

Plasma N-glycome composition associates with chronic low back pain

Trbojević-Akmačić, Irena; Vučković, Frano; Vilaj, Marija; Skelin, Andrea; Karszen, Lennart C.; Krištić, Jasminka; Jurić, Julija; Momčilović, Ana; Šimunović, Jelena; Mangino, Massimo; ...

Source / Izvornik: **Biochimica et Biophysica Acta (BBA) - General Subjects, 2018, 1862, 2124 - 2133**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1016/j.bbagen.2018.07.003>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:184:826642>

Rights / Prava: [Attribution-NonCommercial-NoDerivatives 4.0 International/Imenovanje-Nekomercijalno-Bez prerada 4.0 međunarodna](#)

Download date / Datum preuzimanja: **2024-07-14**



Repository / Repozitorij:

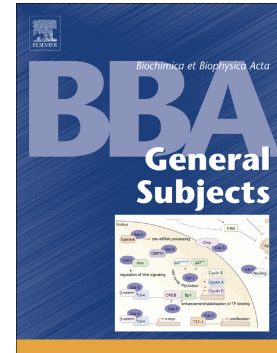
[Repository of the University of Rijeka, Faculty of Medicine - FMRI Repository](#)



Accepted Manuscript

Plasma N-glycome composition associates with chronic low back pain

Irena Trbojević-Akmačić, Frano Vučković, Marija Vilaj, Andrea Skelin, Lennart C. Karssen, Jasminka Krištić, Julija Jurić, Ana Momčilović, Jelena Šimunović, Massimo Mangino, Manuela De Gregori, Maurizio Marchesini, Concetta Dagostino, Jerko Štambuk, Mislav Novokmet, Richard Rauck, Yurii S. Aulchenko, Dragan Primorac, Leonardo Kapural, Klaas Buyse, Dieter Mesotten, Frances M.K. Williams, Jan van Zundert, Massimo Allegri, Gordan Lauc



PII: S0304-4165(18)30186-7
DOI: doi:[10.1016/j.bbagen.2018.07.003](https://doi.org/10.1016/j.bbagen.2018.07.003)
Reference: BBAGEN 29152
To appear in: *BBA - General Subjects*
Received date: 20 March 2018
Revised date: 3 July 2018
Accepted date: 4 July 2018

Please cite this article as: Irena Trbojević-Akmačić, Frano Vučković, Marija Vilaj, Andrea Skelin, Lennart C. Karssen, Jasminka Krištić, Julija Jurić, Ana Momčilović, Jelena Šimunović, Massimo Mangino, Manuela De Gregori, Maurizio Marchesini, Concetta Dagostino, Jerko Štambuk, Mislav Novokmet, Richard Rauck, Yurii S. Aulchenko, Dragan Primorac, Leonardo Kapural, Klaas Buyse, Dieter Mesotten, Frances M.K. Williams, Jan van Zundert, Massimo Allegri, Gordan Lauc , Plasma N-glycome composition associates with chronic low back pain. *Bbagen* (2018), doi:[10.1016/j.bbagen.2018.07.003](https://doi.org/10.1016/j.bbagen.2018.07.003)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Irena Trbojević-Akmačić^a, Frano Vučković^a, Marija Vilaj^a, Andrea Skelin^a, Lennart C. Karssen^b, Jasminka Krištić^a, Julija Jurić^a, Ana Momčilović^{a,c}, Jelena Šimunović^a, Massimo Mangino^{d,e}, Manuela De Gregori^{f,g}, Maurizio Marchesini^{g,h}, Concetta Dagostino^{g,i}, Jerko Štambuk^a, Mislav Novokmet^a, Richard Rauck^j, Yurii S. Aulchenko^b, Dragan Primorac^{k,l,m,n,o}, Leonardo Kapural^j, Klaas Buyse^p, Dieter Mesotten^p, Frances M. K. Williams^d, Jan van Zundert^p, Massimo Allegri^{g,r}, Gordan Lauc^{a,s}

^aGenos Glycoscience Research Laboratory, Zagreb, Croatia (iakmacic@genos.hr, fvuckovic@genos.hr, mvilaj@genos.hr, askelin@genos.hr, jkristic@genos.hr, jjuric@genos.hr, jsimunovic@genos.hr, jstambuk@genos.hr, mnovokmet@genos.hr)

^bPolyOmica, Het Vlaggeschip 61, 5237 PA 's-Hertogenbosch, The Netherlands
(l.c.karssen@polyomica.com, y.s.aulchenko@polyomica.com)

^cThe Glycomics and Glycoproteomics group, Center for Proteomics and Metabolomics, Leiden University Medical Center, The Netherlands (a.momcilovic@lumc.nl)

^dDepartment of Twin Research and Genetic Epidemiology, King's College London, London, UK
(massimo.mangino@kcl.ac.uk, frances.williams@kcl.ac.uk)

^eNIHR Biomedical Research Centre at Guy's and St Thomas' Foundation Trust, London SE1 9RT, UK

^fPain Therapy Service, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
(manuela.degregori@unipv.it)

^gSIMPAR Group, Italy (mmarchesini@parmanesthesia.com, cdagostino@parmanesthesia.com, massimo.allegri@unipr.it)

^hAnesthesia, Intensive Care and Pain Service, Parma Hospital, Parma, Italy

ⁱDepartment of Medicine and Surgery, University of Parma, Parma, Italy

^jCarolinas Pain Institute, Winston Salem, North Carolina, USA (rrauck@ccrpain.com, lkapural@ccrpain.com)

^kSt. Catherine Specialty Hospital, Zabok/Zagreb, Croatia (draganprimorac2@gmail.com)

^lJ J Strossmayer University of Osijek, School of medicine, Osijek, Croatia

^mUniversity of Split, School of Medicine, Split, Croatia

ⁿEberly College of Science, The Pennsylvania State University, University Park

^oChildren's Hospital Srebrnjak, Zagreb, Croatia

^pDepartment of anesthesiology and multidisciplinary pain center, Ziekenhuis Oost-Limburg, Genk, Belgium

(k.buysse@zol.be, d.mesotten@zol.be, jan.vanzundert@zol.be)

^rAnesthesia and intensive care service - IRCCS MultiMedica Hospital, Sesto San Giovanni, Milano, Italy

^sUniversity of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia (glauc@pharma.hr)

* To whom correspondence should be addressed:

Prof. Gordan Lauc

University of Zagreb

Faculty of Pharmacy and Biochemistry

A. Kovačića 1

10 000 Zagreb, Croatia

Phone: +385 1 639 4467

Fax: +385 1 639 4400

E-mail: glauc@pharma.hr

Abstract

BACKGROUND: Low back pain (LBP) is the symptom of a group of syndromes with heterogeneous underlying mechanisms and molecular pathologies, making treatment selection and patient prognosis very challenging. Moreover, symptoms and prognosis of LBP are influenced by age, gender, occupation, habits, and psychological factors. LBP may be characterized by an underlying inflammatory process. Previous studies indicated a connection between inflammatory response and total plasma N-glycosylation. We wanted to identify potential changes in total plasma N-glycosylation pattern connected with chronic low back pain (CLBP), which could give an insight into the pathogenic mechanisms of the disease.

METHODS: Plasma samples of 1128 CLBP patients and 760 healthy controls were collected in clinical centers in Italy, Belgium and Croatia and used for N-glycosylation profiling by hydrophilic interaction ultra-performance liquid chromatography (HILIC-UPLC) after N-glycans release, fluorescent labeling and clean-up. Observed N-glycosylation profiles have been compared with a cohort of 126 patients with acute inflammation that underwent abdominal surgery.

RESULTS: We have found a statistically significant increase in the relative amount of high-branched (tri-antennary and tetra-antennary) N-glycan structures on CLBP patients' plasma glycoproteins compared to healthy controls. Furthermore, relative amounts of disialylated and trisialylated glycan structures were increased, while high-mannose and glycans containing bisecting *N*-acetylglucosamine decreased in CLBP.

CONCLUSIONS: Observed changes in CLBP on the plasma N-glycome level are consistent with N-glycosylation changes usually seen in chronic inflammation.

GENERAL SIGNIFICANCE: To our knowledge, this is a first large clinical study on CLBP patients and plasma N-glycome providing a new glycomics perspective on potential disease pathology.

Keywords: glycan biomarker; low back pain; plasma N-glycosylation; retrospective study

Abbreviations

% Area: percentage of total integrated area of the chromatogram; 2-AB: 2-aminobenzamide; 2-PB: -picoline borane; AbSur: cohort of patients with acute inflammation that had abdominal surgery; ACE: automatic chromatogram extraction; ADCC: antibody-dependent cell-mediated cytotoxicity; ANCOVA: analysis of covariance; B - N-glycans that contain bisecting *N*-acetylglucosamine, BEL: samples from clinical centers in Belgium; CLBP: chronic low back pain; CRO: samples from clinical centers in Croatia; DMSO: dimethyl sulfoxide; FA: formic acid; FDR: false discovery rate; G0: agalactosylated N-glycans; G1: monogalactosylated N-glycans; G2: digalactosylated N-glycans; G3: trigalactosylated N-glycans; G4: tetragalactosylated N-glycans; GlcNAc: *N*-acetylglucosamine; GPs: glycan peaks; GU: glucose unit; HB - highly branched N-glycans; HILIC-SPE: hydrophilic interaction liquid chromatography solid phase extraction; HILIC-UPLC: hydrophilic interaction ultra-performance liquid chromatography; HM - high-mannose N-glycans; IgG: immunoglobulin G; ITA: samples from clinical centers in Italy; LB: low branched N-glycans; LBP: low back pain; LDD: lumbar disc degeneration; META: meta-analysis; MS: mass spectrometry; PBS: phosphate buffered saline; S0: neutral/not sialylated N-glycans; S1: monosialylated N-glycans; S2: disialylated N-glycans; S3: trisialylated N-glycans; S4: tetrasialylated N-glycans; SDS: sodium dodecyl sulfate; UPLC: ultra-performance liquid chromatography

1. Introduction

Low back pain (LBP) includes several mixed pain syndromes being one of the most common health problems in the world. Almost every adult has or will experience an episode of LBP and it is the number one cause of years lived with disability [1]. Prevalence of several frequently overlapping sources of LBP include sacroilitis 25-32 % [2,3], lumbal spondylosis 36-42 % [4–6], spinal stenosis, or lumbar radiculitis/radiculopathy up to 30 % [7,8]. When muscle tension, stiffness or pain are localized above the inferior gluteal folds and below the coastal margin, with or without leg pain, and last for three months or more, the condition is classified as chronic low back pain (CLBP). This is a group of syndromes with heterogeneous variable underlying mechanisms and molecular pathologies that correlate poorly with spine imaging. Additionally, symptoms and prognosis are influenced by many factors (gender [9], age [10], occupation, habits [11]), as well as physiological [12] and psychological factors [13,14], making managing the condition very challenging. The success of interventional techniques, such as epidural steroid injection, facet joints denervation, spinal cord stimulation or laminectomy, depends largely on the specific physiopathology of each patient. However, currently there is little evidence based approach to this disease, as biomarkers and prognostic factors practically do not exist.

In complex organisms, glycans (sugar moieties covalently attached to proteins) play an important role in virtually all processes that involve more than one cell [15]. Nearly all membrane and secreted proteins are modified by glycans with variable site occupancy and glycan composition [16,17]. Absence of N-glycosylation is embryonically lethal [18] and mutations that obstruct proper glycosylation cause debilitating diseases [19]. Inter-individual differences in N-glycosylation are associated with predisposition for and course of different diseases [20]. This is not surprising since the glycan parts of (glyco)proteins are integral elements of the final molecular structure, and together with amino acids in the polypeptide backbone, form a single molecular entity that performs biological functions. For example, glycans attached to immunoglobulin G (IgG) have very profound effects on protein structure and can convert IgG from a pro-inflammatory to an anti-inflammatory mode [21]. Alternative glycosylation (attachment of different glycans) affects binding of IgG to all Fc receptors and is in this way analogous to variation in protein sequence due to genetic variations [22].

