Analysis of low back pain through the methylation process in specific genes. Systematic review

Análise da dor lombar através do processo de metilação em genes específicos. Revisão sistemática

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ABSTRACT

BACKGROUND AND OBJECTIVES: Low back pain is one of the most common complaints. Epigenetics represents a mechanism where the environment can modify gene expression without alterations in the primary DNA sequence. This can be seen in the process of DNA methylation, histone modification, and chromatin reorganization. The objective of this study was to conduct a systematic review on DNA methylation processes related to low back pain.

CONTENTS: Data were collected up to March 2023. The search was conducted on the following article search platforms: Scielo, Pubmed, Regional Portal of BVS, and LILACS. Pre-defined keywords were used in Portuguese or English: low back pain, DNA methylation, epigenomics, and epigenetics. All chosen words were verified through Health Sciences Descriptors (DeCS), and English words were verified in MesHterms. Bias risk analysis was identified. 61 genes were highlighted in the 8 articles that met the inclusion criteria. Only 2 studies presented genes in common, but one of them was in animal samples. Each analyzed gene has its particularity in performing processes, thus presenting differences in how it could generate low back pain. All studies included in this review were assessed for risk of bias.

CONCLUSION: The identified genes contribute significantly to the development of treatments and scientific knowledge. However, as the topic addressed is relatively new, further studies should be developed.

Keywords: DNA, Epigenomics, Low back pain, Methylation.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Os sintomas da dor lombar são algumas das queixas mais comuns. A epigenética representa um mecanismo pelo qual o meio pode modificar a expressão gênica sem que ocorra alterações da sequência primária de DNA. Isso pode ser visto em processos de metilação de DNA, modificação de histonas e reorganização de cromatina. O objetivo deste estudo foi realizar uma revisão sistemática sobre o processo de metilação de DNA relacionado à dor lombar.

CONTEÚDO: A revisão sistemática foi realizada com os dados coletados até março de 2023. A pesquisa foi realizada nas plataformas de busca de artigos: Scielo, Pubmed, Portal Regional da Biblioteca Virtual da Saúde e LILACS. Foram utilizadas palavras-chaves pré-definidas na língua portuguesa ou inglesa: - dor lombar ou *low back pain*, metilação de DNA ou *DNA methylation*, epigenômica ou *epigenetic*; sendo que todas as palavras escolhidas foram verificadas através dos Descritores em Ciências da Saúde (DeCS) e as palavras na língua inglesa foram verificadas no MeSH terms. A análise do risco de viés foi identificada. Nos oito artigos que preencheram os critérios de inclusão foram destacados 61 genes, sendo que apenas dois trabalhos apresentaram genes em comum, porém um deles em amostras animais. Cada gene analisado possui sua particularidade na realização de processos; portanto, apresentando diferenças na forma como poderá gerar a lombalgia. Todos os estudos incluídos nesta revisão tiveram o risco de viés avaliado.

CONCLUSÃO: Os genes identificados podem contribuir para a evolução de tratamentos e conhecimento científico. Porém, como o tema abordado é relativamente novo, mais estudos devem ser desenvolvidos.

Descritores: DNA, Dor lombar, Epigenômica, Metilação.

INTRODUCTION

Low back pain (LBP) symptoms are some of the most common complaints and also the leading cause of disability worldwide, regardless of age group and social class[1-4]. Despite the large number of people affected, most of them cannot accurately establish the cause of the symptoms[5,6].

It is known that most chronic diseases have genetic and environmental influences[7-9]. Among environmental factors,

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HIGHLIGHTS

- Several low back pain-related genes show different levels of DNA methylation;
- There are different genes involved on low back pain in men and women, and their methylation level is also different according to gender and exercise practice;
- There is still no epigenetic biomarker for low back pain, which indicates the need for further studies to clarify the role of epigenetics in low back pain.

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physical and psychological stress has been associated with chronic LBP[10-12].

Although the LBP etiology is multifaceted and not fully understood, genetics, and therefore heredity, can cause anatomical changes in 7-23% of the population[9]. However, current laboratory tests are not able to explain the disease's chronicity, requiring genetic contributions and verification of functional predisposition to low back pain[9].

