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Glycomic markers of inflammatory bowel disease and IgA nephropathy

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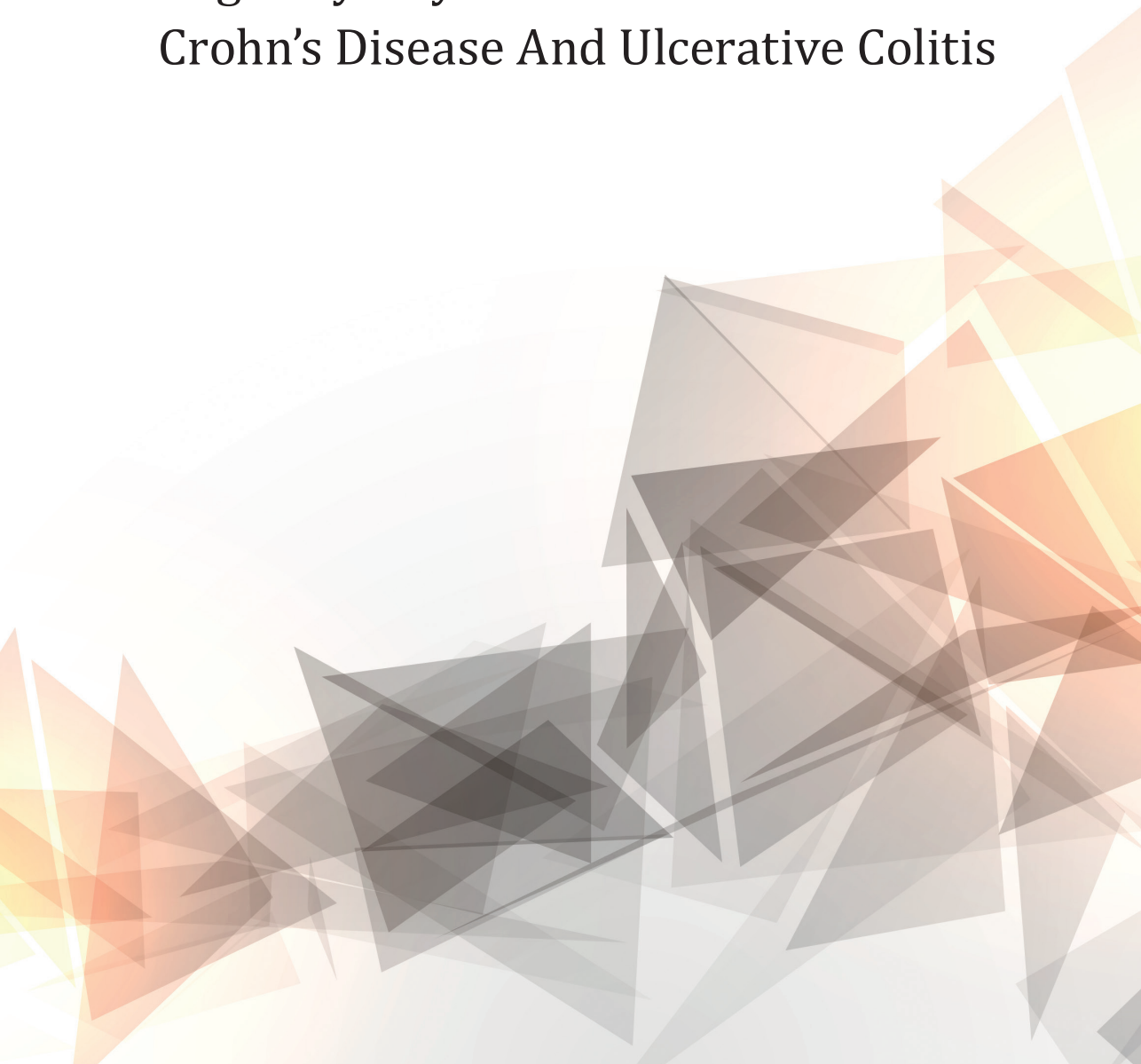
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Chapter 5

IgA Glycosylation Differs Between
Crohn's Disease And Ulcerative Colitis



Immunoglobulin A Glycosylation Differs Between Crohn's Disease And Ulcerative Colitis

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Abstract

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC) are chronic and relapsing inflammations of the digestive tract with increasing prevalence, yet unknown origins or cure. CD and UC have similar symptoms but respond differently to surgery and medication. Current diagnostic tools often involve invasive procedures, while laboratory markers for patient stratification are lacking. Large glycomic studies of immunoglobulin G and total plasma glycosylation have shown biomarker potential in IBD and could help determine disease mechanisms and therapeutic treatment choice. Hitherto, the glycosylation signatures of plasma immunoglobulin A, an important immunoglobulin secreted into the intestinal mucin, have remained undetermined in the context of IBD.