Systemic and/or local inflammatory processes are believed to be an underlying mechanism in at least a subgroup of LBP patients, although this is not generally recognized. Previous studies indicated that inflammatory response associates with changes in total plasma N-glycosylation [23], as well as N-glycosylation of individual serum glycoproteins [24]. Since glycans affect not only protein structure, but also its function, a number of studies have shown that changes in glycosylation are implicated in the pathology of different diseases [25–28].

Glycosylation of individual glycoprotein, IgG, has already been studied in the context of LBP in a large study of 4511 twins from the TwinsUK database [29]. IgG glycans with core fucose (have lower antibody-dependent cell-mediated cytotoxicity, ADCC) and IgG glycans without core fucose (have higher ADCC activity) have been found to correlate with LBP, suggesting the involvement of ADCC and inflammation in LBP pathogenesis. However, there was no correlation found between lumbar disc degeneration (LDD) scores and IgG glycans, implicating different pathogenic mechanisms for specific LBP subtypes and supporting the role of glycans in inflammation-related LBP subtypes.

Considering previous research on inflammatory response association with changes in total plasma N-glycosylation [23], here, we studied the total plasma N-glycome in CLBP patients and controls in order to identify if there is an association between the N-glycosylation pattern on the level of total plasma glycoproteins and risk of CLBP condition. Specific pattern of plasma N-glycosylation could be used as a biomarker of CLBP, and potential indicator of both pathogenic mechanisms and therapy efficiency in future prospective and longitudinal studies.

2. Methods

2.1. Study approval

The study was approved by the ethical committees of the participating clinical centres between December 2013 and March 2014 and written informed consent was obtained from each participant prior to inclusion in the study. Participant samples have been de-identified by code. The study has been registered on clinicaltrials.gov (NCT02037789).

2.2. Clinical samples and data

Retrospective plasma samples of CLBP patients and healthy controls (people without acute or CLBP) were collected in several clinical centers in Italy, Belgium and Croatia that are involved in PainOmics project, following previously established and validated protocol for blood sample collection, storage and shipping [30,31] (Table 1). In short, blood for glycomics analysis has been collected into EDTA containing vacutainer tube and mixed well for balanced clotting. Tube was left at room temperature for 1 hour and plasma was separated by centrifugation at 1620 g for 10 minutes, transferred to a clean tube and centrifuged again at 2700 g for 10 minutes. Volume of 1 mL of plasma after second centrifugation step has been transferred to a clean tube and stored at -80 °C or -20 °C before further processing. CLBP patients were defined according to previously published criteria [31]: all Caucasian adult patients, referred to participant pain clinics, with pain localized to the column between the costal margins and gluteal fold, with or without symptoms into one or both legs, that was lasting more than 12 weeks. We excluded all patients with an acute episode of LBP, any history of spine tumor or infection or recent (less than 12 months) vertebral fractures, or psychiatric disorders. All patients have been classified into 6 major groups: spinal stenosis, discogenic pain, facet joint pain, sacroiliac joint pain, low back pain associated to radicular pain (radicular pain not predominant) and wide-spread LBP. Furthermore, all patients' pain characteristics (intensity and type of pain) have been evaluated with painDETECT [32].

Plasma samples from cohort of patients with acute inflammation that underwent abdominal surgery (AbSur) were collected in several clinical centers in Italy as described earlier [33,34]. Blood samples of 126 patients have been taken in several time points - intraoperatively and 6, 12, 24, and 48 hours after the

surgery. Samples taken intraoperatively and 48 hours after abdominal surgery have been considered as “Case” or “Control”, respectively. In short, blood for glycomics analysis has been collected into EDTA containing vacutainer tube, mixed well for balanced clotting and plasma was separated by centrifugation at room temperature. Samples have been stored at $-20\text{ }^{\circ}\text{C}$ before further processing.

2.3. Deglycosylation of plasma samples

All plasma samples and in-house plasma standards were vortexed after thawing and centrifuged for 3 minutes at $12\ 100\ g$. Each sample ($10\ \mu\text{L}$) was aliquoted to $1\ \text{mL}$ 96-well collection plates (Waters, Milford, MA, USA) following a predetermined experimental design, which was blocked on case-control status, sex and age information (each 96-well plate represents the whole population in terms of case-control status, sex and age distribution), while the rest was randomized. In-house plasma standards were aliquoted in four to five replicates per plate, to control for batch effects, that is plate-to-plate variation.

Plasma proteins were denatured by adding $20\ \mu\text{L}$ of $2\ \%$ (w/v) sodium dodecyl sulfate (SDS, Invitrogen, Carlsbad, CA, USA) and sample incubation at $65\text{ }^{\circ}\text{C}$ for 10 minutes. After cooling to room temperature for 30 minutes, $10\ \mu\text{L}$ of $4\ \%$ (v/v) Igepal CA-630 (Sigma-Aldrich, St. Louis, MO, USA) was added to each sample and the mixture was shaken for 15 minutes at room temperature on a plate shaker. Plasma proteins were deglycosylated by incubation at $37\text{ }^{\circ}\text{C}$ for 18 hours after $1.2\ \text{U}$ of PNGase F ($10\ \text{U}\ \mu\text{L}^{-1}$, Promega, Madison, WI, USA) in $10\ \mu\text{L}$ of $5\times\ \text{PBS}$ was added to each sample. Buffer $5\times\ \text{PBS}$ was made in house: $685\ \text{mmol}\ \text{L}^{-1}\ \text{NaCl}$ (Carlo Erba, Peypin, Italy), $13.5\ \text{mmol}\ \text{L}^{-1}\ \text{Na}_2\text{HPO}_4$ (Acros Organics, Thermo Fisher Scientific, Geel, Belgium), $48.5\ \text{mmol}\ \text{L}^{-1}\ \text{KH}_2\text{PO}_4$ (Sigma-Aldrich), $11\ \text{mmol}\ \text{L}^{-1}\ \text{KCl}$ (Gram-Mol, Zagreb, Croatia), and filtered through $0.2\ \mu\text{m}$ Supor PES filters (Nalgene Thermo Fischer Scientific)..

2.4. Fluorescent labeling and clean-up of released plasma N-glycans

Released plasma N-glycans were subsequently labeled with a fluorescent dye 2-aminobenzamide (2-AB, Sigma-Aldrich). The labeling mixture was freshly prepared by dissolving $0.48\ \text{mg}$ of 2-AB and $1.12\ \text{mg}$ of 2-picoline borane (2-PB, Sigma-Aldrich) in $25\ \mu\text{L}$ of dimethyl sulfoxide (DMSO, Sigma-Aldrich) and glacial acetic acid (Merck, Darmstadt, Germany) ($7:3$, v/v), per sample. Labeling mixture was added to each

sample and the plate was sealed with an adhesive seal. Samples were mixed for 10 minutes at room temperature, and incubated at 65 °C for 2 hours for the labeling reaction to take place.

Released and fluorescently labeled plasma N-glycans were cleaned of excess of reagents and proteins by previously developed and optimized hydrophilic interaction liquid chromatography solid phase extraction (HILIC-SPE) procedure [35]. After labelling, reaction samples were cooled down to room temperature for 30 minutes and 700 µL of cold (4 °C) acetonitrile (Sigma-Aldrich) was added to each sample and gently mixed. The clean-up procedure was performed on a hydrophilic 0.2 µm AcroPrep GHP filter plate (Pall Corporation, Ann Arbor, MI, USA) using a vacuum manifold (Pall Corporation) at around 25 mm Hg. All wells of a GHP filter plate were prewashed with 200 µL of 70 % ethanol in water, 200 µL of ultra-pure water and 200 µL of 96 % cold (4 °C) acetonitrile in water. The samples diluted with cold acetonitrile were loaded to the wells, incubated on a filter for 2 minutes and subsequently washed with 5× 200 µL of 96 % cold (4 °C) acetonitrile in water. Glycans were eluted from a filter plate with 2× 90 µL of ultra-pure water after 15 minutes of shaking at room temperature and centrifugation at 164 g in each step. Combined eluates were stored at -20 °C until the UPLC analysis.

2.5. Plasma N-glycan analysis by HILIC-UPLC

Fluorescently labelled and purified plasma N-glycans were prepared in 75 % acetonitrile (v/v) and analyzed by HILIC-UPLC on an Acquity UPLC instrument (Waters) consisting of a quaternary solvent manager, sample manager and a fluorescence detector. The 2-AB labeled N-glycans were separated on a Waters BEH Glycan chromatography column, 150 × 2.1 mm i.d., 1.7 µm BEH particles, in a linear gradient of 30–47 % 100 mmol L⁻¹ ammonium formate, pH 4.4 (solvent A) and acetonitrile (solvent B) at flow rate of 0.56 mL min⁻¹ and a 23 minutes analytical run. Samples were maintained at 10 °C before injection into the column, the column temperature was 25 °C and separated glycans were detected at excitation wavelengths of 250 nm and emission wavelength of 428 nm. The UPLC system was under the control of the Empower 3 software, build 3471 (Waters). Chromatograms were processed using an automatic processing method and manually corrected to maintain the same intervals of integration across all samples, or processed with a recently developed semi-supervised Automatic Chromatogram Extraction (ACE) method [36] for automated

alignment and detection of glycan peaks in chromatograms. The plasma N-glycan samples were all separated into 39 glycan peaks (GPs) and the amount of glycans in each chromatographic peak was expressed as percentage of total integrated area (% Area). To each detected chromatographic peak a glucose unit (GU) value was assigned according to external standard of hydrolysed and 2-AB labelled glucose oligomers (dextran ladder) which was run with every analytical UPLC batch [37].

2.5.1 Confirmation of the HILIC-UPLC plasma glycome profile

The major glycan present in each of the reported 39 chromatographic peaks was annotated according to GU values, measured m/z value, recorded fragmentation spectra where applicable and according to previously published and annotated serum N-glycome profile [38] (Supplementary Table 1). Several representative samples from control and patient groups were selected and analysed by HILIC-UPLC, as described in previous section, additionally hyphenated to the Bruker compact Q-ToF mass spectrometer (MS), both controlled using HyStar software (Bruker, Bremen, Germany) version 4.1. UPLC was coupled to MS via Ion Booster (Bruker) ion source with additional flow of 100 $\mu\text{L min}^{-1}$ of acetonitrile introduced into analytical flow using a t-piece. Capillary voltage was set to 2250 V with nebulizing gas at pressure of 5.5 Bar. Drying gas was applied to source at a flow rate of 4 L min^{-1} and temperature of 100 °C, while vaporiser temperature was set to 220 °C and flow rate of 5 L min^{-1} . Nitrogen was used as a source gas, while argon was used as collision gas. Ion energy was set to 5 eV, transfer time was 100 μs . Spectra were recorded in mass range from 50 m/z to 4000 m/z at a rate of 0.5 Hz. For MS acquisition collision energy was set to 4 eV and Rf 4200 Vpp. Fragment spectra were acquired using auto MS/MS mode using a precursor ion list of detected m/z values corresponding to free 2-AB labelled plasma glycans (Supplementary Table 1). Active exclusion was set to one minute after three spectra and number of precursor ions was 1. All recorded spectra were analysed using Bruker Data Analysis software version 4.4 (Bruker). Glycan compositions and certain structural features were determined using GlycoMod [39] and GlycoWorkbench [40].