Additionally, chronic pain is known to be associated with long-term changes in gene expression, which is a genetically and epigenetically controlled mechanism[10]. Epigenetics represents the modulation of gene expression without changes in the primary DNA sequence[11]. This can be seen in the processes of DNA methylation, histone modification and chromatin reorganization, as well as microRNAs[12].

DNA methylation stands out for being involved in cell regulation and differentiation, considered as two of the main factors that regulate gene activities[13]. Previous studies have shown the relationship of changes in DNA methylation patterns in animal models and in patients with chronic low back pain[10,14,15,16].

In addition to the identification of genetic predisposition, knowledge of epigenetic changes involved in chronic nonspecific low back pain may contribute to the understanding and development of new treatments[17].

Thus, this study sought to highlight, through a systematic review, the expression of genes modulated by different methylation patterns involved in low back pain.

CONTENTS

Article selection criteria

For this work, were included all articles related to LBP and DNA methylation found, so that situations in which there is LBP, but without a defined cause, could be clarified. Thus, all selected articles should present concepts of epigenetics from the point of view of DNA methylation. Therefore, presenting methylation of specific genes for LBP.

Articles were selected regardless of the concept of LBP, collection instruments, date of publication, sample size and sample gender. Articles that were not clear in the following requirements were excluded: relation to the established theme, genetic analysis not related to the methylation process or gene specification.

Search strategy

The following article search platforms were considered: Scielo, PubMed, BVS Regional Portal, LILACS. The following predefined keywords were used in Portuguese and English: *dor lombar* or low back pain, *metilação de DNA* or DNA methylation, *epigenômica* or epigenetic; all the words chosen were verified through the Health Sciences Descriptors (DeCS) and the words in English were verified in MesHterms (Table 1). The search period was until March 2023. The articles and data were extracted by two independent reviewers (L.R.M.P. and R.C.P), using the pre-established search and analysis strategies.

A literature review was conducted, including quantitative articles, according to the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) methodology. The research question construction was based on the acronym PICO: Patient/problem (description of the problem or population), Intervention (proposed intervention), Control/comparison (description of the intervention), Outcome (effect of the intervention). Articles were selected by reading the title or abstract. After selecting the articles that would be included in this review, full readings were performed and the main points of the works were highlighted.

Risk of bias

To ensure better quality control and early identification of possible sources of bias, the instrument developed by the authors[18] was used. This instrument classifies the risk of bias as low, moderate or high according to the internal and external validity of each study. This instrument takes into account the following criteria: 1 - representativeness of the study sample in relation to the national population, 2 - a sampling system that represents the target population, 3 - sample selection method, 4 - probability of non-response bias, 5 - way of obtaining the response of interest, 6 - definition of the LBP concept used to define the sample, 7 - reliability and validity of the tools used, 8 - standardization of the collection process, 9 - appropriate prevalence period of interest, 10 - presence of calculation error and/or reporting of the parameter of interest's numerator and denominator values[18].

External validity was assessed taking into account the first 4 items, while the remaining items assessed the internal validity of each article. Finally, each article was classified according to its score, with 10-9 classified as low risk and 8-7 classified as moderate risk; lower scores were classified as high risk[18].

Data extraction and analysis

The variables of interest, such as first author, date of publication of the article, type of study, sample size and result of the study were transferred to an electronic spreadsheet (Excel), for better visualization and interpretation of the data obtained (Table 1).

Table 1. Database search strategy

LILACS, Scielo, BVS Regional Portal	Low back pain AND DNA methylation; low back pain AND epigenetic; low back pain AND DNA methylation AND epigenetic – research also carried out in the Portuguese language
Pubmed	Low back pain AND DNA methylation; low back pain AND epigenetic; low back pain AND DNA methylation AND epigenetic

RESULTS

From the searches performed, a total of 92 articles were found. Of these articles, 43 were extracted from BVS Regional Portal and 49 from Pubmed platform. Scielo and LILACS platforms, despite using different forms of search, did not present any article available related to this research.

After screening 54 articles were excluded due to duplication and one article was excluded because it was blocked for full reading. After analyzing the titles, seven articles were discarded because they were not related to the research theme. Another 11 articles also did not meet the inclusion criteria and were therefore excluded after analyzing the abstract. Finally, 19 articles were selected for full reading.

