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Association between total plasma N-glycan levels and chronic back pain: a prospective study

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Abstract — **Current prospective study identifies variation of N-glycosylation patterns in total blood plasma associated with chronic back pain.**

Keywords — low back pain, N-glycans, total plasma N-glycome, glycosylation

Introduction

Low back pain (LBP) is one of the most prevalent and disabling health problems worldwide, incorporating different pain syndromes [1]. One of the probable causes of LBP is local inflammation,. Inflammatory reactions have been reported to be associated with alterations in N-glycosylation of several serum glycoproteins [2] and total blood plasma protein [3]. Given that glycans modify the protein structure and affect their function [4], changes in glycosylation are implicated in the pathogenesis of different diseases and may be considered as biomarkers in some [5–8].

Our previous retrospective case-control study identified an association between total plasma N-glycome and chronic LBP (CLBP) [9]. The current prospective study addresses the question of temporal variation of total plasma N-glycome in the development of CLPB and whether plasma N-glycome can serve as a predictive biomarker for CLBP.

Subjects and methods

Clinical samples and data

The sample comprised 1,114 Europeans recruited at baseline (T0) when they experienced a serious episode of acute LBP and followed-up at 3 months (T1). Total sample size was 1,085 people at T0 and 549 people at T1. For n=520 participants data were obtained at both time points.

Blood samples and clinical data were collected in five centers located in the United Kingdom, Belgium, Croatia (2 centers) and Italy in the EU FP7 PainOmics project [10, 11].

Glycan profiles were measured for 39 original glycan traits and summarised into 14 derived traits. Cases and controls were defined according to presence or absence of LBP at T1: those who reported LBP at T1 were considered to have evolved into CLBP. Information about pain type (spontaneous or after injury), probable pain generator and neuropathic pain was also collected.

Statistics

The analysis was carried out in each center cohort separately followed by the meta-analysis. For each cohort, glycan profiles were adjusted for age, sex and body mass index (BMI) and the inverse rank-transformation to normality was performed. Case-control comparisons were performed using generalized linear model. To estimate the predictive power of total plasma N-glycome we calculated the area under the ROC-curve, AUC value. Meta-analysis of the results was performed using metaphor v2.1.0 R package. Paired t-test was applied to detect alterations in glycan levels between T0 and T1 in cases and controls; the results were meta-analyzed using metap v1.1 R package. Additionally, we ran canonical correlation analysis to obtain correlation matrices within and between cases and controls in each cohort for both time points.

We checked for pain generator effect using ANOVA and repeated all the analyses in the subsets of data split by different pain generators and pain types. Some tests were also run on principle components of glycan level traits and neuropathic pain data. All the analyses were performed in R programming language in RStudio, R versions 3.5.3 and 3.6.1. Significance was taken as $p\text{-value}<0.05$ and correction for multiple testing was $0.05/14\approx0.00357$.

Results

1. We addressed three questions: 1) is there a difference between cases and controls at T0?; 2) is there a difference in glycome profile between cases and

controls at T1?; and 3) is there a difference between T0 and T1 in cases and controls?

2. No statistically significant association between levels of total plasma N-glycan at T1 and CLBP was revealed; however, we detected negative association under nominally significant threshold for neutral, monogalactosylated and high-mannose glycans, consistent with our previous retrospective study [9].

3. We detected statistically significant differences in N-glycosylation pattern between T0 and T1 in controls for low- and high-branching glycans, trisialylated, di- and trigalactosylated glycans. No such differences were found for cases, suggesting that people who develop CLBP keep their pro-inflammatory profile of plasma N-glycans.

4. We did not find evidence of predictive capacity of N-glycan profile for future development of CLBP. We also did not reveal interactions between pain-generator and N-glycome of plasma. Results obtained on principle components and in different subsets of data split by pain types and generators were consistent with those obtained in original data.

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