2.6. Statistics

In order to remove experimental variation from the measurements, normalization and batch correction were performed on the UPLC glycan data. To make measurements across samples comparable, normalization by total area was performed where the peak area of each of the 39 glycan containing peaks was divided by total area of the corresponding chromatogram. Prior to batch correction, normalized glycan measurements were log-transformed due to right-skewness of their distributions and the multiplicative nature of batch effects. Batch correction was performed on log-transformed measurements using the ComBat method (R package “svay3.6.0”) [41], where the technical source of variation (which sample was analyzed on which plate) was modeled as batch covariate. To correct the measurements for experimental noise, estimated batch effects were subtracted from log-transformed measurements. In addition to the 39 directly measured glycan containing peaks, 14 derived traits were calculated from the directly measured glycans (Table 2). These derived traits average particular glycosylation features across different individual glycan structures and consequently they are more closely related to individual enzymatic activities and underlying genetic polymorphisms.

Association analyses between disease status and glycan traits were performed using an analysis of covariance (ANCOVA) model with age and gender included as covariates. Analyses were first performed for each cohort separately and then combined using inverse-variance weighted meta-analysis (META, R package “metaphor v1.9-4”) [42]. Prior to the analyses, the glycan variables were all transformed to the standard Normal distribution (mean = 0, sd = 1) by inverse transformation of ranks to Normality (R package “GenABELv1.8-0”, function `rtransform`) [43]. Using rank transformed variables in analyses makes estimated effects of different glycans in different cohorts comparable as transformed glycan variables have the same standardized variance. In case-control regression analysis, coefficients of binary predictors refer to a change in a glycan variable between 2 classes of binary predictors expressed in SDs. The false discovery rate (FDR) was controlled using the Benjamini-Hochberg procedure, and p-values corrected for multiple testing (with FDR cutoff set at 0.05) are shown throughout.

Data was analyzed and visualized using R programming language (version 3.0.1).

3. Results

3.1. Glycosylation changes in three cohorts

Patients and healthy volunteers were enrolled in the study through clinical centers in Italy (ITA), Belgium (BEL) and Croatia (CRO). Total plasma N-glycosylation was analysed by ultra-performance liquid chromatography (UPLC) (Figure 1) in 1128 CLBP patients and 760 healthy controls (Table 1). This type of analysis represents the first step in a biomarker discovery – a snapshot of the differences in N-glycosylation pattern between CLBP patients and controls

We observed statistically significant differences in relative abundance of several major structurally similar glycan groups represented as derived glycosylation traits (Figure 2, Table 2 and 3) between CLBP patients and healthy controls of the ITA and BEL cohorts. A similar trend was also seen in the CRO cohort, although not significant after correction for multiple comparisons. (Figure 2, Table 3). All glycan groups (derived glycosylation traits) that were shown to be significantly changed change in the same direction (column “effect” in Table 3) regardless of the clinical center of study participants’ enrolment, demonstrating consistent changes of total plasma N-glycosylation in patients with CLBP compared to controls.

On the other hand, some differences in relative abundance of several derived glycosylation traits can be seen between controls enrolled in different clinical centers and the same is observed for patients (Figure 2). Since extreme care has been taken to standardize all steps of the study [30] – from blood sample collection to sample processing, storage and shipping, these changes can probably be attributed to differences in populations (e.g. different genetic and environment background), which are known to affect plasma glycome composition [44].

3.2. Changes in derived N-glycan structural features between CLBP and controls

The relative area of chromatographic peaks corresponding to highly branched – denoted as “HB” in Table 3 – glycan structures is increased (and, concomitantly, the % Area of chromatographic peaks corresponding to glycan structures with low branching – denoted as “LB” in Table 3 – is decreased) in CLBP patients compared to controls, which is consistent with previous findings of increased branching in chronic inflammation [45–47].

Interestingly, while the % Area of chromatographic peaks corresponding to monosialylated (S1) and tetrasialylated (S4) glycan structures were similar or lower in CLBP patients compared to controls, the percentage areas of disialylated (S2) and trisialylated (S3) glycan structures were increased in CLBP patients compared to controls (Table 3, Figure 2).

Unsurprisingly, changes in galactosylation levels were similar to sialylation, reflecting the fact that galactose is a prerequisite for glycan sialylation. A different trend can only be observed in monogalactosylated (G1) structures, which are lower in CLBP patients compared to controls, while monosialylated (S1) structures are similar or slightly lower in patients compared to controls.

High-mannose glycans (HM) and glycans that contain bisecting *N*-acetylglucosamine (B) have shown the same pattern of change between CLBP patients and controls (Figure 2). This decrease in % Areas of chromatographic peaks corresponding to HM and B glycan structures in CLBP patients compared to controls was statistically significant in ITA and CRO cohorts, but did not reach the statistical significance in BEL cohort (Table 3).

3.3. Glycosylation in CLBP and inflammation

Since similar changes in the glycome were observed in a previously analyzed AbSur cohort, we have compared total plasma N-glycosylation changes from AbSur study with the total plasma N-glycosylation changes in CLBP study (Figure 2). For the majority of derived glycosylation traits the changes in the CLBP patients were in the same direction as in patients with acute inflammation compared to controls, but the extent of the change was somewhat smaller in CLBP. This finding indicates that CLBP is associated with systemic inflammation in at least a subgroup of patients. However, due to the cross-sectional nature of this study (plasma samples collected after CLBP development), we could not investigate longitudinal changes of plasma N-glycome through CLBP condition development and progression, and whether a changed N-glycomic profile during inflammation is a pre-requisite for CLBP condition development, or merely a consequence of CLBP progression.

4. Discussion

Phenotypic diversity caused by differences in glycosylation has not been investigated to a large extent. We pioneered high-throughput glycomics screening of human populations in 2009 [48] and since then analysed over 50,000 individual glycomes through numerous clinical and epidemiological studies. Our large-scale glycomics studies revealed very high inter-individual variability of both, the plasma N-glycome [44] and the IgG glycome [49]. However, within an individual, the composition of the N-glycome was found to have significant short-term (in the order of weeks) stability in homeostatic conditions [50].

In a previous study [29] we found, in a large cohort of twins, a correlation between LBP and IgG glycan modules featuring glycans that either promote or block ADCC. On the other hand, we have not found any correlation between LDD and glycan structures suggesting that inflammation is not connected with this specific subtype of LBP. Although in this study we explored one aspect of LBP, complex pathophysiology of this condition still remains poorly understood and biomarker potential of N-glycome not yet defined. Additionally, a genome wide association study of LBP in over 400,000 individuals revealed only three replicable associations between genetic variants (not related explicitly to glycosylation) and LBP, indicating that genetics has very limited potential for stratification of LBP patients (manuscript in submission).

Therefore, the disease mechanisms underlying the syndrome of CLBP remain incompletely understood, making patient prognosis and therapy challenging. Previous studies implicated protein glycosylation as one of the players involved in inflammation [29]. To deepen our understanding of CLBP pathology and potentially improve therapy efficiency on an individual patient basis, we explored changes in total plasma N-glycosylation in 1888 healthy individuals and CLBP patients in an international multi-center retrospective clinical study.

We found statistically significant differences in plasma N-glycosylation between patients and matching (by age, gender and clinical centre) healthy controls consistent with changes in glycosylation previously reported for chronic inflammation – an increase in the level of high-branched glycan structures [45–47]. Increase in N-glycan branching is a result of *N*-acetylglucosaminyl (GlcNAc) transferase IV (transfers β 1,4-linked GlcNAc to tri-mannosyl core creating a third antenna) and GlcNAc transferase V (transfers β 1,6-linked GlcNAc to the tri-mannosyl core creating a fourth antenna) function [51]. One of the

plasma glycoproteins that contributes to majority of tri-antennary and tetra-antennary sialylated (highly branched) glycans is α -1-acid glycoprotein [52], which is an acute phase protein whose concentration significantly increases as a response to inflammatory stimuli. Interestingly, it has been shown that the relative abundance of total bi-antennary glycans and α 1,3-fucosylated bi-, tri- and tetra-antennary α -1-acid glycoprotein glycan structures are increasing in acute and chronic inflammation compared to healthy individuals, while the relative abundance of total tri-antennary and total tetra-antennary glycans are decreasing [53]. Fucose bound in an α 1,3 linkage to the antenna is part of a sialyl Lewis X (SLe^x) structure that consists of a terminal α 2,3-sialic acid residue linked to galactose that is attached to GlcNAc. Increase in SLe^x structures is another previously identified signature of chronic inflammation [54].

Increased levels of glycans with bisecting GlcNAc has been previously connected with inflammation, mostly on the level of IgG [55]. On the other hand, it is known that presence of bisecting GlcNAc during glycan synthesis prevents formation of tri-antennary and tetra-antennary glycan structures, causing a decrease in glycan branching [51]. Since the relative amount of high-branched glycan structures on the level of total plasma glycoproteins was increased in CLBP patients compared to controls, it is not surprising that glycans containing bisecting GlcNAc were decreased in CLBP.

Although high-mannose glycans are usually not very abundant in secreted proteins relative to other glycan types and are considered to be incomplete products of the N-glycosylation process, its importance via interactions with mannose receptors on macrophages and dendritic cells as part of the innate immune response is well recognized [56]. Additionally it has been shown that high-mannose glycans increase serum clearance of IgG [57] and can initiate complement pathway by binding to mannose-binding lectin [58].

Observed increase in disialylated and trisialylated glycan structures in CLBP patients potentially indicate an increase in relative abundance of these specific glycan structures or an amount of specific glycoproteins carrying disialylated and trisialylated glycans, instead of a systematic increase in level of total plasma glycoproteins sialylation. These targeted changes in disialylated and trisialylated glycan structures in CLBP are a novel finding.

To the best of our knowledge, this is a first large clinical study on CLBP patients and healthy individuals providing a total plasma N-glycomics perspective and a potential biomarker for this phenotype.

The next step would be population stratification according to their inflammatory response. We are currently evaluating patients with acute LBP observing if they develop or do not develop chronic pain. With this prospective study we plan to go a step further and evaluate a biomarker potential of glycome in acute systemic inflammation.

ACCEPTED MANUSCRIPT

Author contributions

MM, RR, DP, LK, FMKW, JvZ, MA, and GL designed the research study. AS, MDG, MM, CD, KB and DM acquired samples and research study participants' data. IT-A, MV, AS, JK, JJ, AM, JŠ, JŠ and MN performed the experiments. FV, LCK, and YSA performed quality control and analyzed the data. IT-A, FV, and GL drafted the manuscript and all authors edited the final version of the manuscript.

Acknowledgements

This research was supported by the European Commission FP7 "PainOmics" project (contract No. 602736), as well as funding from the European Structural and Investments funds for project "Croatian National Centre of Research Excellence in Personalized Healthcare" (contract No. KK.01.1.1.01.0010). AM and JŠ are part of the "GlyCoCan" project, which is funded from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 676421.

We would like to thank all participants enrolled in this study through clinical centers in Italy (PainTherapy Service, Fondazione IRCCS Policlinico San Matteo, Pavia; and Anesthesia, Critical Care and Pain Medicine Unit, Division of Surgical Sciences, Department of Medicine and Surgery, University of Parma, Parma), Belgium (Department of anesthesiology and multidisciplinary pain center, Ziekenhuis Oost-Limburg, Genk, Belgium), and Croatia (St. Catherine Specialty Hospital, Zabok/Zagreb, Croatia).