Of the 19 articles selected, 8 addressed the topic of global methylation, one presented only protocols for research development, one was based solely on another article already included in the review and one article used exclusively a bioinformatics platform for data verification. Therefore, these articles were excluded from this study. Figure 1 shows the selection process.

Among the selected studies, the oldest articles were published in 2016[19] and 2019[14]. Thus, it was observed that the topic addressed, besides being current, evidences the evolution of genetics, providing clarifications not previously addressed.

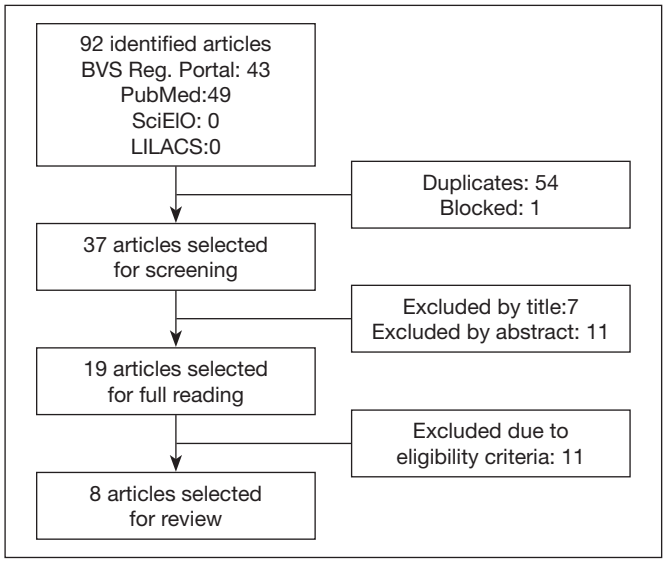

Figure 1. Study selection

Of the eight articles selected[10,14,15,16,19-22] at the end of the analyzes, a systematic review article[22] was included, and due to the present study different objective and data discrepancy there was a need to analyze each of its articles; only the works that met the selection criteria of this study were verified; therefore, clarity in the methodology and identification of specific genes in the methylation process related to low back pain were necessary. In addition, articles that were already included in this study were excluded; only one article[23] from the review needed to be verified. Finally, the need to analyze a single article meant that the data explanation was only related to this one and not to the review as a whole.

After analyzing all the articles included in this work, it was found that two articles[14,16] used mice for their investigation. Of these two articles, one performed only analysis through mice[16]; while the other obtained a mixed sample[14], composed of mice and humans. A total of 75 animals were used, all of them male and with controlled environments. Only these studies were of longitudinal analysis, besides the study[20] that pointed out scarcity and difficulty in obtaining data in these circumstances.

In the other 6 studies[10,15,19,20,21,23], human biological samples were collected for analysis using peripheral blood, whole blood, ligamentum flavum and nucleus pulposus cells. A total of 271 people participated in the studies, with a mean age close to 51.7 years, of both genders. Low back pain was identified through self-report, medical record analysis, questionnaires and due to the need for surgery. In 2 of the articles, the way of identifying pain was not clarified[16,23].

Information on authors, year of publication, type of study, biological material used, sample size, mean age of sample, genetic analyses used, and LBP identification is shown in table 2.

All articles presented genetic analysis by methylation; as well as all of them also identified specific genes. The main genes cited were: CELSR1, MFGE8, NR2F2, GPAT2, WDR5, CIof127, DYRK3, PAX5, HTRA3, MINK1, K1F11, COL4A3, MACC1, PIGC, ACSM5, HSPA6, LGALS8, FRMD4A, KLRC4-KLRK1, RAMP1, COL21A1, TACSTD2, RPH3AL, NINJ2,

Table 2. General characteristics of the selected articles

Authors	Type of study	Biological sample	Sample size (n)	Gender	Age (years)	Genetic analysis	LBP identification	Significance
Aroke et al.[15]	Transversal	Human peripheral blood	50	Both	52	DNA methylation by bisulfite	Self-report, medical records - American Colleges of Physicians e American Pain Society, Brief Pain Inventory (BPI) – Short Form	Yes
Cao et al.[24]	Transversal	Human ligamentum flavum	10	Both	63.5	EZ DNA methylation kit, cell culture in DMEM medium	Examinations and surgical needs	Yes
Grégoire, et al.[10]	Transversal	Human peripheral blood	75	Both	43.4	DNA methylation by bisulfite	Self-report - Canadian adaptation of the NIH Low Back Pain Taskforce	Yes

Continue...

