Exercise Attenuates Low Back Pain and Alters Epigenetic Regulation in Intervertebral Discs in a Mouse Model.

Yuya Kawarai1,2, Seon Ho Jang 1, Seung Hwan Lee 1, Magali Millecamps 1, Hyung Mo Kang 1, Stephanie Gregoire 1, Miyako Suzuki-Narita 1,2, Seiji Ohtori 2, Laura Stone 1,3

1McGill University, Quebec, Canada, 2Chiba University, Chiba, Japan, 3University of Minnesota, Minneapolis, MN

Email: yuya.kawarai@mail.mcgill.ca

Disclosures: Nothing to disclose

INTRODUCTION: Chronic low back pain (LBP) is a leading cause of disability in adults in the US. The poor rates of recovery and high rates of recurrence contribute to extreme social and economic costs. LBP is complex and multifaceted, and current treatments are often either ineffective or are limited by undesired side-effects.

Intervertebral disc (IVD) pathology is one of the major contributors to chronic LBP. We have previously characterized the SPARC-null mouse model of LBP associated with IVD degeneration which is associated with progressive age-dependent disc degeneration and pathological disc innervation.

Epigenetics refers to several mechanisms, including DNA methylation, that have the ability to change gene expression without changing the underlying DNA sequence. DNA methylation can alter the entire state of a tissue for an extended period of time and thus could potentially be harnessed for long-term pain relief. Lifestyle factors, such as diet and physical activity, have a strong influence on epigenetic regulation.

DNA methyltransferases (DNMTs) are responsible for adding a methyl group to cytosine at the 5'-carbon position of the pyrimidine ring in DNA while enzymes of the trans-tenelecans (TET) family catalyze the stepwise oxidation of 5-methylcytosine (5-mC) in DNA to 5-hydroxymethylcytosine and further oxidation products. Given DNA methylation most commonly leads to inhibition of gene transcription, these enzymes modify gene transcription. McP2 and MBD1-3 are members of the methyl-CpG binding domain (MBD) protein family and are critical players in determining the transcriptional state of the genome.

Exercise is a commonly prescribed treatment for chronic LBP, and sex-specific epigenetic adaptations in response to endurance exercise have been reported. However, whether exercise interventions that attenuate LBP are associated with epigenetic alterations in degenerating IVDs has not been evaluated. We hypothesize that the therapeutic efficacy of physical activity is mediated at the epigenetic level. Using the SPARC-null mouse model of LBP associated with IVD degeneration, we therefore investigated a) impact of disc degeneration on epigenetic regulatory genes in the IVDs, b) the sensitivity of the epigenetic regulatory machinery to therapeutic environmental change, and c) sex-specific effects of exercise on epigenetic regulatory machinery.

METHODS: All experiments were approved by the Animal Care Committee at McGill University and conformed to the ethical guidelines of the Canadian Council on Animal Care and the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues. 8 month-old male and female SPARC-null and age-matched control (WT C57BL/6) mice were assigned to exercise (n=56) or sedentary (n=52) groups. Animals in the exercise group received a circular plastic running wheel in their home cage on which they could run freely. Animals in the sedentary group received an identical home cage that was secured in place to prevent rotation. After 6 months of intervention, voluntary physical activity was quantified using a voluntary running test with a wheel that counted revolutions for 1 hour in a separate cage for each mouse. Behavioral signs of axial discomfort, locomotor capacity, and radiating pain were determined, and epigenetic regulatory gene mRNA expression and global DNA methylation (5-mC: 5-methylcytosine) in IVDs were assessed. Statistics: Two-way analysis of variance (ANOVA) followed by the Sidak’s multiple comparisons post-hoc test.

RESULTS SECTION: Voluntary running was significantly higher in exercise than in sedentary groups (male: p<0.0001, female: p<0.0001, n = 11–16/ group). Male and female SPARC-null mice were hyper-sensitive to cold stimuli and showed signs of axial discomfort compared to WT controls (Cold sensitivity: p<0.0001; Axial discomfort: p<0.01; n = 7–10/ group). Lumbar IVDs from WT sedentary and SPARC-null sedentary mice presented similar global DNA methylation (%5-mC) and mRNA expression of epigenetic regulatory genes in both sexes. Exercise reduced LBP-related behaviors and the mRNA expression of Mecp2 in SPARC-null mice (p < 0.05, n = 4–7/ group) and significantly decreased global DNA methylation in both WT (p < 0.05, n = 4–5/ group) and SPARC-null mice (p < 0.01, n = 4–5/ group). mRNA expression analysis revealed that exercise resulted in sex-specific changes in genes related to epigenetic regulation.

DISCUSSION: This is the first study to investigate the impact of IVD degeneration on local epigenetic regulatory machinery and its sensitivity to long-term increases in physical activity. Our results demonstrated that WT sedentary and SPARC-null sedentary mice presented similar global DNA methylation (%5-mC) and mRNA expression of epigenetic regulatory genes in both sexes, suggesting IVD degeneration does not significantly affect the epigenetic regulatory machinery in IVDs. Therefore, epigenetics in degenerating IVDs could be targeted by therapeutic intervention such as exercise therapy.

In the present study, voluntary running exercise improved sensory symptoms associated with LBP, reduced global DNA methylation in healthy and pathologic IVDs, and produced sex-specific differences in the mRNA expression of epigenetic regulatory genes. Indeed, discs extracted from exercising mice presented reduced mRNA expression of Dnmt3a, Mecp2, and Tete1 in males and Dnmt3b and Mecp2 in females, whereas it increased the expression of Mbd2 in females. The mRNA expression in the SPARC-null mice, exercise significantly reduced the mRNA expression of Mecp2 in both sexes. Accumulating evidence indicates that Mcp2 is related to chronic pain and involved in DNMT1-mediated maintenance of DNA methylation. Given that exercise reduced the mRNA expression of Mecp2 and global DNA methylation in IVDs, we hypothesized that a decrease in Mecp2 expression commits the alteration of global DNA methylation via DNMT-mediated maintenance of DNA methylation. Finally, sex-specific effects of exercise on mRNA expression of Dnmt3a, Mbd2b, and Tete1 were observed.

There are some limitations. First, samples used in this study are from mouse IVDs. While acquiring human IVDs is challenging, further research with human IVDs is necessary. Second, these data cannot directly link the epigenetic changes to the therapeutic improvement in LBP. Third, the methylation and transcription status of pro-inflammatory and pro-nociceptive genes associated with IVD degeneration and chronic LBP should be examined in a future study.

In summary, voluntary running exercise improves sensory symptoms associated with LBP, decreases global DNA methylation in IVDs and sex-specific differences in the mRNA expression of epigenetic regulatory genes.

SIGNIFICANCE/CLINICAL RELEVANCE: Epigenetic modifications are at the interface between environment and genetics, creating a mechanism by which experience can lead to long-lasting changes in gene expression. Given DNA methylation is related to chronic pain conditions, including chronic LBP, exercise might alleviate LBP, in part, through epigenetic mechanisms. Elucidating epigenetic changes after exercise will provide insights into the mechanisms underlying the effects of exercise effects on chronic LBP. Targeting epigenetic alterations with lifestyle change could have therapeutic impact on tissue homeostasis in IVDs and lead to novel long-lasting therapeutic possibilities impacted by lifestyle.