Conflict of interest statement

G. Lauc is the founder and owner of Genos Ltd, a private research organization that specializes in high-throughput glycomic analysis and has several patents in this field. I. Trbojević-Akmačić, F. Vučković, M. Vilaj, A. Skelin, J. Krištić, J. Jurić, A. Momčilović, J. Šimunović, J. Štambuk, M. Novokmet are employees of Genos Ltd. Y. S. Aulchenko and L. C. Karssen are owners of Maatschap PolyOmica, a private organization providing services and doing research and development in the field of computational and statistical (gen)omics. All other authors declare no conflicts of interest.

FIGURE LEGENDS

Figure 1. A) Representative plasma N-glycans chromatographic profile. Glycans labeled with 2-aminobenzamide have been separated by ultra-performance liquid chromatography (UPLC) based on hydrophilic interactions (HILIC). Major glycan structures are shown for each glycan containing chromatographic peak (GP1–GP39). **B) Different plasma N-glycosylation profiles of a healthy individual (Control) and a chronic low back pain patient (Case).** Chromatographic profiles have been overlaid and scaled to the highest peak.

Figure 2. Changes in total plasma N-glycosylation in chronic low back pain (CLBP). Relative abundance (%Area, the percentage of total plasma N-glycan pool) of major plasma N-glycan groups (derived traits, cf. Table 2) between healthy individuals (Control) and chronic low back pain patients (Case) collected in three clinical centers in Italy (ITA), Belgium (BEL) and Croatia (CRO), as well as comparison with patients that underwent abdominal surgery (AbSur) has been shown. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.

References

- [1] T. Vos, R.M. Barber, B. Bell, A. Bertozzi-Villa, S. Biryukov, I. Bolliger, F. Charlson, A. Davis, L. Degenhardt, D. Dicker, L. Duan, H. Erskine, V.L. Feigin, A.J. Ferrari, C. Fitzmaurice, T. Fleming, N. Graetz, C. Guinovart, J. Haagsma, G.M. Hansen, S.W. Hanson, K.R. Heuton, H. Higashi, N. Kassebaum, H. Kyu, E. Laurie, X. Liang, K. Lofgren, R. Lozano, M.F. MacIntyre, M. Moradi-Lakeh, M. Naghavi, G. Nguyen, S. Odell, K. Ortblad, D.A. Roberts, G.A. Roth, L. Sandar, P.T. Serina, J.D. Stanaway, C. Steiner, B. Thomas, S.E. Vollset, H. Whiteford, T.M. Wolock, P. Ye, M. Zhou, M.A. Ávila, G.M. Aasvang, C. Abbafati, A.A. Ozgoren, F. Abd-Allah, M.I.A. Aziz, S.F. Abera, V. Aboyans, J.P. Abraham, B. Abraham, I. Abubakar, L.J. Abu-Raddad, N.M.E. Abu-Rmeileh, T.C. Aburto, T. Achoki, I.N. Ackerman, A. Adelekan, Z. Ademi, A.K. Adou, J.C. Adsuar, J. Arnlov, E.E. Agardh, M.J. Al Khabouri, S.S. Alam, D. Alasfoor, M.I. Albittar, M.A. Alegretti, A. V. Aleman, Z.A. Alemu, R. Alfonso-Cristancho, S. Alhabib, R. Ali, F. Alla, P. Allebeck, P.J. Allen, M.A. AlMazroa, U. Alsharif, E. Alvarez, N. Alvis-Guzman, O. Ameli, H. Amini, W. Ammar, B.O. Anderson, H.R. Anderson, C.A.T. Antonio, P. Anwari, H. Apfel, V.S.A. Arsenijevic, A. Artaman, R.J. Asghar, R. Assadi, L.S. Atkins, C. Atkinson, A. Badawi, M.C. Bahit, T. Bakfalouni, K. Balakrishnan, S. Balalla, A. Banerjee, S.L. Barker-Collo, S. Barquera, L. Barregard, L.H. Barrero, S. Basu, A. Basu, A. Baxter, J. Beardsley, N. Bedi, E. Beghi, T. Bekele, M.L. Bell, C. Benjet, D.A. Bennett, I.M. Bensenor, H. Benzian, E. Bernabe, T.J. Beyene, N. Bhala, A. Bhalla, Z. Bhutta, K. Bienhoff, B. Bikbov, A. Bin Abdulhak, J.D. Blore, F.M. Blyth, M.A. Bohensky, B.B. Basara, G. Borges, N.M. Bornstein, D. Bose, S. Boufous, R.R. Bourne, L.N. Boyers, M. Brainin, M. Brauer, C.E.G. Brayne, A. Brazinova, N.J.K. Breitborde, H. Brenner, A.D.M. Briggs, P.M. Brooks, J. Brown, T.S. Brugha, R. Buchbinder, G.C. Buckle, G. Bukhman, A.G. Bulloch, M. Burch, R. Burnett, R. Cardenas, N.L. Cabral, I.R. Campos-Nonato, J.C. Campuzano, J.R. Carapetis, D.O. Carpenter, V. Caso, C.A. Castaneda-Orjuela, F. Catala-Lopez, V.K. Chadha, J.C. Chang, H. Chen, W. Chen, P.P. Chiang, O. Chimed-Ochir, R. Chowdhury, H. Christensen, C.A. Christophi, S.S. Chugh, M. Cirillo, M. Coggeshall, A. Cohen, V. Colistro, S.M. Colquhoun, A.G. Contreras, L.T. Cooper, C. Cooper, K. Cooperrider, J. Coresh, M. Cortinovis, M.H. Criqui, J.A. Crump, L. Cuevas-Nasu, R. Dandona, L. Dandona, E. Dansereau, H.G.

Dantes, P.I. Dargan, G. Davey, D. V. Davitoiu, A. Dayama, V. De La Cruz-Gongora, S.F. De La Vega, D. De Leo, B. Del Pozo-Cruz, R.P. Dellavalle, K. Deribe, S. Derrett, D.C. Des Jarlais, M. Dessalegn, G.A. DeVeber, S.D. Dharmaratne, C. Diaz-Torne, E.L. Ding, K. Dokova, E.R. Dorsey, T.R. Driscoll, H. Duber, A.M. Durrani, K.M. Edmond, R.G. Ellenbogen, M. Endres, S.P. Ermakov, B. Eshrati, A. Esteghamati, K. Estep, S. Fahimi, F. Farzadfar, D.F.J. Fay, D.T. Felson, S.M. Fereshtehnejad, J.G. Fernandes, C.P. Ferri, A. Flaxman, N. Foigt, K.J. Foreman, F.G.R. Fowkes, R.C. Franklin, T. Furst, N.D. Futran, B.J. Gabbe, F.G. Gankpe, F.A. Garcia-Guerra, J.M. Geleijnse, B.D. Gessner, K.B. Gibney, R.F. Gillum, I.A. Ginawi, M. Giroud, G. Giussani, S. Goenka, K. Goginashvili, P. Gona, T.G. De Cosio, R.A. Gosselin, C.C. Gotay, A. Goto, H.N. Gouda, R.L. Guerrant, H.C. Gughani, D. Gunnell, R. Gupta, R. Gupta, R.A. Gutierrez, N. Hafezi-Nejad, H. Hagan, Y. Halasa, R.R. Hamadeh, H. Hamavid, M. Hammami, G.J. Hankey, Y. Hao, H.L. Harb, J.M. Haro, R. Havmoeller, R.J. Hay, S. Hay, M.T. Hedayati, I.B.H. Pi, P. Heydarpour, M. Hijar, H.W. Hoek, H.J. Hoffman, J.C. Hornberger, H.D. Hosgood, M. Hossain, P.J. Hotez, D.G. Hoy, M. Hsairi, H. Hu, G. Hu, J.J. Huang, C. Huang, L. Huiart, A. Hussein, M. Iannarone, K.M. Iburg, K. Innos, M. Inoue, K.H. Jacobsen, S.K. Jassal, P. Jeemon, P.N. Jensen, V. Jha, G. Jiang, Y. Jiang, J.B. Jonas, J. Joseph, K. Juel, H. Kan, A. Karch, C. Karimkhani, G. Karthikeyan, R. Katz, A. Kaul, N. Kawakami, D.S. Kazi, A.H. Kemp, A.P. Kengne, Y.S. Khader, S.E.A.H. Khalifa, E.A. Khan, G. Khan, Y.H. Khang, I. Khonelidze, C. Kieling, D. Kim, S. Kim, R.W. Kimokoti, Y. Kinfu, J.M. Kinge, B.M. Kissela, M. Kivipelto, L. Knibbs, A.K. Knudsen, Y. Kokubo, S. Kosen, A. Kramer, M. Kravchenko, R. V. Krishnamurthi, S. Krishnaswami, B.K. Defo, B.K. Bicer, E.J. Kuipers, V.S. Kulkarni, K. Kumar, G.A. Kumar, G.F. Kwan, T. Lai, R. Lalloo, H. Lam, Q. Lan, V.C. Lansingh, H. Larson, A. Larsson, A.E.B. Lawrynowicz, J.L. Leasher, J.T. Lee, J. Leigh, R. Leung, M. Levi, B. Li, Y. Li, Y. Li, J. Liang, S. Lim, H.H. Lin, M. Lind, M.P. Lindsay, S.E. Lipshultz, S. Liu, B.K. Lloyd, S.L. Ohno, G. Logroscino, K.J. Looker, A.D. Lopez, N. Lopez-Olmedo, J. Lortet-Tieulent, P.A. Lotufo, N. Low, R.M. Lucas, R. Lunevicius, R.A. Lyons, J. Ma, S. Ma, M.T. MacKay, M. Majdan, R. Malekzadeh, C.C. Mapoma, W. Marcenes, L.M. March, C. Margono, G.B. Marks, M.B. Marzan, J.R. Masci, A.J. Mason-Jones, R.G. Matzopoulos, B.M. Mayosi, T.T. Mazorodze, N.W. McGill, J.J. McGrath, M.