Table 2. General characteristics of the selected articles – continuation

Authors	Type of study	Biological sample	Sample size (n)	Gender	Age (years)	Genetic analysis	LBP identification	Significance
Jiang et al.[20]	Longitudinal	Cartilaginous end plate (CEP) mice/ human - when performed surgery	15 mice 18 human	Male	6 weeks	Quantitative RT-PCR, Western blotting, metilação de DNA	Mice - Pfirrmann grade - to check impact Human - sample through surgery in the lumbar region	Yes
Luo et al.[25]	Longitudinal	Lumbar disc samples from mice	60 mice	Male	2 months	RT-qPCR, Western blot, flow cytometry, methylation, ELISA	None - performed surgical techniques exclusively for analysis	Yes
Adhikari et al.[20]	Longitudinal	Human peripheral blood	11	Both	34	bisulfite sequencing, EZ DNA methylation kit, RNA sequencing by Illumina NextSeq 550 system	Self-report - brief 40-iten byTaskforce on Research Standards for cLBP; Brief Pain Inventory (BPI); Emotion Regulation Questionnaire (ERQ)	No
Li et al. [27]	Transversal	Tissue of nucleus pulposus	95	Both	55	Intervertebral disc RNA-seq, RNA scope, RT-PCR, Western blot	None - performed surgical techniques, due to operative need	Yes
Sukenaga et al.[19]	Transversal	Total human blood	12	Both	62.5	Illumina HumanMethylation450 BeadChip, SurePrint G3 Human Gene Expression 8x60K v2 microarray kit	DN4 (Douleur Neuropathique 4), SF--MPQ (Short-Form McGill Pain Questionnaire)	Yes

MR1, DTHD1, MYT1L, MYO1D, PTPRE, ACBD5, EDIL3, PGAM2, NUP35, NOTCH1, ZNF718, PLD6, Sox-9, EZH2, DNMT3B, TRPA1, COX2, YAP, PSMD1, PSMD12, PSMA3, PSMB6, PSMB10, KLRK1, KPNB1, LAPTM5, ICAM-3, RPL23A, ALKBH5, and TRPA1.

Only one study[10] considered gender differences for genetic analysis. In addition to genetic analysis through DNA methylation, other means such as cell culture in DMEM medium, RT-PCR, western blotting, flow cytometry, and ELISA were used.

The main genes found, according to each article, are shown in Table 3.

In the risk of bias assessment, the articles analyzed obtained a score between 8 and 9; 2 articles were classified as medium risk of bias due to score 8, and the remaining articles were classified as low risk of bias.

In the initial criteria of external validity, such as national representation of the target population, all articles presented a negative response. For the criterion that the sampling system is a true or close representation of the target population, only two articles were included. The only specific reason was because the samples were from mice.

Table 4 presents risk of bias assessment according to each article included in this work.

Table 3. List of genes by authors

Authors	Genes
Aroke et al.[15]	CELSR1, MFGE8, NR2F2, GPAT2, WDR5, Clof127, DYRK3, PAX5, HTRA3, MINK1, K1F11, NAV1,DAD1, FAM101B, TCF25, OLFM1, FBRS
Cao et al.[21]	COL4A3, MACC1, PIGC, ACSM5, HSPA6
Grégoire et. [10]	Woman- LGALS8, FRMD4A, KLRC4-KLRK1, RAMP1, COL21A1, TACSTD2, RPH3AL, NINJ2, MR1, DTHD1 Man - MYT1L, MYO1D, PTPRE, ACBD5, EDIL3, PGAM2, NUP35, NOTCH1, ZNF718, PLD6
Jiang et al.[14]	Sox-9, EZH2
Luo et al.[16]	DNMT3B, TRPA1,COX2,YAP
Adhikari et al.[20]	PSMD1, PSMD12, PSMA3, PSMB6, PSMB10, KLRK1, KPNB1, LAPTM5, ICAM-3 e RPL23A
Li et al.[23]	ALKBH5, DNMT3B
Sukenaga et al.[19]	TRPA1

