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Understanding Pathophysiological Mechanisms of Chronic Pain Resolution in the Presence of Widespread Pain via Transcriptomics-Wide Analysis

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INTRODUCTION

Pain serves an **adaptive survival function**, an inner warning system pointing to harm or a treatment. Pain management and loss of productivity during chronic pain result in substantial societal costs. In 2018 it was reported that the **prevalence** of chronic pain is more than **30**% in America alone, with chronic low back pain being the **most reported condition** (1).

It has been shown that people with widespread pain are less likely to resolve local chronic pain compared to people without it (6). **Drugs** have shown **minimal effectiveness**, moreover, some of them have substantial side effects, and **exercise** is the current **first-line treatment** prescribed to patients with chronic pain. Many studies suggest that physical exercise might contribute to chronic pain improvement (2).

Therefore, we aimed to **investigate** the **molecular pathophysiological mechanisms** underlying the **improvement of chronic low back pain** (LBP) and how presence of widespread pain is correlated chronic low back pain improvement following **exercise** through a **transcriptome-wide analysis** of the peripheral immune cells.

METHODS

Study design and Grouping: The blood samples were collected from 32 low back pain patients who underwent 14 weeks of physical exercise. The statuses of improved and persistent pain group were assigned based on the pain score difference of at least two between the first and second time points. Same patients were also divided into "Widespread (-)" or "Widespread (+)" based on the presence of the widespread pain at the baseline.

Statistical analysis: FASTQ files of the sequencing data were mapped on the human genome GRCh38 using STAR (3). Five specific contrasts both on gene and pathways levels were performed. Differential expression of genes was detected with the help of the "DESeq2" R package (4). The differential expression gene analysis results were given for input to "fgsea" R package for pathways analysis.

CONCLUSIONS

The results of our transcriptomics analyses of the cohort of LPB patients undergoing physical exercise provided few insights. Our findings suggest that active biological processes underlie pain resolution in chronic LBP patients during the course of physical exercise, with a significant contribution from active downregulation of inflammation. This active downregulation of inflammation is absent in patients with widespread pain.

RESULTS

Differential Gene Expression:

We assessed genome-wide transcriptomics differences in a cohort of 32 low back pain patients at the first blood collection time point, 2 weeks after the start of exercise (T1), at the second blood collection time point, after the last exercise session on week 14th (T2), and differences within each group over time. This analysis did not yield any significant results, but Kolmogorov-Smirnov test analysis indicated that distributions of changes in gene expression betas were significantly different between the improved and persistent groups (D = 0.04, P-value = 2.258e-10), and between the widespread (-) and widespread (+) groups (D = 0.05, P-value = 1.532e-14), suggesting that there are more regulations of the gene expression in the improved and widespread (-) pain groups than in the persistent and widespread (+) pain groups.

General Pathway Analysis:

We next calculated the correlation of changes in transcriptionally based biological pathways over the time of exercise between persistent and improved pain groups and between groups with present or absent widespread pain. We found that the majority of transcriptional changes over time have a positive correlation.

Most of the pathways, located at the top right and bottom left corners, have similar directionality reflecting the similar biological responses of both groups to the exercise. However, there were a number of pathways that were located at the top left and bottom right quadrants of the plot, indicating the opposite directions (Fig. 1). The inflammatory pathways (marked in yellow) were found abundantly enriched in the anticorrelated cluster characterized by biological pathways downregulated overtime by the improved and the widespread (-) pain groups and up-regulated by the persistent and the widespread (+) groups, which are located at the bottom right quadrant (Fig. 1 A, B).

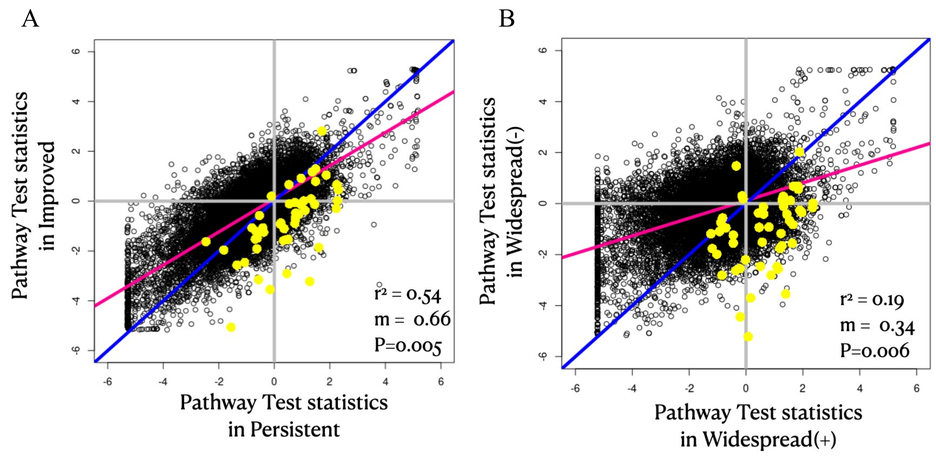

Fig. 1 **Pathway Trajectories in Time Between the Four Pain Groups**
(A) - Pathway trajectories in time between the people in improved pain group and persistent pain group, (B) - Pathway trajectories in time between the people in widespread (-) pain group and widespread (+) pain group. Each dot is a pathway. Pathways coordinates are in test statistics space, obtained from pathway analyses by fgsea. The pink line was obtained from linear regression of the data, whereas the blue line is from theoretical expectation of equal trajectories. Yellow dots represent inflammatory pathways. Percent variance explained (Pearson's r^2), slope (m), and P-value of regression are reported.