Our study investigated the associations of immunoglobulin A1 and A2 glycosylation with IBD in 442 IBD cases (188 CD and 254 UC) and 120 healthy controls by reverse-phase liquid chromatography electrospray-ionization mass spectrometry of tryptic glycopeptides. Differences of IgA *O*- and *N*-glycosylation (including galactosylation, bisection, sialylation and antennarity) between patients groups were associated with the diseases and these findings led to the construction of a statistical model to predict the disease group of the patients without the need of invasive procedures. This study expands the current knowledge about CD and UC and could help the development of noninvasive biomarkers and better patient care.

Keywords: Inflammatory bowel disease, Crohn's Disease, ulceratives colitis, IgA1, glycosylation, biomarker

Introduction

Interest in large glycomics studies is rapidly increasing and associations of plasma protein glycosylation with disease have been demonstrated for cancers, autoimmune diseases and inflammatory bowel disease (IBD)¹⁻⁵.

IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC) are lifelong, chronic and include relapsing inflammatory conditions of the digestive tract with yet unknown etiology⁶. CD and UC have similar symptoms but respond differently to medication and surgical treatments and the distinction between the two often requires invasive procedures which might include intestinal tissue sampling⁷. Early and non-invasive patient stratification from readily available body fluids is needed to best target therapy.

Immunoglobulin G (IgG) is of particular interest as an inflammatory marker in IBD because it is widely involved in autoimmunity. Having established the biomarker potential of the IgG glycosylation and total plasma *N*-glycome (TPNG) in IBD, IgA1 seemed to be an ideal additional candidate to receive attention due to its presence in mucosal fluids of the intestinal tract and its established role in immunological responses and mucosal protection against microorganisms such as bacteria and viruses. The latter happens by binding or agglutinating them, thus promoting clearance and preventing infections and inflammations^{3, 4, 8-12}. However, the function of IgA glycosylation in health and disease is still an active area of research.

The *O*- and *N*-glycosylation of IgA1 and IgA2 have previously been monitored for 29 women during pregnancy and in the post-partum period using matrix-assisted laser desorption/ionization-Fourier-transform ion-cyclotron resonance (MALDI-FTICR)¹³. The same MALDI-FTICR method has again been used for the analysis of plasma IgA from 284 individuals comprising both rheumatoid arthritis (RA) patients and controls¹⁴. However, the complex and technically challenging sample handling used in these studies is an obstacle in large-scale clinical studies. Recently, an alternative LC-MS(/MS) approach with reduced benchwork sample preparation and a more streamlined workflow has been successfully applied to analyze IgA glycopeptides in a cohort of IgA nephropathy patients and healthy controls¹⁵. The same method was selected for this research.

Next to its important functions in intestine immunity, IgA was also implicated as potential target glycoprotein based on previous TPNG analysis. Specifically, the IgA glycans expectedly contribute to a large extent to specific plasma glycoforms affected by IBD, including elevated bisection levels of the diantennary fucosylated sialylated glycans^{4, 16}. IgA1 and IgA2 glycoforms have previously been associated with CD, UC and IgA nephropathy (IgAN) where the general glycan size was reduced

(lower levels of sialylation, galactosylation and antennarity of *N*-glycans, and reduced sialylation and galactosylation of *O*-glycans) as well as with secondary IgA nephropathy where nephropathy follows CD, indicating the potential involvement of IgA in these diseases^{15, 17-21}. Small-scale studies have also demonstrated changes in the *O*-glycosylation traits of IgA such as reduced levels of GalNAc in IBD^{22, 23}. Still, the overall role of IgA glycosylation in disease remains largely unclear. It is mostly unexplored to which extent IgA glycosylation affects FcαR receptor binding triggering pro- or anti-inflammatory responses, or clearance from the circulation by the asialoglycoprotein receptor^{16, 24, 25}.

In this study, the IgA glycosylation profiles of 442 IBD patients and 120 healthy controls were analyzed by LC-MS/MS with glycosylation site-specificity. The main goal was to find associations between IgA glycosylation and IBD characteristics to discriminate CD, UC and HC, and subsequently, in a secondary analysis, to find associations with disease behavior, and surgical and medical treatments, for improved patient care.