Table 4. Risk of bias assessment

Authors	Aroke et al.[15]	Cao et al.[21]	Grégoire et al.[10]	Jiang et al.[14]	Luo et al.[25]	Adhikari et al.[201]	Li et al.[27]	Sukenaga et al.[19]
Was the study's target population a close representation of the national population in relation to relevant variables?	No	No	No	No	No	No	No	No
Was the sampling frame a true or close representation of the target population?	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Was some form of random selection used to select the sample, OR was a census undertaken?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the likelihood of nonresponse bias minimal?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were data collected directly from the subjects (as opposed to a proxy)?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was an acceptable case definition used in the study?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the study instrument that measured the parameter of interest shown to have validity and reliability?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the same mode of data collection used for all subjects?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was the length of the shortest prevalence period for the parameter of interest appropriate?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

DISCUSSION

This review evaluated the methodological quality of existing articles on the proposed theme, highlighting the growing evolution of genetic analysis of diseases present in everyday life and enabling a greater understanding of the disease.

Epigenetics takes on the role of analysis, seeking to identify possible genes that trigger pain or that may alter some function or structure responsible for maintaining or even preventing pain.

One study[15] brought the possibility of non-specific LBP related to epigenetics, taking into account the modifications that alter the ossification of chondrocytes into osteoblasts. Differential methylation of the gene encoding the extracellular matrix protein SPARC was associated with LBP severity. The study also analyzed spinal disc degeneration, pain severity, SPARC (secreted acidic cysteine-rich protein) protein expression and DNA methylation in the SPARCs promoter region, where they observed that when DNA methylation is increased there is a reduction in gene expression.

CELSR1, K1F11, MINK1, NAV1, MFGE8, VISION1 genes were highlighted. It was noted that NAV1 and K1F11 genes play a role in neuronal migration and act in microtubule regulation and axonal growth, respectively. They may affect the development and transmission of CELSR1 and MINK1 nociceptors[15], which act in cell growth and the skeletal system. The latter, together with MFGE8, WDR5, PAX5 and DAD1 genes, when hypomethylated are associated with immunological disease processes[19]. In addition, it was pointed out that TBX21 and IFNG genes are related to chronic pain condition. TAC1, on the other hand, plays a role in pain modulation, generating a state of chronic inflammation. Therefore, it is important to consider a possible inflammation related to the spinal matrix integrity[15].

Still regarding the lumbar region structures, one study[21] considered some known causes for LBP development, such as spinal canal stenosis, spondylolisthesis of the lumbar spine, and disc herniation. These diseases were related to changes in the ligamentum flavum. The justification for the analysis of the ligamentum flavum was due to its physiology, as it is responsible for the spinal cord mobility and protection, being composed of 80% elastic fibers and 20% collagen fibers.

In the above mentioned analysis, the following genes stood out: COL4A3, MACC1, HSPA6, PIGC and ACSM5. All genes highlighted participate in the process of glucose and lipid metabolism via PI3K (collagen-related pathway), being positively correlated with the yellow ligament thickness. With the exception of MACC1, which showed no difference[21].

It has also been noted that hypermethylation of DNMT1 (DNA methyltransferases type 1) mediator in ACSM5 leads to its hypomethylation; thus, downregulation inhibits fibrosis proliferation and promotes apoptosis of cells in the ligamentum flavum of patients with hypertrophy in this ligament[21].

Another point to be discussed is the sample gender. One study[10], which was the only one to consider a possible difference in this genetic profile, aimed to correlate DNA methylation profiles in human T cells from LBP cases. 2496 differentially methylated positions (DmPs) were identified in women, while 490 DmPs were observed in men.

In the study mentioned above the main hypermethylated genes in women were: LGALS8, FRMD4A, KLRC4-KLRK1, RAMP1, COL21A, TACSTD2 and RPH3AL; in men MYT1L, MYO1D, NUP31 and NOTCH1 stood out. Among the hypomethylated genes in women, the following stood out: NINJ2, MRI1, DTHD1 and MRI1; in men: ZNF718 and PLD6[10].

Although low back pain affects both genders, the study in question[10] showed the existence of marked differences in methylation, suggesting fundamentally different mechanisms and the possibility of gender-specific epigenetic biomarkers and distinct therapeutic approaches; however, the existence of only one article in which this correlation was performed hinders an in-depth analysis and brings a perspective to be observed in future works.