McKee, A. McLain, B.J. McMahon, P.A. Meaney, M.M. Mehndiratta, F. Mejia-Rodriguez, W. Mekonnen, Y.A. Melaku, M. Meltzer, Z.A. Memish, G. Mensah, A. Meretoja, F.A. Mhimbira, R. Micha, T.R. Miller, E.J. Mills, P.B. Mitchell, C.N. Mock, T.E. Moffitt, N.M. Ibrahim, K.A. Mohammad, A.H. Mokdad, G.L. Mola, L. Monasta, M. Montico, T.J. Montine, A.R. Moore, A.E. Moran, L. Morawska, R. Mori, J. Moschandreas, W.N. Moturi, M. Moyer, D. Mozaffarian, U.O. Mueller, M. Mukaigawara, M.E. Murdoch, J. Murray, K.S. Murthy, P. Naghavi, Z. Nahas, A. Naheed, K.S. Naidoo, L. Naldi, D. Nand, V. Nangia, K.M.V. Narayan, D. Nash, C. Nejjari, S.P. Neupane, L.M. Newman, C.R. Newton, M. Ng, F.N. Ngalesoni, N.T. Nhung, M.I. Nisar, S. Nolte, O.F. Norheim, R.E. Norman, B. Norrving, L. Nyakarahuka, I.H. Oh, T. Ohkubo, S.B. Omer, J.N. Opio, A. Ortiz, J.D. Pandian, C.I.A. Panelo, C. Papachristou, E.K. Park, C.D. Parry, A.J.P. Caicedo, S.B. Patten, V.K. Paul, B.I. Pavlin, N. Pearce, L.S. Pedraza, C.A. Pellegrini, D.M. Pereira, F.P. Perez-Ruiz, N. Perico, A. Pervaiz, K. Pesudovs, C.B. Peterson, M. Petzold, M.R. Phillips, D. Phillips, B. Phillips, F.B. Piel, D. Plass, D. Poenaru, G. V. Polanczyk, S. Polinder, C.A. Pope, S. Popova, R.G. Poulton, F. Pourmalek, D. Prabhakaran, N.M. Prasad, D. Qato, D.A. Quistberg, A. Rafay, K. Rahimi, V. Rahimi-Movaghar, S.U. Rahman, M. Raju, I. Rakovac, S.M. Rana, H. Razavi, A. Refaat, J. Rehm, G. Remuzzi, S. Resnikoff, A.L. Ribeiro, P.M. Riccio, L. Richardson, J.H. Richardus, A.M. Riederer, M. Robinson, A. Roca, A. Rodriguez, D. Rojas-Rueda, L. Ronfani, D. Rothenbacher, N. Roy, G.M. Ruhago, N. Sabin, R.L. Sacco, K. Ksoreide, S. Saha, R. Sahathevan, M.A. Sahraian, U. Sampson, J.R. Sanabria, L. Sanchez-Riera, I.S. Santos, M. Satpathy, J.E. Saunders, M. Sawhney, M.I. Saylan, P. Scarborough, B. Schoettker, I.J.C. Schneider, D.C. Schwebel, J.G. Scott, S. Seedat, S.G. Sepanlou, B. Serdar, E.E. Servan-Mori, K. Shackelford, A. Shaheen, S. Shahraz, T.S. Levy, S. Shangguan, J. She, S. Sheikhabaei, D.S. Shepard, P. Shi, K. Shibuya, Y. Shinohara, R. Shiri, K. Shishani, I. Shiue, M.G. Shrime, I.D. Sigfusdottir, D.H. Silberberg, E.P. Simard, S. Sindi, J.A. Singh, L. Singh, V. Skirbekk, K. Sliwa, M. Soljak, S. Soneji, S.S. Soshnikov, P. Speyer, L.A. Sposato, C.T. Sreeramareddy, H. Stoeckl, V.K. Stathopoulou, N. Steckling, M.B. Stein, D.J. Stein, T.J. Steiner, A. Stewart, E. Stork, L.J. Stovner, K. Stroumpoulis, L. Sturua, B.F. Sunguya, M. Swaroop, B.L. Sykes, K.M. Tabb, K. Takahashi, F. Tan, N. Tandon, D. Tanne, M. Tanner, M. Tavakkoli, H.R. Taylor, B.J. Te Ao, A.M.

- Temesgen, M. Ten Have, E.Y. Tenkorang, A.S. Terkawi, A.M. Theadom, E. Thomas, A.L. Thorne-Lyman, A.G. Thrift, I.M. Tleyjeh, M. Tonelli, F. Topouzis, J.A. Towbin, H. Toyoshima, J. Traebert, B.X. Tran, L. Trasande, M. Trillini, T. Truelsen, U. Trujillo, M. Tsilimbaris, E.M. Tuzcu, K.N. Ukwaja, E.A. Undurraga, S.B. Uzun, W.H. Van Brakel, S. Van De Vijver, R. Van Dingenen, C.H. Van Gool, Y.Y. Varakin, T.J. Vasankari, M.S. Vavilala, L.J. Veerman, G. Velasquez-Melendez, N. Venketasubramanian, L. Vijayakumar, S. Villalpando, F.S. Violante, V. V. Vlassov, S. Waller, M.T. Wallin, X. Wan, L. Wang, J. Wang, Y. Wang, T.S. Warouw, S. Weichenthal, E. Weiderpass, R.G. Weintraub, A. Werdecker, K.R. Wessells, R. Westerman, J.D. Wilkinson, H.C. Williams, T.N. Williams, S.M. Woldeyohannes, C.D.A. Wolfe, J.Q. Wong, H. Wong, A.D. Woolf, J.L. Wright, B. Wurtz, G. Xu, G. Yang, Y. Yano, M.A. Yenesew, G.K. Yentur, P. Yip, N. Yonemoto, S.J. Yoon, M. Younis, C. Yu, K.Y. Kim, M.E.S. Zaki, Y. Zhang, Z. Zhao, Y. Zhao, J. Zhu, D. Zonies, J.R. Zunt, J.A. Salomon, C.J.L. Murray, Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013, *Lancet*. 386 (2015) 743–800. doi:10.1016/S0140-6736(15)60692-4.
- [2] V. Katz, J. Schofferman, J. Reynolds, The sacroiliac joint: a potential cause of pain after lumbar fusion to the sacrum., *J. Spinal Disord. Tech.* 16 (2003) 96–9.
- [3] S.P. Cohen, Sacroiliac joint pain: A comprehensive review of anatomy, diagnosis and treatment, *Anesth. Analg.* 101 (2005) 1440–1453. doi:10.1213/01.ANE.0000180831.60169.EA.
- [4] L. Manchikanti, V. Pampati, B. Fellows, A.G. Baha, The inability of the clinical picture to characterize pain from facet joints, *Pain Physician*. 3 (2000) 158–166.
- [5] A. Schwarzer, C. Aprill, R. Derby, J. Fortin, G. Kine, N. Bogduk, Clinical features of patients with pain stemming from the lumbar zygapophysial joints. Is the lumbar facet syndrome a clinical entity?, *Spine (Phila Pa 1976)*. 19 (1994) 1132–1137.
- [6] L. Manchikanti, V. Pampati, B. Fellows, C.E. Bakhit, The diagnostic validity and therapeutic value of lumbar facet joint nerve blocks with or without adjuvant agents, *Curr Rev Pain*. 4 (2000) 337–344.
- [7] S.P. Cohen, C.E. Argoff, E.J. Carragee, Management of low back pain., *BMJ*. 337 (2008).

doi:10.1136/bmj.a2718.

- [8] L. Kapural, A. Ng, J. Dalton, E. Mascha, M. Kapural, M. de La Garza, N. Mekhail, Intervertebral disc biacuplasty for the treatment of lumbar discogenic pain: Results of a six-month follow-up, *Pain Med.* 9 (2008) 60–67. doi:10.1111/j.1526-4637.2007.00407.x.
- [9] A.Y. Wong, J. Karppinen, D. Samartzis, Low back pain in older adults: risk factors, management options and future directions, *Scoliosis Spinal Disord.* 12 (2017) 14. doi:10.1186/s13013-017-0121-3.
- [10] E. Fehrmann, S. Kotulla, L. Fischer, T. Kienbacher, K. Tuechler, P. Mair, G. Ebenbichler, B. Paul, The impact of age and gender on the ICF-based assessment of chronic low back pain, *Disabil. Rehabil.* 0 (2018) 1–10. doi:10.1080/09638288.2018.1424950.
- [11] I. Heuch, I. Heuch, K. Hagen, J.-A. Zwart, Physical activity level at work and risk of chronic low back pain: A follow-up in the Nord-Trøndelag Health Study, *PLoS One.* 12 (2018) e0175086.
- [12] G. Lippi, C. Dagostino, R. Buonocore, R. Aloe, C. Bonaguri, G. Fanelli, M. Allegri, The serum concentrations of leptin and MCP-1 independently predict low back pain duration, *Clin. Chem. Lab. Med.* 55 (2017) 1368–1374. doi:10.1515/cclm-2016-0942.
- [13] M.M. Wertli, R. Eugster, U. Held, J. Steurer, R. Kofmehl, S. Weiser, Catastrophizing - A prognostic factor for outcome in patients with low back pain: A systematic review, *Spine J.* 14 (2014) 2639–2657. doi:10.1016/j.spinee.2014.03.003.
- [14] M. Le Borgne, A.H. Boudoukha, A. Petit, Y. Roquelaure, Chronic low back pain and the transdiagnostic process: How do cognitive and emotional dysregulations contribute to the intensity of risk factors and pain?, *Scand. J. Pain.* 17 (2017) 309–315. doi:10.1016/j.sjpain.2017.08.008.
- [15] A. Varki, Biological roles of oligosaccharides: all of the theories are correct., *Glycobiology.* 3 (1993) 97–130. doi:10.1093/glycob/3.2.97.
- [16] A.J. Hülsmeier, P. Paesold-Burda, T. Hennet, *N*-Glycosylation Site Occupancy in Serum Glycoproteins Using Multiple Reaction Monitoring Liquid Chromatography-Mass Spectrometry, *Mol. Cell. Proteomics.* 6 (2007) 2132–2138. doi:10.1074/mcp.M700361-MCP200.
- [17] S.L. King, H.J. Joshi, K.T. Schjoldager, A. Halim, T.D. Madsen, M.H. Dziegiel, A. Woetmann, S.Y. Vakhrushev, H.H. Wandall, Characterizing the O-glycosylation landscape of human plasma, platelets,

and endothelial cells, *Blood Adv.* 1 (2017) 429–442. doi:10.1182/bloodadvances.2016002121/.

- [18] K.W. Marek, I.K. Vijay, J.D. Marth, A recessive deletion in the GlcNAc-1-phosphotransferase gene results in peri-implantation embryonic lethality, *Glycobiology.* 9 (1999) 1263–1271.
- [19] H.H. Freeze, Genetic defects in the human glycome, *Nat. Rev. Genet.* 7 (2006) 537–551.
- [20] G. Lauc, M. Pezer, I. Rudan, H. Campbell, Mechanisms of disease: The human N-glycome, *Biochim. Biophys. Acta - Gen. Subj.* 1860 (2016) 1574–1582.
- [21] G.P. Subedi, A.W. Barb, The Structural Role of Antibody N-Glycosylation in Receptor Interactions, *Structure.* 23 (2015) 1573–1583.
- [22] G.P. Subedi, A.W. Barb, The immunoglobulin G1 N-glycan composition affects binding to each low affinity Fc γ receptor, *MAbs.* 8 (2016) 1512–1524. doi:10.1080/19420862.2016.1218586.
- [23] M. Novokmet, E. Lukić, F. Vučković, Ž. Đurić, T. Keser, K. Rajšl, D. Remondini, G. Castellani, H. Gašparović, O. Gornik, G. Lauc, Changes in IgG and total plasma protein glycomes in acute systemic inflammation, *Sci. Rep.* 4 (2014) 1–10.
- [24] O. Gornik, G. Lauc, Glycosylation of serum proteins in inflammatory diseases., *Dis. Markers.* 25 (2008) 267–278.
- [25] R. Goulabchand, T. Vincent, F. Batteux, J.F. Eliaou, P. Guilpain, Impact of autoantibody glycosylation in autoimmune diseases, *Autoimmun. Rev.* 13 (2014) 742–750.
- [26] F. Vučković, J. Krištić, I. Gudelj, M. Teruel, T. Keser, M. Pezer, M. Pučić-Baković, J. Štambuk, I. Trbojević-Akmačić, C. Barrios, T. Pavic, C. Menni, Y. Wang, Y. Zhou, L. Cui, H. Song, Q. Zeng, X. Guo, B. a. Pons-Estel, P. McKeigue, A. Leslie Patrick, O. Gornik, T.D. Spector, M. Harjaček, M. Alarcon-Riquelme, M. Molokhia, W. Wang, G. Lauc, Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome, *Arthritis Rheumatol.* 67 (2015) 2978–2989.
- [27] I. Trbojević Akmačić, N.T. Ventham, E. Theodoratou, F. Vučković, N.A. Kennedy, J. Krištić, E.R. Nimmo, R. Kalla, H. Drummond, J. Štambuk, M.G. Dunlop, M. Novokmet, Y. Aulchenko, O. Gornik, H. Campbell, M. Pučić Baković, J. Satsangi, G. Lauc, Inflammatory Bowel Disease Associates with Proinflammatory Potential of the Immunoglobulin G Glycome, *Inflamm. Bowel Dis.*