Materials and methods

Clinical samples

The plasma samples used for this analysis were received from the University Hospital Careggi (Florence, Italy) and consisted of 442 IBD cases (188 CD and 254 UC) and 120 healthy controls (HC) as previously described^{4, 26}. Written informed consent was obtained from the study participants and the ethical approval was obtained from the Ethics Review Board of the Casa Sollievo della Sofferenza Hospital (Italy). The clinical information of CD and UC patients was collected following the rules of the Montreal classification of inflammatory bowel disease²⁷. The demographics of the individuals included in the statistical analysis are detailed in **Table 1**.

Table 1: Demographics of the individuals included for statistical analysis.

Sample numbers	HC	CD	UC
Total (males)	120 (72)	188 (110)	254 (159)
Age, mean $\pm$ SD			
Males	48.6 $\pm$ 16.1	36.0 $\pm$ 13.6	42.2 $\pm$ 16.1
Females	60.0 $\pm$ 17.8	37.3 $\pm$ 15.6	42.1 $\pm$ 16.7
All	53.2 $\pm$ 17.7	36.5 $\pm$ 14.4	42.1 $\pm$ 16.3
Patient information			
Disease group (counts (males))		CD	UC
Disease Location (CD)			
Ileum $\pm$ UpperGI* (L1 $\pm$ L4)		65 (39) $\pm$ 1 (1)	
Colon $\pm$ UpperGI (L2 $\pm$ L4)		67 (44) $\pm$ 6 (4)	
Ileum+Colon $\pm$ UpperGI (L3 $\pm$ L4)		47 (20) $\pm$ 1 (1)	
Upper GI (L4)		8 (6)	
Disease Location (UC)			
Rectum (E1)			29 (15)
Sigmoid + Left colon (E2)			127 (79)
Entire colon (E3)			93 (61)
Disease behaviour (CD)			
Inflammatory (B1)		106 (64)	
Stenosing (B2)		55 (27)	
Fistulizing (B3)		26 (18)	
Surgery			
No		127 (74)	243 (151)
Before sample collection		49 (28)	8 (5)
After sample collection		8 (5)	2 (2)
Drugs			
Mesalazine		38 (19)	75 (46)
Steroids		42 (25)	79 (49)
Azathioprine/6-mercaptopurine		43 (26)	58 (38)
Anti-TNF α		51 (31)	22 (14)

*GI = Gastro-Intestinal (tract)

Glycoproteomics analysis

Capture of IgA

IgA1 and IgA2 were captured from five μL of plasma (containing approx. 350 μg total protein and 13 μg IgA) with camelid antibodies domains immobilized on agarose beads (CaptureSelect IgA Affinity Matrix, Thermo Fisher Scientific, Waltham, MA) as previously described¹³. Ten μL of bead slurry were pipetted into each well of a 96-well Orochem filter plate (AcroPrep, Pall Corporation, Ann Arbor, MI, USA). The beads were washed three times with 200 μL ultrapure water (prepared at 18.2 M Ω with a Purelab Ultra, Veolia Water Technologies Netherlands B.V., Ede, The Netherlands). and three times with PBS 1x solution on vacuum manifold. Five μL of sample were added to 100 μL of PBS in each well and incubated with shaking for one hour at room temperature (RT). The beads were washed on vacuum manifold with 3x200 μL PBS 1x and three times 200 μL water. The captured IgA was released from the beads with 100 μL of 100 mM formic acid, eluted in PP V-bottom plates by centrifugation (1 min, 100 g), and dried at 50 °C in a vacuum centrifuge.

Trypsin digestion

The samples were reconstituted in 100 μL 20 mM ammonium bicarbonate buffer and reduced with 2 μL dithiothreitol (DTT) 125 mM for 30 min at 60 °C. 4 μL of 125 mM of iodoacetamide (IAA) was added for alkylation and the samples incubated with shaking for 30 min at RT wrapped in aluminum foil. The alkylation was quenched by adding 2 μL 100 mM DTT. The digestion was performed overnight at 37 °C with 210 ng of TPCK-treated trypsin and the samples were frozen at -20°C until measurement.