Another line of research was addressed in a study[20] that sought to identify the methylation of specific genes in the process of low

back pain in relation to physical exercise. In this case, it was observed that the genes PSMD1, PSMD12, PSMA3, PSMB6 and PSMB10 – in the NIK/NF-kB pathway – were hypomethylated in post-yoga samples. Participants reported greater pain reduction after performing the exercises. Antisense transcripts of the genes KLRK1, KPNB1, LAPTM5, ICAM-3 and RPL23A stood out. KLRK1 is an activator of NK cell cytotoxicity; similarly, KPNB1 is a key regulator of NF-κB signaling in chronic peripheral neuropathic pain. In contrast, LAPTM5 is up-regulated in neuropathic pain, as well as ICAM-3 and RPL23A. They have also been reported to be downregulated in rheumatoid arthritis synovium.

NF-κB can be activated via the canonical pathway through stimuli including tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and toll-like receptors, or via the non-canonical pathway through NIK. The NIK pathway is activated by β-cell activating factor, which is a TNF family receptor, by NF-κB activating receptor and by lymphotoxin. NF-κB activation is identified in glutamatergic neurons and may protect neurons by regulating neuronal inflammatory reactions and the surrounding neuronal environment.

The nociceptive fibers A-delta and C are glutamatergic, thus providing a direct pathway to influence pain processing; therefore, they are related to the reduction of generalized pain sensitivity seen before and after the application of yoga. The survey in question was a pilot study and therefore did not have statistical significance, but it is possible to verify different genes acting in the pain process and their proximity to physical exercise. Thus, it is possible to realize that changes in the environment, as well as the regular exercise, can influence different genes[20].

Some studies required greater control over the variables. Two researchs[14,16] used mice to perform their analysis. The advantage of conducting research with animals is the amount of information available due to the laboratory and surgical practices performed on these sample groups. In addition, the biological material is available at the discretion of the researcher and the study environment can be thoroughly controlled, further facilitating the collection of reliable information. It was observed that this happened in both studies; however, as the sample was animal, even if created through surgical techniques, it did not have proximity to the target population (when analyzing the bias criteria). As seen in previous studies, gender, as well as environmental circumstances, exercise practice, among other factors can affect the methylations of specific genes; therefore, the evaluation and analysis of human samples would be necessary to obtain further clarification and guide practical applications.

However, one of the studies mentioned above[14] provided important information by linking cartilaginous endplate degeneration (CEP) to one of the main causes of intervertebral disc degeneration. As described, Sox-9 is essential for cartilage development and is a transcription factor required for chondrogenesis and in various stages of the chondrocyte. It has also been elucidated that the histone methyltransferase enhancer of zeste homolog2 (EZH2) assists in epigenetic regulation responsible for inflammation, autoimmunity and several malignancies.

The mentioned study identified the regulatory effects of EZH2 on degeneration-related genes and Sox-9 expression in mouse endplate chondrocytes. Sox-9 overexpression can reverse the effect of EZH2 and inhibition of EZH2 decreases cartilage endplate degeneration and attenuates the progression of intervertebral disc degeneration through Sox-9 demethylation[14].

In the same study, in addition to animal samples, humans were also used for data collection. The investigated patients showed that the level of EZH2 was hyperactivated in chondrocytes of osteoarthritis patients, being induced through IL-1β. A high nterver of EZH2 to the Sox-9 promoter was observed in degenerated human CEPs, and silencing of EZH2 may trigger demethylation of H3K27me3 at Sox-9 promoter sites, nterve to high Sox-9 expression. However, overexpression of EZH2 deactivated Sox-9, catalyzing methylation of H3K27me3 at Sox-9 promoter sites. EZH2 inhibition may exert its protective role by removing H3K27me3 from the Sox-9 promoter[14].

One study[16] also performed analyses with animal samples, but without further analysis in humans. The study investigated the nucleus pulposus (NP) cells: aggrecan, type II collagen and other components of the extracellular matrix (ECM), verifying that they maintain the intervertebral disc (IVD) integrity.

The mentioned analysis took into account that DNA Methyltransferase 3 Beta (DNMT3B) can methylate centromeric, pericentromeric and subtelomeric repeats. DNMT3B protein plays ntervert roles in diseases depending on the nterver. Na inhibitor of DNMT3B enhances the nterverteb of the nterverte receptor ankyrin 1 (TRPA1) during erythroid and megakaryocyte differentiation. In addition, TRPA1 is a nterv channel found in the plasma membrane of numerous cell types and is a possible identifier of inflammatory pain[16].