- [28] F.H. Routier, E.F. Hounsell, P.M. Rudd, N. Takahashi, A. Bond, F.C. Hay, A. Alavi, J.S. Axford, R. Jefferis, Quantitation of the oligosaccharides of human serum IgG from patients with rheumatoid arthritis : a critical evaluation of different methods, *J. Immunol. Methods*. 2013 (1998) 113–130.
- [29] M.B. Freidin, T. Keser, I. Gudelj, J. Stambuk, D. Vucenovic, M. Allegri, T. Pavic, M. Simurina, S.M. Fabiane, G. Lauc, F.M.K. Williams, The Association between Low Back Pain and Composition of IgG Glycome, *Sci. Rep.* 6 (2016) 26815.
- [30] C. Dagostino, M. De Gregori, C. Gieger, J. Manz, I. Gudelj, G. Lauc, L. Divizia, W. Wang, M. Sim, I.K. Pemberton, J. MacDougall, F. Williams, J. Van Zundert, D. Primorac, Y. Aulchenko, L. Kapural, M. Allegri, PainOmics Group, Validation of standard operating procedures in a multicenter retrospective study to identify -omics biomarkers for chronic low back pain., *PLoS One*. 12 (2017) e0176372.
- [31] M. Allegri, M. De Gregori, C.E. Minella, C. Klersy, W. Wang, M. Sim, C. Gieger, J. Manz, I.K. Pemberton, J. MacDougall, F.M. Williams, J. Van Zundert, K. Buyse, G. Lauc, I. Gudelj, D. Primorac, A. Skelin, Y.S. Aulchenko, L.C. Karssen, L. Kapural, R. Rauck, G. Fanelli, PainOMICS Group, “Omics” biomarkers associated with chronic low back pain: protocol of a retrospective longitudinal study., *BMJ Open*. 6 (2016) e012070.
- [32] R. Freynhagen, R. Baron, U. Gockel, T.R. Tölle, pain *DETECT* : a new screening questionnaire to identify neuropathic components in patients with back pain, *Curr. Med. Res. Opin.* 22 (2006) 1911–1920. doi:10.1185/030079906X132488.
- [33] M. De Gregori, L. Diatchenko, P.M. Ingelmo, V. Napolioni, P. Klepstad, I. Belfer, V. Molinaro, G. Garbin, G.N. Ranzani, G. Alberio, M. Normanno, F. Lovisari, M. Somaini, S. Govoni, E. Mura, D. Bugada, T. Niebel, M. Zorzetto, S. De Gregori, M. Molinaro, G. Fanelli, M. Allegri, Human Genetic Variability Contributes to Postoperative Morphine Consumption, *J. Pain*. 17 (2016) 628–636. doi:10.1016/j.jpain.2016.02.003.
- [34] I. Gudelj, M. Baciarello, I. Ugrina, M. De Gregori, V. Napolioni, P.M. Ingelmo, D. Bugada, S. De Gregori, L. Đerek, M. Pučić-Baković, M. Novokmet, O. Gornik, G. Saccani Jotti, T. Meschi, G. Lauc,

- M. Allegri, O. Gornik, T. Pavic, G. Lauc, G. Lauc, A. Vojta, V. Zoldoš, G. Lauc, J. Krištić, V. Zoldoš, R.B. Parekh, M.A. Wolfert, G.-J. Boons, J.N. Arnold, R. Saldova, U.M.A. Hamid, P.M. Rudd, C. a Reis, H. Osorio, L. Silva, C. Gomes, L. David, S.G. Raja, G.D. Dreyfus, H.L. Rittner, A. Brack, C. Stein, D. Bugada, C.L. Wu, S.N. Raja, M. De Gregori, Z.Y. Ren, I.C. Hwang, A. Knezevic, M. Pucic, F. Vučković, G. Thanabalasingham, M. Novokmet, I.T. Akmačić, V. Vanhooren, J.C. Kalff, A. Cruickshank, W. Fraser, H. Burns, J. Van Damme, A. Shenkin, A. Gonzalez-Quintela, A. Shenkin, J. V. Castell, H. Ohzato, G. Lauc, O. Gornik, B. der L. EC, P.F. de Haan, E.C. Havenaar, D.W. Van, M. Sperandio, J.B. Lowe, J. Mitoma, H.E. Miwa, Y. Song, R. Alvarez, R.D. Cummings, P. Stanley, R.S. Tirumalai, J.L. Dage, B.L. Ackermann, H.B. Halsall, T. Imre, D. Kolarich, A. Weber, P.L. Turecek, H.P. Schwarz, F. Altmann, K. Mills, J. Nilsson, H. Hwang, A. Harazono, F. Clerc, Y. Satomi, Y. Shimonishi, T. Takao, Y. Satomi, Y. Shimonishi, T. Hase, T. Takao, N.L. Wilson, B.L. Schulz, N.G. Karlsson, N.H. Packer, J.N. Arnold, K.R. Reiding, D. Blank, D.M. Kuijper, A.M. Deelder, M. Wuhrer, B. Adamczyk, W.B. Struwe, A. Ercan, P. a. Nigrovic, P.M. Rudd, S. Zhang, K. Jiang, C. Sun, H. Lu, Y. Liu, P. Pompach, W. van Dijk, E.C. Havenaar, E.C.B. der Linden, E.C.B. der Linden, E.C. van Ommen, W. van Dijk, E.C. Havenaar, W. JM, B. Fournet, D. Konan, D. Biou, G. Durand, M. De Gregori, I.T. Akmačić, D. Bates, M. Mächler, B. Bolker, S. Walker, Y. Benjamini, Y. Hochberg, Changes in total plasma and serum N-glycome composition and patient-controlled analgesia after major abdominal surgery, *Sci. Rep.* 6 (2016) 31234. doi:10.1038/srep31234.
- [35] I. Trbojević Akmačić, I. Ugrina, J. Štambuk, I. Gudelj, F. Vučković, G. Lauc, M. Pučić Baković, High Throughput Glycomics: Optimization of Sample Preparation, *Biochem.* 80 (2015) 934–942.
- [36] A. Agakova, F. Vučković, L. Klarić, G. Lauc, F. Agakov, Automated Integration of a UPLC Glycomic Profile, in: G. Lauc, M. Wuhrer (Eds.), *High-Throughput Glycomics Glycoproteomics. Methods Mol. Biol.*, 1503rd ed., Humana Press, New York, 2017: pp. 217–233.
- [37] L. Royle, C.M. Radcliffe, R. a Dwek, P.M. Rudd, Detailed structural analysis of N-glycans released from glycoproteins in SDS-PAGE gel bands using HPLC combined with exoglycosidase array digestions, *Methods Mol. Biol.* 347 (2006) 125–143.
- [38] R. Saldova, A. Asadi Shehni, V.D. Haakensen, I. Steinfeld, M. Hilliard, I. Kifer, Å. Helland, Z.

- Yakhini, A.L. Børresen-Dale, P.M. Rudd, Association of N-glycosylation with breast carcinoma and systemic features using high-resolution quantitative UPLC, *J. Proteome Res.* 13 (2014) 2314–2327. doi:10.1021/pr401092y.
- [39] C.A. Cooper, E. Gasteiger, N.H. Packer, GlycoMod - A software tool for determining glycosylation compositions from mass spectrometric data, *Proteomics.* 1 (2001) 340–349. doi:10.1002/1615-9861(200102)1:2<340::AID-PROT340>3.0.CO;2-B.
- [40] A. Ceroni, K. Maass, H. Geyer, R. Geyer, A. Dell, S.M. Haslam, GlycoWorkbench: A tool for the computer-assisted annotation of mass spectra of glycans, *J. Proteome Res.* 7 (2008) 1650–1659. doi:10.1021/pr7008252.
- [41] J.T. Leek, W.E. Johnson, H.S. Parker, A.E. Jaffe, J.D. Storey, The SVA package for removing batch effects and other unwanted variation in high-throughput experiments, *Bioinformatics.* 28 (2012) 882–883. doi:10.1093/bioinformatics/bts034.
- [42] W. Viechtbauer, Conducting Meta-Analyses in R with the metafor Package, *J. Stat. Softw.* 36 (2010) 1–48. doi:10.1103/PhysRevB.91.121108.
- [43] Y.S. Aulchenko, S. Ripke, A. Isaacs, C.M. van Duijn, GenABEL: An R library for genome-wide association analysis, *Bioinformatics.* 23 (2007) 1294–1296. doi:10.1093/bioinformatics/btm108.
- [44] A. Knežević, O. Polašek, O. Gornik, I. Rudan, H. Campbell, C. Hayward, A. Wright, I. Kolčić, N. O'Donoghue, J. Bones, P.M. Rudd, G. Lauc, Variability, heritability and environmental determinants of human plasma N-glycome, *J. Proteome Res.* 8 (2009) 694–701.
- [45] J.N. Arnold, R. Saldoval, U.M. Abd Hamid, P.M. Rudd, Evaluation of the serum N-linked glycome for the diagnosis of cancer and chronic inflammation, *Proteomics.* 8 (2008) 3284–3293. doi:10.1002/pmic.200800163.
- [46] K. Fassbender, W. Zimmerli, A. Aeschlimann, M. Kellner, W.M. It, Glycosylation of α 1-acid glycoprotein in relation to duration of disease in acute and chronic infection and inflammation, *Clinica Chim. Acta.* 203 (1991) 315–328.
- [47] A. Mackiewicz, K. Mackiewicz, Glycoforms of serum alpha 1-acid glycoprotein as markers of inflammation and cancer, *Glycoconj. J.* 12 (1995) 241–247.

- [48] A. Knežević, J. Bones, S.K. Kračun, O. Gornik, P.M. Rudd, G. Lauc, High throughput plasma N-glycome profiling using multiplexed labelling and UPLC with fluorescence detection, *Analyst*. 136 (2011) 4670.
- [49] M. Pučić, A. Knežević, J. Vidič, B. Adamczyk, M. Novokmet, O. Polašek, O. Gornik, S. Šupraha-Goreta, M.R. Wormald, I. Redžić, H. Campbell, A. Wright, N.D. Hastie, J.F. Wilson, I. Rudan, M. Wuhrer, P.M. Rudd, D. Josić, G. Lauc, High Throughput Isolation and Glycosylation Analysis of IgG-Variability and Heritability of the IgG Glycome in Three Isolated Human Populations, *Mol. Cell. Proteomics*. 10 (2011) M111.010090-M111.010090.
- [50] O. Gornik, J. Wagner, M. Pučić, A. Knežević, I. Redžić, G. Lauc, Stability of N-glycan profiles in human plasma, *Glycobiology*. 19 (2009) 1547–1553.
- [51] I. Brockhausen, S. Narasimhan, H. Schachter, The biosynthesis of highly branched N-glycans: studies on the sequential pathway and functional role of N-acetylglucosaminyltransferases I, II, III, IV, V and VI., *Biochimie*. 70 (1988) 1521–33.
- [52] F. Clerc, K.R. Reiding, B.C. Jansen, G.S.M. Kammeijer, A. Bondt, M. Wuhrer, Human plasma protein N-glycosylation, *Glycoconj. J.* 33 (2016) 309–343.
- [53] K. Higai, Y. Aoki, Y. Azuma, K. Matsumoto, Glycosylation of site-specific glycans of α 1-acid glycoprotein and alterations in acute and chronic inflammation, *Biochim. Biophys. Acta - Gen. Subj.* 1725 (2005) 128–135.
- [54] E.C.M. Brinkman-Van Der Linden, P.F. De Haan, E.C. Havenaar, W. Van Dijk, Inflammation-induced expression of sialyl Lewis(x) is not restricted to α 1-acid glycoprotein but also occurs to a lesser extent on α 1- antichymotrypsin and haptoglobin, *Glycoconj. J.* 15 (1998) 177–182.
- [55] M. Wuhrer, M.H.J. Selman, L.A. McDonnell, T. Kümpfel, T. Derfuss, M. Khademi, T. Olsson, R. Hohlfeld, E. Meinl, M. Krumbholz, Pro-inflammatory pattern of IgG1 Fc glycosylation in multiple sclerosis cerebrospinal fluid, *J. Neuroinflammation*. 12 (2015) 1–14. doi:10.1186/s12974-015-0450-1.
- [56] Y. van Kooyk, G.A. Rabinovich, Protein-glycan interactions in the control of innate and adaptive immune responses, *Nat. Immunol.* 9 (2008) 593–601. doi:10.1038/ni.f.203.
- [57] A.M. Goetze, Y.D. Liu, Z. Zhang, B. Shah, E. Lee, P. V. Bondarenko, G.C. Flynn, High-mannose

glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans,

Glycobiology. 21 (2011) 949–959. doi:10.1093/glycob/cwr027.