RP-LC-ESI-MS(/MS)

Reverse-phase liquid chromatography electrospray-ionization mass spectrometry (RP-LC-ESI-MS) experiments were performed with an Ultimate 3000 RSLCnano system (Thermo Fisher Scientific) fitted with a Pepmap100 C18, 5 μm 0.3x5 mm precolumn and a custom-made Acquity BEH130 C18 75 μm x 100 mm UPLC M-Class analytical column. This was coupled to a Maxis Impact HD quadrupole-time-of-flight (qTOF) mass spectrometer fitted with a captive spray nanoBooster (Capillary voltage: 1200V; nanoBooster pressure 0.2 bar; dry gas flow: 3.0 L/min; dry gas temperature: 180 °C) (Bruker Daltonics, Bremen, Germany). One μL of digested sample was injected, with a flow rate of 500 $\mu\text{L}/\text{min}$, eluted with the following gradient: 3% solvent B at 0 min, 30% B at 6.5 min, 95% B at 10 min, hold for two min, back to 3% B at 13 min, hold for eight min. Solvent A consisted of 0.1% TFA (v/v), solvent B of 95% ACN (v/v). Mass spectra were recorded from 550 to 1800 Th with a frequency of 1 Hz.

To confirm the structures of certain analytes, high-resolution fragmentation spectra of selected glycopeptides were recorded on a different instrument. The material used for the MS/MS glycopeptide identification was a pooled sample of captured IgA taken from the first 96-well plate of the prepared samples including material from HC, CD, UC and control plasma (VisuconF, Affinity Biologicals, Canada). For this, a nanoLC (Thermo Fisher Scientific) was equipped with an Acclaim PepMap 100 trap column (100 μm $\times$ 20 mm, particle size 5 μm , Thermo Fisher Scientific) and an Acclaim PepMap RSLC C18 nano-column (75 μm $\times$ 150 mm, particle size 2 μm , Thermo Fisher Scientific). The separation gradient started from 97% solvent A (0.1% formic acid in water) and 3% solvent B (95% ACN) to 53% solvent B over 30 min, with a flow rate of 700 nL/min. The system was coupled to a maxis quadrupole time-of-flight-MS (q-TOF-MS; Bruker Daltonics) equipped with a nanoBooster (Bruker Daltonics). Ionization was enhanced by applying acetonitrile-enriched gas at 0.2 bar used as a dopant. The source parameters were set to a flow of nitrogen drying gas of 3.0 L/min at 180°C and a capillary voltage of 1200 V. The MS was operated in stepping-energy CID mode and acquired from m/z 50-2800 with the precursor selection above m/z value 680 as previously described²⁸.

Data processing

Raw LC-MS data were converted to mzXML with MSConvert. LacyTools²⁹ was used to align the retention time, calibrate the mass spectra, define time windows around each glycopeptide cluster extraction and for the integration of the sum spectra for a list of analytes determined by manual exploration of the data of each disease group using sum spectra and theoretical glycosylation sites based on the peptide sequence (Uniprot # P01876 for IgA1 and P01877 for IgA2). For time alignment, the most abundant glycopeptide of each cluster was used (search windows of 20 s) and the maximum mass error of the highest isotope ± 0.15 Th. A time window of ± 8 s per cluster (10 s for the *O*-glycopeptide) was summed, and each extracted glycoform was reported as relative percentage per cluster. The optimal time windows for analyte extraction were determined for each cluster by monitoring the elution profile and retention time of all extracted analytes per glycopeptide cluster. The average retention time of the cluster was then calculated and the sum windows fixed to include all analytes. The extracted glycopeptide data was curated on average signal-to-noise ratio threshold >9 , absolute error on exact mass <20 ppm, and deviation compared to the calculated isotopic pattern <0.2 . This curation was applied for all detected charge states. Per sample, the data was kept when not more than one peptide cluster was rejected due to low-abundance of analytes. A final list of 51 glycopeptides was retained (26 *N*-linked and 25 *O*-linked glycopeptides (**Supplementary table 1, Supplementary table 2**) and from these, 45 derived traits were calculated (**Supplementary table 3**).

The identity of glycopeptides was verified by MS/MS using Byonic. The search parameters included the protein sequences of secreted IgA1 (P01876-1) and IgA2 (P01877-1) from Uniprot in FASTA format, cysteine alkylation as fixed peptide modification, two possible missed cleavages, and a semi-specific (N-ragged) digestion specificity. Furthermore, a list of 318 *N*-glycan compositions and 9 *O*-glycan compositions was included based on IgA glycoforms previously identified (based on monosaccharides HexNAc, Hex, Fuc, NeuAc and NeuGc)^{15, 30, 31}. The precursor mass tolerance was set at 10 ppm, the fragment mass tolerance at 30 ppm. The results of the Byonic search were filtered based on the identification score (Pep2D) to be below 0.001, manually expected (at least one glycoform per peptide portion) and are reported in **Supplementary table 2** and **Supplementary Figure 1-4**. The identity of the IgA glycoforms that were not reported by Byonic were deduced by exact mass differences in the high-resolution MS1 data, including retention time and isotope pattern matching (**Supplementary Figures 5-9**).