TRPA1 exerts anti-inflammatory and protective effects, and may also promote the development of degenerative cartilage changes and joint pain in osteoarthritis, when the expression of a potential mediator, cyclooxygenase 2 (COX-2), is suppressed[16].

COX-2 is induced by pro-inflammatory cytokines and is also a key link in triggering subsequent inflammatory responses. Hypoxia is known to increase COX2 expression in mesenchymal stem cells, and then activates Yes-associated protein (YAP) in hepatoma carcinoma cells and leads to increased cell proliferation. Downregulation of YAP is involved in intervertebral disc disease (IVDD) and cellular aging. Thus, DNMT3B may regulate the TRPA1/COX2/YAP axis to alleviate IVDD[16].

Induction of DNMT3B expression was found to increase proliferation and reduce NP cell apoptosis, ECM degradation and inflammation. DNMT3B, methylated TRPA1 and TRPA1/COX2/YAP mediated the protective effect of DNMT3B on NP cells. In the mice with ntervertebral disc changes, overexpression of DNMT3B could alleviate the number and structure of NP cells. Thus, the findings indicated that DNMT3B/TRPA1/COX2/YAP may be a novel therapeutic target for certain intervertebral disc changes[16].

This is consistent with the results of a study[23] that worked with NP tissues of human origin and made important considerations regarding senescence in these cells, suggesting that there is an increase in ALKBH5 expression through KDM4A, which reduces

trimethylation of histone H3 lysine 9 in the ALKBH5 promoter, which increases ALKBH5 expression. Increased ALKBH5 in turn reduces m6A methylation of DNMT3B mRNA, increasing its expression and stability.

DNMT3B inhibition could partially abolish E4F1 promoter methylation and re-establish E4F1 expression. Significantly, when E4F1 expression was decreased in NP cells by siRNA, these cells became senescent, whereas overexpression of E4F1 in NP cells can reverse TNFα-induced senescence and forced E4F1 expression can partially abolish the pro-senescence effects of ALKBH5 and DNMT3B. These results reveal a critical role for m6A modification of DNMT3B mRNA in NP cell senescence and intervertebral disc degeneration[23].

One study[19] highlighted the importance of TRPA1 methylation, a gene also mentioned by another research[16], in relation to the epigenetics of chronic pain. The first study showed a significant correlation between increased TRPA1 gene methylation level in blood cells and increased DN4 (*Douleur Neuropathique* 4) scores, which represent the diversity of neuropathic pain symptoms. Moreover, a significant correlation between reduced TRPA1 expression and increased DN4 scores was described. TRPA1 gene was then associated with a key role in the development of chronic pain in humans and is related to functional changes in neuroimmune interactions. Such analyses are in line with the findings of a research[16] that pointed to the gene as a possible marker of inflammatory pain.

Parte superior do formulário

Although LBP is one of the most common complaints worldwide[1], few studies on epigenetics identifying specific genes in methylation was found. Thus, it can be seen the need for studies in this area, contributing to the improvement and clarification on the subject.

In addition, the results found showed heterogeneity in the mode of data collection, type of biological material analyzed, objective and results of the study, making any meaningful grouping of the data impossible.

Another point to be taken into account is the ethnicity of the population, in addition to a more rigorous criterion regarding age, gender, drug therapy used, physical activity, among other variables; since it is understood that epigenetics can be influenced by several factors and the non-control of these variables could influence the result obtained, also justifying the different genes found[7,8].

Regarding the external validity criteria, none of the studies had a significant sample size in relation to the target population. However, as the research addresses a relatively new topic, the genes identified may contribute to the evolution of treatments and scientific knowledge, showing the need for further studies on this topic.

CONCLUSION

This work contributed by demonstrating the need for further studies on the subject, exposing considerations regarding sample size and control of variables and strongly recommending that robust studies with low levels of bias be carried out.

AUTHORS' CONTRIBUTIONS

Layse Rafaela Moroti-Perugini
Data Collection, Research, Methodology, Writing - Preparation of the Original
Isadora Fernandes Cônsolo
Data Collection
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Conceptualization, Supervision, Visualization
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