- [58] R. Malhotra, M.R. Wormald, P.M. Rudd, P.B. Fischer, R. a Dwek, R.B. Sim, Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein., *Nat. Med.* 1 (1995) 237–243. doi:10.1038/nm0395-237.

ACCEPTED MANUSCRIPT

Table 1. Information about participants enrolled in a chronic low back pain study.

| | ITA | | BEL | | CRO | |
|----------------------|-------------|-------------|-------------|-------------|-------------|------------|
| | Case | Control | Case | Control | Case | Control |
| Sample Number | 375 | 379 | 567 | 191 | 186 | 190 |
| Age (med/IQR) | 67 (51-76) | 68 (50-77) | 56 (49-67) | 56 (48-65) | 55 (48-65) | 56 (41-65) |
| Sex (F) (n/%) | 201 (53.6%) | 205 (54.1%) | 309 (54.5%) | 108 (56.5%) | 120 (64.5%) | 79 (41.6%) |

Plasma N-glycosylation analysis has been performed for participants from three clinical centers in Italy (ITA), Belgium (BEL) and Croatia (CRO). Case - chronic low back pain patients, Control - healthy individuals, med - median value, IQR - interquartile range.

Table 2. Derived glycan traits defined from 39 directly measured chromatographic peaks corresponding to glycans with similar structural features (Figure 1).

| Structural feature | Chromatographic peaks corresponding to glycans with designated feature |
|--|--|
| LB (Low-branching) | GP1+GP2+GP3+GP4+GP5+GP6+GP8+GP9+GP10+GP11+GP12+GP13+GP14+GP15+GP16+GP17+GP18+GP20+GP21+GP22+GP23 |
| HB (High-branching) | GP24+GP25+GP26+GP27+GP28+GP29+GP30+GP31+GP32+GP33+GP34+GP35+GP36+GP37+GP38+GP39 |
| S0 (Neutral/not sialylated glycans) | GP1+GP2+GP3+GP4+GP5+GP6+GP7+GP8+GP9+GP10+GP11 |
| S1 (Monosialylated glycans) | GP12+GP13+GP14+GP15+GP16+GP17 |
| S2 (Disialylated glycans) | GP18 +GP20+GP21+GP22+GP23+GP24+GP25+GP26 |
| S3 (Trisialylated glycans) | GP27+GP28+GP29+GP30+GP31+GP32+GP33+GP34+GP35 |
| S4 (Tetrasialylated glycans) | GP36+GP37+GP38+GP39 |
| G0 (Agalactosylated glycans) | GP1+GP2 |
| G1 (Monogalactosylated glycans) | GP3+GP4+GP5+GP6+GP12+GP13 |
| G2 (Digalactosylated glycans) | GP8+GP9+GP10+GP11+GP14+GP15+GP16+GP17+GP18+GP20+GP21+GP22+GP23 |
| G3 (Trigalactosylated glycans) | GP24+GP25+GP26+GP27+GP28+GP29+GP30+GP31+GP32+GP35 |
| G4 (Tetragalactosylated glycans) | GP33+GP34+GP36+GP37+GP38+GP39 |
| HM (High-mannose glycans) | GP2+GP7+GP19 |
| B (bisecting <i>N</i> -acetylglucosamine containing glycans) | GP2+GP3+GP6+GP9+GP11+GP12+GP15+GP17+GP21+GP23 |

GP - glycan peak. LB - monoantennary and biantennary glycans, HB - tri-antennary and tetra-antennary glycans, S0 - glycans with no sialic acid, S1 - glycans with one sialic acid, S2 - glycans with two sialic acids, S3 - glycans with three sialic acids, S4 - glycans with four sialic acids, G0 - glycans with no galactose, G1 - glycans with one galactose, G2 - glycans with two galactoses, G3 - glycans with three galactoses, G4 - glycans with four galactoses, HM - high-mannose glycans, B - glycans that contain bisecting *N*-acetylglucosamine.

Table 3. Differences in derived structural features (TRAITS, defined in Table 2) of plasma protein N-glycans (Figure 1) between chronic low back pain cases and healthy controls in the three populations - Italy (ITA), Belgium (BEL) and Croatia (CRO), together with meta-analysis of all three populations (META).

| TRAIT | ITA | | | | BEL | | | | CRO | | | | META | | | |
|-------------------------|--------|-----|-------|-------|--------|-----|-------|----------|--------|-----|-------|-------|--------|-----|-------|-------|
| | effect | SE | p.val | q.val | effect | SE | p.val | q.val | effect | SE | p.val | q.val | effect | SE | p.val | q.val |
| LB (Low-branching) | -0.2 | 0.0 | 2.23 | 3.47 | -0.3 | 0.0 | 2.81 | 3.94E-04 | -0.1 | 0.1 | 3.39 | 5.05 | -0.2 | 0.0 | 5.14 | 1.20 |
| | 20 | 72 | E-03 | E-03 | 40 | 81 | E-05 | 04 | 15 | 21 | E-01 | E-01 | 47 | 49 | E-07 | E-06 |
| HB (High-branching) | 0.2 | 0.0 | 2.18 | 3.47 | 0.2 | 0.0 | 4.76 | 1.11E-03 | 0.0 | 0.1 | 4.48 | 5.23 | 0.2 | 0.0 | 6.25 | 9.72 |
| | 21 | 72 | E-03 | E-03 | 84 | 81 | E-04 | 03 | 91 | 21 | E-01 | E-01 | 23 | 49 | E-06 | E-06 |
| S0 (Neutral) | -0.4 | 0.0 | 5.45 | 3.44 | -0.3 | 0.0 | 1.23 | 7.26E-04 | -0.1 | 0.1 | 1.23 | 3.05 | -0.3 | 0.0 | 4.22 | 5.91 |
| | 42 | 71 | E-10 | E-09 | 19 | 83 | E-04 | 04 | 86 | 21 | E-01 | E-01 | 56 | 49 | E-13 | E-12 |
| S1 (Monosialylated) | 0.0 | 0.0 | 7.05 | 7.05 | 0.0 | 0.0 | 9.27 | 9.27E-01 | -0.1 | 0.1 | 1.71 | 3.41 | -0.0 | 0.0 | 8.03 | 8.03 |
| | 27 | 72 | E-01 | E-01 | 08 | 83 | E-01 | 01 | 63 | 20 | E-01 | E-01 | 12 | 49 | E-01 | E-01 |
| S2 (Disialylated) | 0.3 | 0.0 | 1.36 | 3.17 | 0.1 | 0.0 | 5.56 | 8.65E-02 | 0.1 | 0.1 | 1.16 | 3.05 | 0.2 | 0.0 | 2.79 | 7.82 |
| | 44 | 71 | E-06 | E-06 | 60 | 84 | E-02 | 02 | 90 | 21 | E-01 | E-01 | 54 | 49 | E-07 | E-07 |
| S3 (Trisialylated) | 0.2 | 0.0 | 4.53 | 5.76 | 0.2 | 0.0 | 3.11 | 1.09E-03 | 0.1 | 0.1 | 1.31 | 3.05 | 0.2 | 0.0 | 2.25 | 3.93 |
| | 05 | 73 | E-03 | E-03 | 97 | 82 | E-04 | 03 | 82 | 21 | E-01 | E-01 | 35 | 50 | E-06 | E-06 |
| S4 (Tetrasialylated) | 0.1 | 0.0 | 1.07 | 1.25 | 0.0 | 0.0 | 5.59 | 7.12E-01 | -0.0 | 0.1 | 4.31 | 5.23 | 0.0 | 0.0 | 2.52 | 2.71 |
| | 14 | 71 | E-01 | E-01 | 48 | 83 | E-01 | 01 | 96 | 23 | E-01 | E-01 | 57 | 50 | E-01 | E-01 |
| G0 (Agalactosylated) | -0.3 | 0.0 | 1.85 | 2.59 | -0.2 | 0.0 | 4.03 | 1.11E-03 | -0.1 | 0.1 | 3.11 | 5.05 | -0.3 | 0.0 | 1.93 | 1.35 |
| | 98 | 62 | E-10 | E-09 | 71 | 77 | E-04 | 03 | 14 | 13 | E-01 | E-01 | 12 | 44 | E-12 | E-11 |
| G1 (Monogalactosylated) | -0.4 | 0.0 | 3.18 | 1.11 | -0.3 | 0.0 | 1.56 | 7.26E-04 | -0.1 | 0.1 | 3.60 | 5.05 | -0.3 | 0.0 | 1.58 | 7.36 |
| d) | 23 | 71 | E-09 | E-08 | 11 | 82 | E-04 | 04 | 08 | 19 | E-01 | E-01 | 30 | 49 | E-11 | E-11 |

| | | | | | | | | | | | | | | | | |
|---|-----|-----|------|------|-----|-----|------|--------|-----|-----|------|------|-----|-----|------|------|
| G2 | 0.2 | 0.0 | 2.97 | 5.94 | 0.0 | 0.0 | 3.51 | 4.91E- | 0.0 | 0.1 | 5.28 | 5.69 | 0.1 | 0.0 | 1.91 | 2.43 |
| (Digalactosylated) | 85 | 68 | E-05 | E-05 | 76 | 82 | E-01 | 01 | 74 | 18 | E-01 | E-01 | 79 | 48 | E-04 | E-04 |
| G3 | 0.2 | 0.0 | 2.71 | 3.80 | 0.2 | 0.0 | 3.54 | 7.07E- | - | 0.1 | 8.70 | 8.70 | 0.1 | 0.0 | 1.65 | 2.30 |
| (Trigalactosylated) | 12 | 71 | E-03 | E-03 | 28 | 78 | E-03 | 03 | 0.0 | 22 | E-01 | E-01 | 82 | 48 | E-04 | E-04 |
| G4 | 0.0 | 0.0 | 3.04 | 3.27 | 0.1 | 0.0 | 3.91 | 6.85E- | 0.2 | 0.1 | 6.12 | 2.86 | 0.1 | 0.0 | 7.35 | 8.58 |
| (Tetragalactosylated) | 70 | 69 | E-01 | E-01 | 65 | 80 | E-02 | 02 | 26 | 21 | E-02 | E-01 | 28 | 48 | E-03 | E-03 |
| HM (High-mannose glycans) | - | 0.0 | 8.51 | 2.38 | 0.0 | 0.0 | 6.99 | 8.15E- | - | 0.1 | 3.00 | 2.10 | - | 0.0 | 1.68 | 3.35 |
| | 0.3 | 64 | E-08 | E-07 | 31 | 79 | E-01 | 01 | 0.3 | 14 | E-03 | E-02 | 0.2 | 46 | E-06 | E-06 |
| | 45 | | | | | | | | 40 | | | | 19 | | | |
| B (bisecting <i>N</i> - acetylglucosamine containing glycans) | - | 0.0 | 7.37 | 3.44 | - | 0.0 | 7.85 | 8.45E- | - | 0.1 | 1.83 | 2.10 | - | 0.0 | 8.20 | 2.87 |
| | 0.4 | 70 | E-10 | E-09 | 0.0 | 83 | E-01 | 01 | 0.3 | 18 | E-03 | E-02 | 0.2 | 49 | E-09 | E-08 |
| | 33 | | | | 22 | | | | 69 | | | | 81 | | | |