Statistical analysis

The associations of the IgA glycopeptides and calculated derived traits with CD, UC, and IBD-specific clinical traits, *i.e.*, extent of the disease, drug intake and surgical intervention, were tested as previously described in the software R studio for R (Version 1.0.136)⁴. In short, the data was batch corrected (on logarithmically transformed data) to account for the different preparation days, position on row and column of the randomized samples on the 96-well plates. Associations with age and sex were determined in the three patient groups with linear and logistic regressions, included further as covariates in the logistic regression model. The associations of the IgA glycosylation with age, sex, Crohn's disease, ulcerative colitis and IBD-related parameters such as disease location, surgery and medication, were tested by linear and logistic regression on scaled data (subtraction of the mean and division by the SD). Age, sex and their interaction were included as covariates in the models for the disease-related tests. The odds ratios (OR) were calculated with their 95% confidence intervals (CI) assuming a Student's *t*-distribution, and are thus representative of single standard deviation increases in the tested traits.

For the receiver operating characteristic (ROC) analysis, the glycopeptides most significantly associated with IBD (*p*-values and effect sizes) were selected for the initial model. The ones found to not significantly contribute to the model were removed step-by-step considering similarities of derived traits (which can share effects in the model and lower their individual significance while only reflect a single biological trait) and percentage of effect on the prediction until only significant traits were left, thus achieving maximal prediction power with only the necessary selection of traits. The model was trained 20 times on a random selection of 75% of the

samples and the prediction was calculated for the respective 25% remaining. The standard error (SE) was also calculated for all predictions.

The Montreal classification of inflammatory bowel disease was followed to categorize disease localization, extent and behavior for CD and UC patients²⁷. The different localization and extent of the disease were compared in CD patients affected either in the ileum (L1), the colon (L2), or both the ileocolon (L3) and in the upper gastro-intestinal (GI) tract (L4) (L1 vs L2 vs L3+L4). Association with disease behavior was tested by comparing the inflammatory (B1), the stenosing (B2), and the fistulizing variant (B3) (B1 vs B2 vs B3). In UC, the patients groups affected in the rectum + sigmoid + left colon (E1+E2) were compared to the ones affected in the entire colon (E3), (E1+E2 vs E3). The hypothesis tested here is that a more extensive or aggressive disease behavior would associate with more extreme glycosylation profiles compared to HC.

We also tested for association of profiles with surgery and medication. According to the guidelines of the respective countries to treat IBD diseases, patients were given mesalazine, steroids, azathioprine/6-mercaptopurine or anti-TNF α . Patients were stratified according to escalation of drug class used at the time of sample collection according to the practices of the medical center and the glycosylation profiles respective to each drug class were compared to each other^{32, 33}. The hypothesis tested here is that patients suffering from more severe disease exhibit more extreme IgA glycosylation profiles and these patients can be identified based on their need to use more potent drugs. The groups of patients not requiring surgery were opposed to those who received surgery after sample collection, again using surgical intervention as a proxy for disease severity. Moreover, we compared the profiles of patients who received surgery before or after sample collection to evaluate the treatment effect.

The commonly accepted significance threshold of $\alpha = 0.05$ was adjusted for multiple testing with a Bonferroni correction when evaluating the associations with age, sex, CD and UC. As the relative intensities of the glycopeptide signals are intimately related, the number of tests performed on the derived traits was used as correction factor.