Inflammatory and Cell Activation Pathway Analysis:

We next focused on the differences in the improved and persistent as well as widespread (-) and widespread (+) pain groups for the Inflammatory and Cell Activation biological pathways. We found that patients from the improved and widespread (-) pain groups had inflammatory response pathway downregulated over the time of exercises (Fig. 2A,C), when the persistent and widespread (+) pain group did not show any significant differences (Fig. 2B,D).

When analyzed blood cells activation pathways, we found that all of the detected significantly expressed cell type activation pathways were downregulated overtime in all pain groups (Fig. 3). More pathways were downregulated in the improved and even more in the widespread (-) pain groups (Fig. 3A, C), while either less or none were downregulated in the persistent and widespread (+) pain groups (Fig. 3B,D).

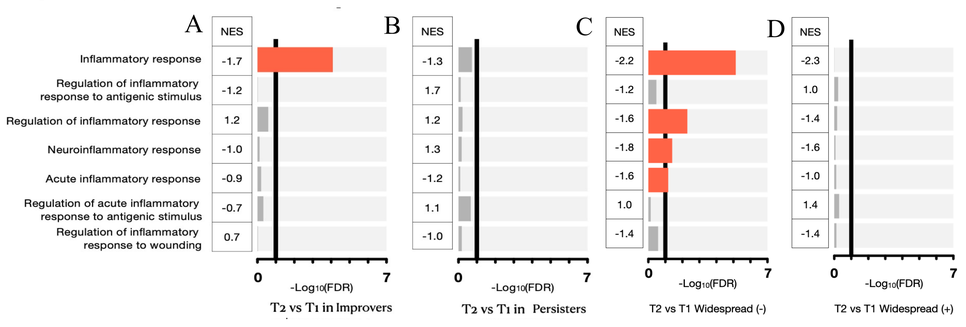

Fig. 2 **Functional Difference in the Inflammatory Pathways.**
Functional differences assessed for selected pathways in Gene Ontology's (GO) biological processes, overarching under "inflammatory response" (GO: 0006954). Statistically significant pathways at the FDR 10% level are highlighted in orange. NES column shows normalized enrichment scores for every pathway. (A,B) - Differentially expressed pathways over the time of exercise for improved pain group (A) or persistent pain group (B). (C, D) - Differentially expressed pathways over the time of exercise for widespread (-) pain group (C) or widespread (+) pain group (D).

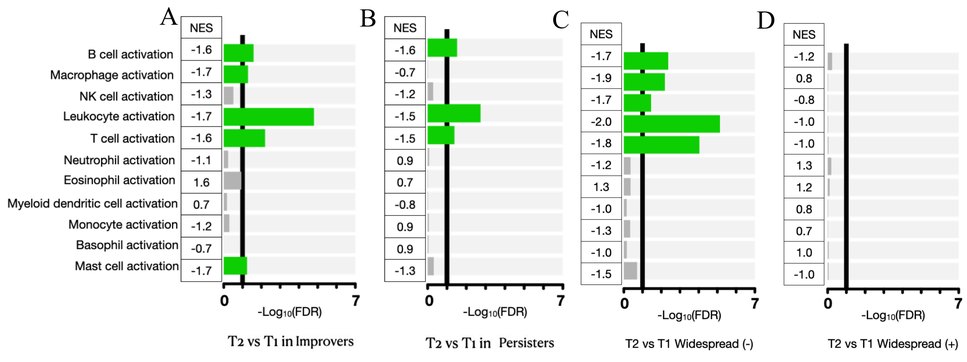

Fig. 3 **Functional Difference in the Cell Activation Pathways.**
Functional differences assessed for selected pathways in Gene Ontology's (GO) biological processes overarching pathway of "leukocyte activation" (GO:0045321). Statistically significant pathways at the FDR 10% level are highlighted in green. NES column shows normalized enrichment scores for every pathway. (A,B) - Differentially expressed pathways over the time of exercise for improved pain group (A) or persistent pain group (B). (C, D) - Differentially expressed pathways over the time of exercise for widespread (-) pain group (C) or widespread (+) pain group (D).

REFERENCES

1.Wahrman, A. (2018, September 24). Chronic pain: How we're losing the battle. Healthline. https://www.healthline.com/health-news/america-is-losing-the-war-on- chronic-pain

2.Qaseem, A., Wilt, T. J., McLean, R. M., & Forciea, M. A. (2017). Noninvasive treatments for acute, subacute, and chronic low back pain: A clinical practice guideline from the American College of Physicians. Annals of Internal Medicine, 166(7), 514. https://doi.org/10.7326/m16-2367

3.Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2012). Star: Ultrafast universal RNA-seq aligner. Bioinformatics, 29(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

4.Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with deseq2. Genome Biology, 15(12). https://doi.org/10.1186/s13059-014-0550-8

5.Parisien, M., Lima, L. V., Dagostino, C., El-Hachem, N., Drury, G. L., Grant, A. V., Huising, J., Verma, V., Meloto, C. B., Silva, J. R., Dutra, G. G., Markova, T., Dang, H., Tessier, P. A., Slade, G. D., Nackley, A. G., Ghasemlou, N., Mogil, J. S., Allegri, M., & Diatchenko, L. (2022). Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Science Translational Medicine, 14(644). https://doi.org/10.1126/scitranslmed.abj9954

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