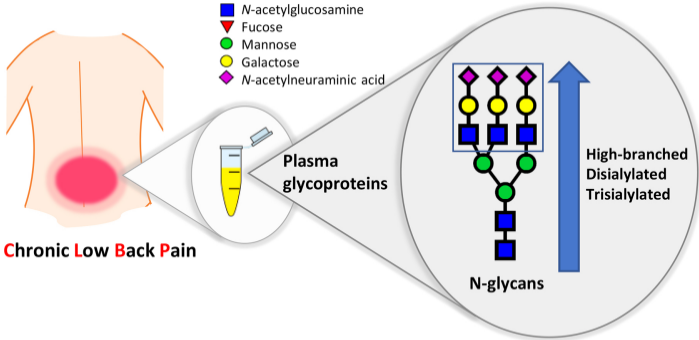
Effect - beta coefficient estimated based on regression model, SE - standard error of estimated beta

coefficient, p.val - p-value, q.val - adjusted p-value using a FDR approach. Each TRAIT represents a sum of chromatographic peaks corresponding to glycans with similar structural features. LB - monoantennary and biantennary glycans, HB - tri-antennary and tetra-antennary glycans, S0 - glycans with no sialic acid, S1 - glycans with one sialic acid, S2 - glycans with two sialic acids, S3 - glycans with three sialic acids, S4 - glycans with four sialic acids, G0 - glycans with no galactose, G1 - glycans with one galactose, G2 - glycans with two galactoses, G3 - glycans with three galactoses, G4 - glycans with four galactoses, HM - high-mannose glycans, B - glycans that contain bisecting *N*-acetylglucosamine

Graphical Abstract**Highlights**

- Relative occurrences of high-branched plasma *N*-glycans are increased in chronic low back pain
- Plasma protein *N*-glycosylation changes are consistent with chronic inflammation
- Relative occurrences of disialylated and trisialylated glycans change in chronic low back pain
- Specific glycan changes have a potential as biomarker for chronic low back pain

ACCEPTED MANUSCRIPT



Graphics Abstract

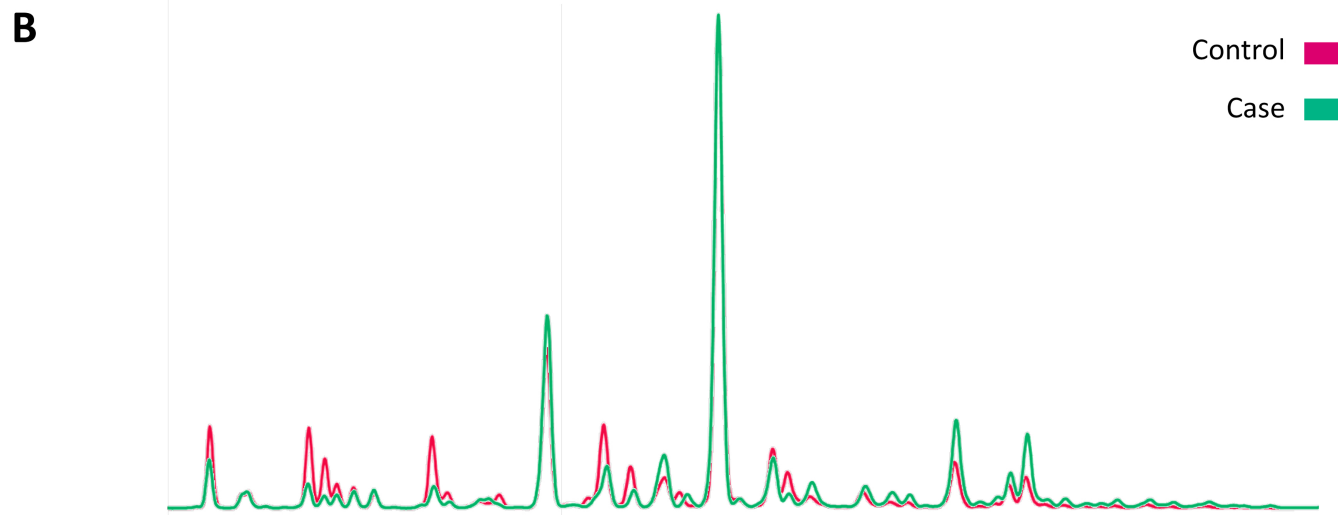
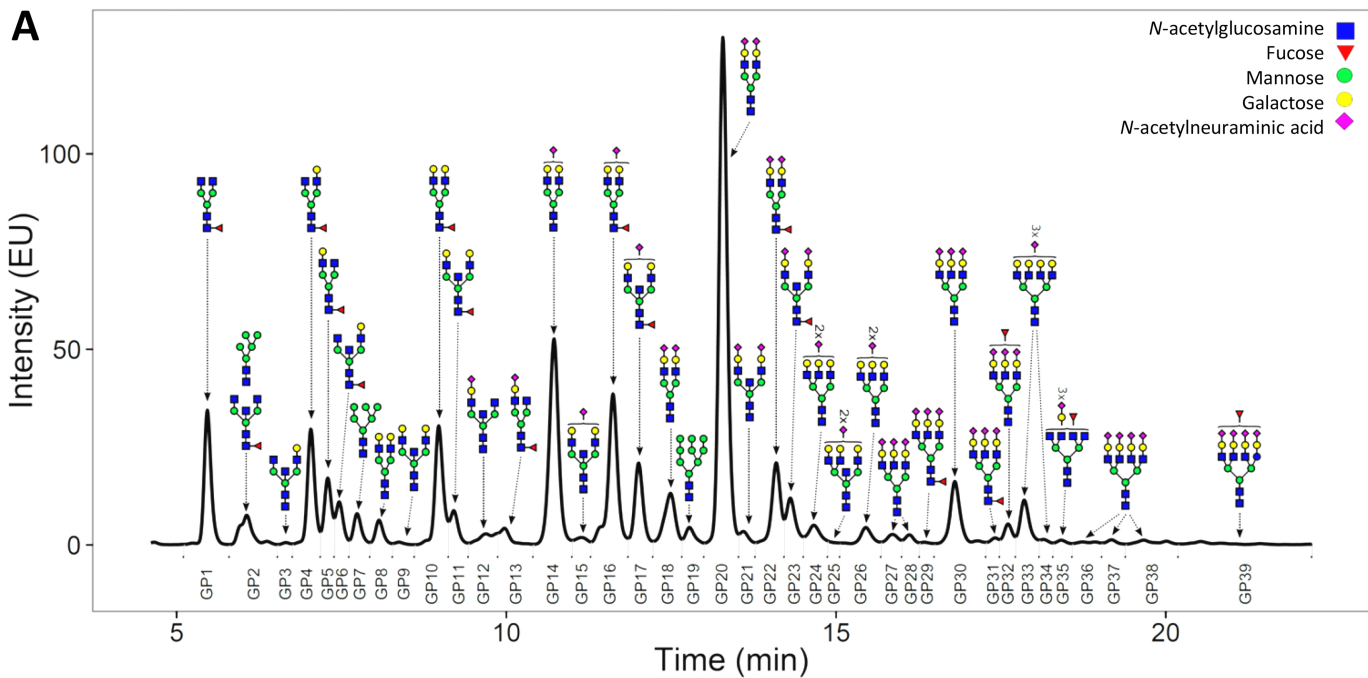


Figure 1

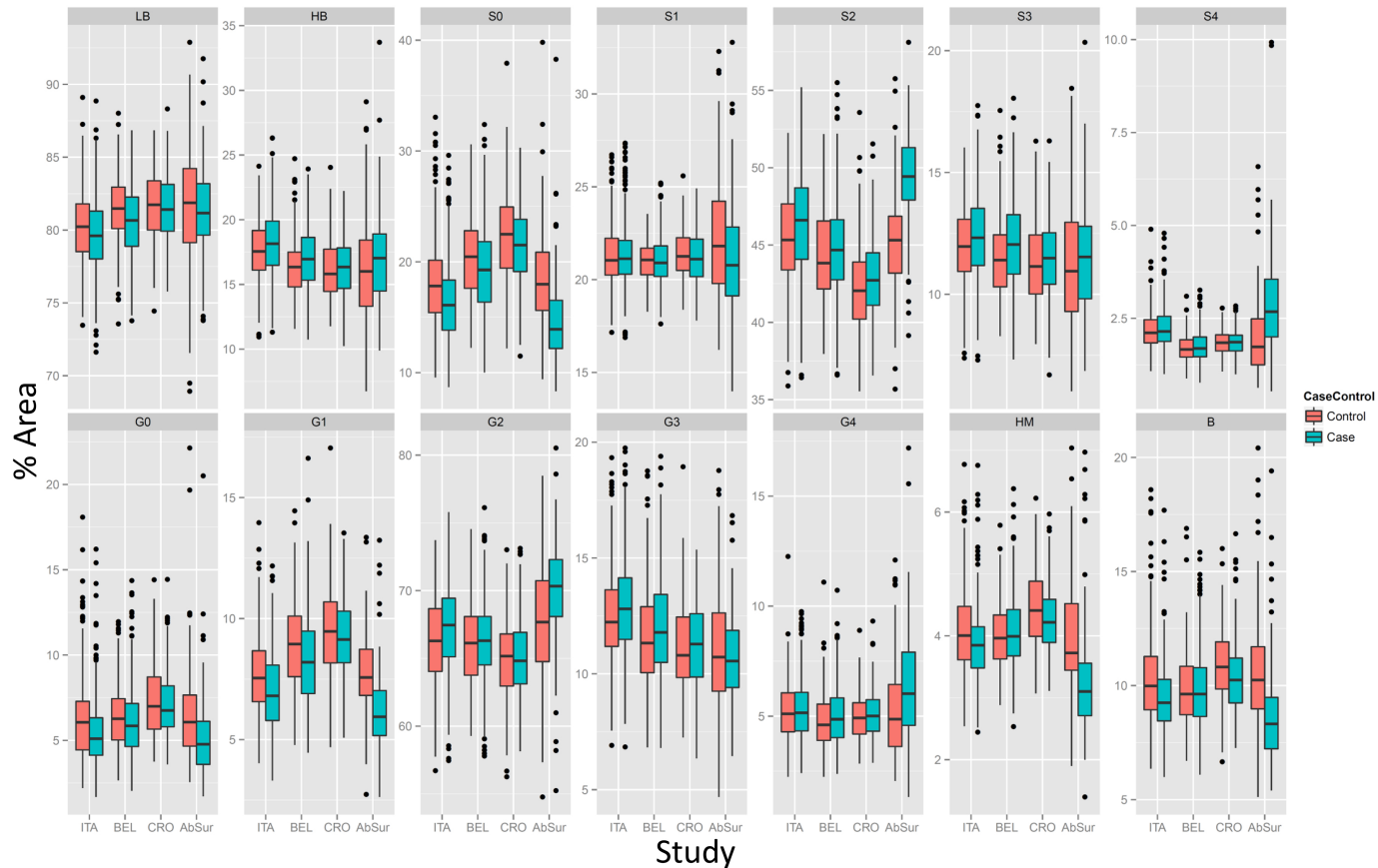


Figure 2