Results

Following site-specific IgA1/IgA2 glycosylation analysis by LC-MS/MS, a total of six *N*-linked glycoforms was characterized on Asn₁₄₄, eight on Asn₂₀₅, seven on Asn₃₄₀, five on the two different clusters of the truncated peptide containing Asn₃₄₀, and 25 *O*-linked glycoforms on the peptide portion containing Ser₈₉₋₁₂₆ (**Figure 1**, **Figure 2**). Some glycopeptides similar to those found on Asn₃₄₀ of IgA1 were observed on Asn₃₂₇ of IgA2 (10x less abundant than IgA1) during the manual exploration of the data but the list of potential analytes did not pass the curation step during data extraction due to a low S/N ratio, also impacting their isotopic pattern match compared to the calculated pattern so they were rejected from the statistical analysis. No confident identifications for the IgA2 glycopeptides covering Asn₃₂₇ were found starting with amino acids MAG instead of LAG (IgA2 isoform #P01877-1) in the Byonic workflow. No glycopeptides passing our quality criteria were observed for the theoretical glycosylation site of Asn₄₇ of IgA2 nor for Asn₉₂ of the low abundant allotype A2M of IgA2. Trace amounts of the what could be VFP glycopeptide (Asn₄₇) fragments with a different cleavage at the *N*-terminal of its sequence (SES) were detected, but not further evaluated due to their low abundance. For clarity of the discussion, the peptides will be referred to by the one-letter codes of the first three amino acids (and last for the peptides with a truncated variant); amino acid sequences are shown in **Figure 2**.

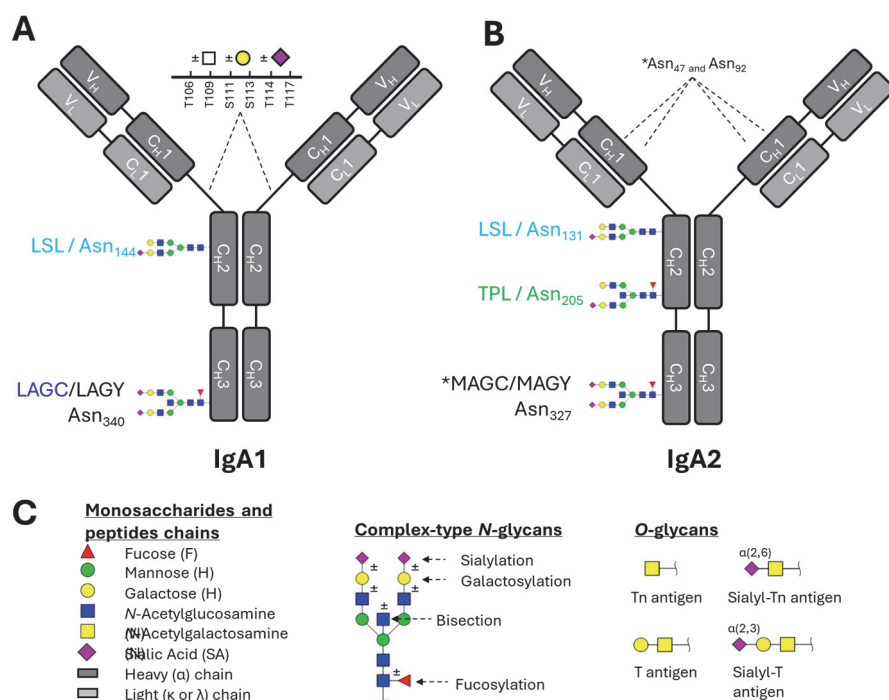


Figure 1: Symbolic representation of IgA1 and IgA2 glycosylation. The acronyms HYT, LSL, TPL, LAG(C/Y) and MAG(C/Y), which are referring to the different glycopeptides, come from the first three amino acids of the corresponding tryptic digest sequence of IgA1 and IgA2. A) IgA1 has two N-glycosylation sites at Asn₁₄₄ and Asn₃₄₀ which are occupied by complex-type N-glycans. IgA1 also contains six O-glycosylation sites in the hinge region at Thr₁₀₆, Thr₁₀₉, Ser₁₁₁, Ser₁₁₃, Thr₁₁₄ and Thr₁₁₇. The O-glycosylation of IgA1 is represented as one combined monosaccharide composition, as no information on specific glycan structures and attachment sites was obtained. B) The observed glycosylation sites of IgA2 at Asn₁₃₁, Asn₂₀₅ and Asn₃₂₇ are occupied with complex-type N-glycans. * The IgA2 glycosylation sites Asn₄₇ and Asn₉₂ and Asn₃₂₇ did not pass quality curation criteria due the low intensity of analytes. C) Symbols used for representing the glycans and their general antennary structure (N-linked glycans) and example structures for the O-linked glycans.

