggrecan degradation is primarily regulated by two members of the disintegrin and metalloproteinase (ADAMTS) family, ADAMTS4 and ADAMTS5.[36] Previous studies have shown that while proteases play various roles in processes involving bone and cartilage,[37] certain MMPs and ADAMTSs have specific roles in the process of IDD.[11,12] Thus, while MMPs and ADAMTSs are important in IDD, their roles in different stages of IDD, as well as their responses to running, are not fully known.[38] No significant differences were observed in the enzymes examined between SPARC-null and WT in either sex (Figure 6). This result was unexpected given other reports of altered MMPs and ADAMTSs in degenerating compared to normal tissues.[39] However, the use of 14-month-old mice could explain the lack of difference in *MMPs* and *ADAMTSs* in our results. Studies in younger mice are needed to address this hypothesis.

Consistent with the collagen and aggrecan findings, there was an overall tendency toward mRNA upregulation in runners. It has been shown that loss of MMPs leads to impaired healing processes.[40] Thus,

increased mRNA expression, potentially resulting in increased expression and activity of degrading enzymes, may be a physiological attempt to degrade damaged IVDs to rebuild the ECM in runners. Alternatively, increases in MMPs may be maladaptive.

4.4 | Effect of *SPARC* deletion and long-term running activity on TIMPs and CCN2

TIMPs inhibit MMPs and are important regulators of ECM homeostasis. Expression and regulation of MMPs and TIMPs have been determined to be crucial in ECM maintenance and dynamics in relations to various pathologies and biological processes.[41] In normal conditions, TIMPs are expressed in balance with MMPs.[42] In abnormal conditions, including IDD[11] and other pathologies like tumor development,[43] this balance between MMPs and TIMPs is compromised. Consistent with the MMP and ADAMTS results, no effects of the SPARC-null deletion were observed in TIMP mRNAs in either sex (Table 3). Voluntary wheel running also had no significant effects in either strain or sex. While expression of several degrading enzymes was higher in the exercise groups, changes in these inhibiting enzymes were not observed. These results suggest that exercise may accelerate ECM remodeling by upregulating mRNA, and potentially protein levels, of the degrading enzymes while keeping the inhibiting enzymes' levels regulated.

CCN2, connective tissue growth factor, is involved in the regulation of chondrogenesis.[44] Deletion of *CCN2* in mice notochord has been shown to impair intervertebral disc development and develop IDD.[45] No significant effects of strain, running or sex were observed on *CCN2* expression; CCN2 may therefore not play a critical role in ECM remodeling in response to either deletion of *SPARC* or to physical activity.

4.5 | Sex differences

Awareness of sex differences is increasing exponentially, including in the study of LBP and IDD. In humans, a recent study showed that female patients undergoing surgery for lumbar degenerative disease (disc degeneration, discherniation, spondylolisthesis, and spinal canal stenosis) have worse preoperative pain, disability, and health-related quality of life.[46] In mice, a recent study showed histological and behavioral sex-differences associated with IDD.[47] Data from the current study suggests that males and females use different strategies to regulate and maintain IVD ECM homeostasis in WT mice, in SPARC-null mice, and in response to long-term physical activity.

For example, mRNA expressions of *Col2a1*, *Col10a1*, and *ACAN* were significantly greater in female SPARC-null mice compared to males. *Col1a1*,*MMP13*, *ADAMTS4*, and *ADAMTS5* expressions were increased in both WT and SPARC-null female mice compared to male mice (Table 2). Although a previous study showed no difference in ECM collagen levels between males and females using mass spectrometry-based proteomics,[48] it is possible that females have higher mRNA levels than males. This increased expression in females is suggestive of augmented remodeling in females.

Given that *Col1a1*, *Col2a1*, *Col10a1*, and *MMP13* mRNA expression were lower in WT males than in females, it is interesting that all three collagens examined and *MMP13* were upregulated in male but not female SPARC-null runners. Thus, the physiological response in males to running may be to increase ECM turnover as a recovery strategy. Given the sex differences in fundamental ECM proteins and enzymes, it is crucial to consider males and females separately when exploring interventions in the future.