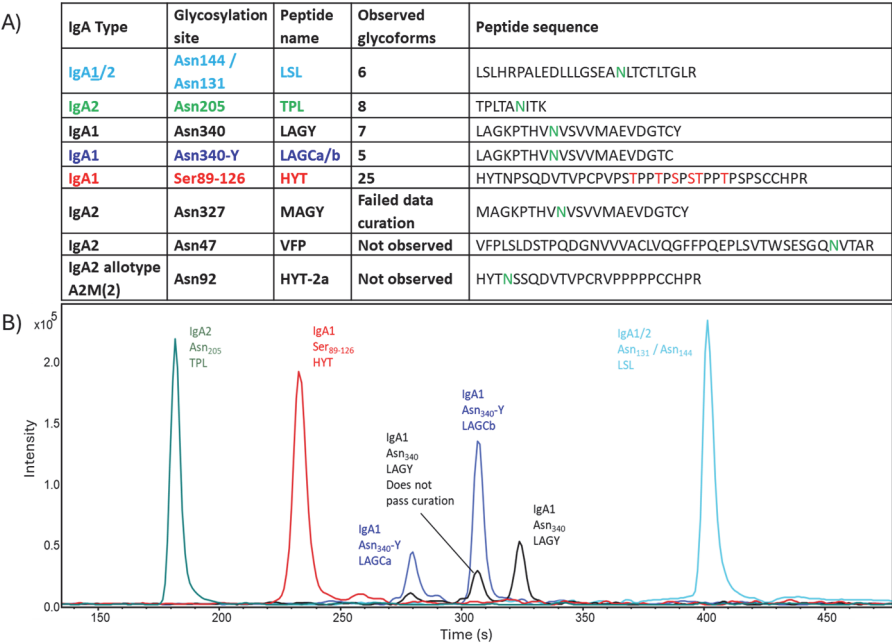


Figure 2: Description of the glycopeptide clusters. A) IgA1 and IgA2 glycopeptides overview. Glycosylation sites, short peptide name, number of extracted glycoforms, and peptide sequence are given for each glycopeptide cluster. B) Extracted Ion Chromatograms (EIC) of one selected glycopeptide per cluster with representative retention time of the whole glycopeptide cluster.

The identification of the glycoforms of the different detected peptides is depicted in **Figure 3**, **Figure 4** and in the **supplementary figures 1-9**. The peptide portion was annotated based on MS/MS data of high abundant parent ions as shown in **the supplementary material**. The supplementary material is available at <https://pubs.acs.org/doi/10.1021/acs.jproteome.3c00260>.