4.6 | Limitations and future directions

The focus on mRNA expression without parallel measurement of protein contentor disc biomechanics is a limitation of this study. Since expression levels of mRNA may not directly reflect protein levels, the effect of the *SPARC* deletion and the impact of running on ECM protein expression and disc function are needed.

In this study, only one model of IDD was used and only one time point was examined. SPARC-null mouse model is advantageous when studying long-term interventions such as running activity since the IDD údevelops naturally with age. However, one model is unlikely to

TABLE 3 Summary of mRNA expression. Schematic summarizing changes in mRNA expressions

	WT vs SPARC-null		WT sed vs run		SPARC-null sed vs run	
	M	F	M	F	M	F
Col1,2,10	– – –	– – –	– – –	– – –	↑↑↑	– – –
ACAN	**↓**	–	–	–	–	–
MMP3,9,13	– – –	– – –	– – –	– – –	**↑** – **↑**	**↑** – –
ADAMTS4,5	– **↓**	– –	– **↓**	**↑** –	**↑** –	– –
TIMP1,2,3,4	– – – –	– – – **↓**	– – – –	– – – –	– – – –	– – – –
CCN2	**↓**	–	–	–	–	–

Note: Bolded arrows represent statistically significant comparisons and non-bolded arrows represent nonsignificant trends with adjusted *P* values of <.1. Two-way ANOVA followed by Tukey multiple comparisons test.

reflect the many potential mechanisms that may drive LBP or IDD in individuals. More studies are needed to determine if these results extend to other models (e.g., disc puncture or SM/J mice[27,49,50]), are conserved across age, and are relevant to clinical settings.

Although collagen, aggrecan, MMPs (MMP3, MMP9, MMP13), ADAMTSs (ADAMTS4, ADAMTS5), and their inhibitors are major players of the ECM dynamics, many other ECM components that were not evaluated here are likely to have a role in the changing ECM in SPARC-null mice and after running. Therefore, expanding these studies to other ECM components and/or enzymes is necessary to fully understand the complex mechanisms of ECM involvement in LBP and IDD.

Finally, while increased running was observed in mice that had exposure to functional compared to sedentary wheels, information on home cage activity, such as total duration, speed and circadian rhythm over the 6-month exposure were not examined in this study.

4.7 | Implications

Our study shows that increased physical activity increases mRNA expression of key ECM genes, primarily in males. This suggests that the body's attempt to restore ECM integrity in degenerating discs may involve an overall increase in ECM dynamics. Furthermore, the response to running was more pronounced in degenerating SPARC-null disc compared to age-matched WT controls, suggesting that the environment in degenerating discs differs from normal tissue in its response to remodelling.[14] Thus, when assessing therapeutic interventions, studying responses in healthy tissues or naïve animals may be misleading.

In terms of sex differences, this study highlights differences in mRNA expression of ECM genes between males and females in both healthy and degenerating discs and in response to exercise. While the underlying causes of the differences are unclear, these data emphasize the importance of including both males and females in research studies and underscore the potential that therapies may be sex-specific.

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AUTHOR CONTRIBUTIONS

Seon Ho Jang contributed to the conception of the study, experimental planning, data acquisition, data analysis and interpretation, statistical analysis, drafting and revision of the manuscript. Seunghwan Lee contributed to the conception of the study, experimental planning, data acquisition, data analysis and interpretation, statistical analysis, drafting and revision of the manuscript. Magali Millecamps contributed to the conception of the study, experimental planning, data acquisition, data analysis and interpretation, statistical analysis, drafting and revision of the manuscript, funding acquisition and management of resources. Alexander Danco contributed to the conception of the study, experimental planning, data acquisition, data analysis, and interpretation. HyungMo Kang contributed to experimental planning and data acquisition. Stéphanie Grégoire contributed to experimental planning, data acquisition, data analysis and interpretation, statistical analysis, drafting and revision of the manuscript. Miyako Suzuki-Narita contributed to experimental planning, data acquisition, drafting, and revision of the manuscript. Laura S. Stone contributed to the conception of the study, experimental planning, data analysis and interpretation, statistical analysis, drafting and revision of the manuscript, funding acquisition and management of resources.

CONFLICT OF INTERESTS

The authors declare no potential conflict of interest.

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