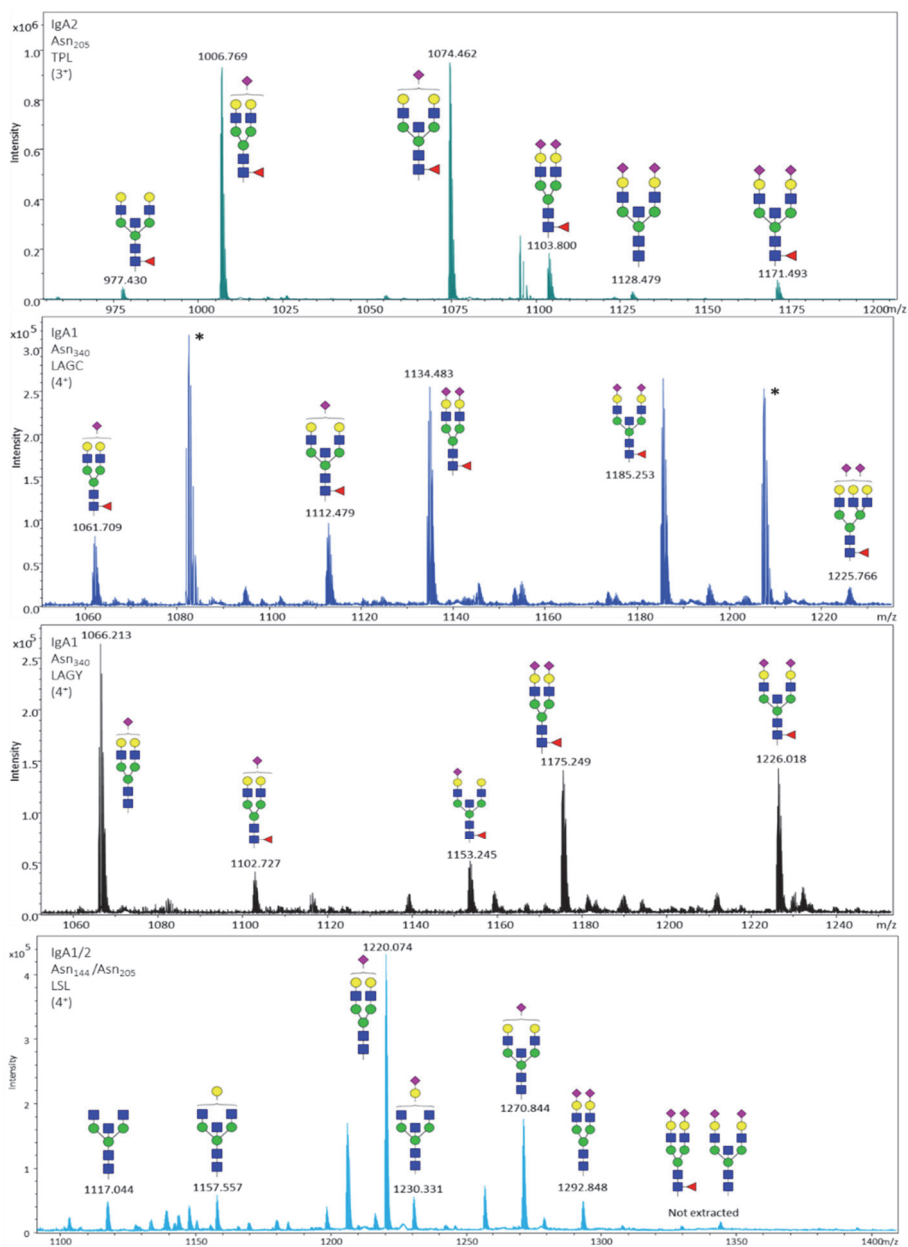


Figure 3: Assignment of the major N-glycoforms of IgA1 and IgA2. A) Peptide cluster TPL at Asn₂₀₅ of IgA2, zoom-in off the 3+ charged glycopeptides. B) Truncated peptide cluster LAGC at Asn₃₄₀ of IgA1, zoom-in off the 4+ charged glycopeptides. The two peaks marked with an asterisk are residues from another peptide without glycan eluting at the same time and in charge state 2+. C) Peptide cluster LAGY at Asn₃₄₀ of

*IgA1, zoom-in of the 4+ charged glycopeptides. D) Peptide cluster LSL at Asn₁₄₄/Asn₂₀₅ of IgA1 and IgA2 and zoom-in of the 4+ charged glycopeptides. All the glycoforms are described in **the supplementary material**.*

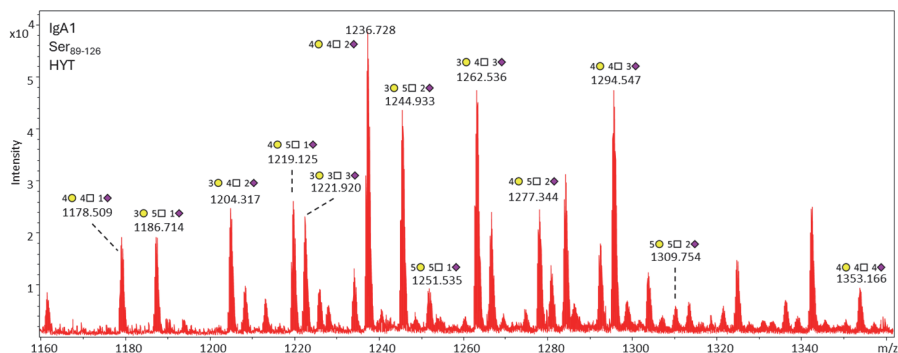


Figure 4: Assignment of O-glycoforms of peptide cluster HYT. Assignment of O-glycoforms of peptide cluster HYT at Ser₈₉₋₁₂₆ of IgA1 and zoom-in of the 5⁺ charged glycopeptides. The pictograms represent the combined O-glycopeptide monosaccharide composition from all sites and does not convey information on specific glycan structures of attachment sites. Overall, the glycopeptides covered a wide range of compositions ($H_{3-5}N_{3-6}S_{1-5}$) containing up to 6N. Not all glycoforms are labeled on the figure as the complete list of the 25 IgA1 hinge region O-glycopeptide species identified and quantified is reported in **the supplementary material**.

IgA glycosylation is associated with age and sex in healthy controls and IBD patients

In HC, CD and UC, an increase of bisection, a decrease of galactosylation and sialylation per galactose were observed with age on all N-glycosylation sites (TPL, LAGY and the truncated LAGC glycopeptides) as reported in **Supplementary table 4**. A decrease of antennarity was observed in the glycans of the TPL peptide of HC and on the glycans of the LAGY peptide of HC and UC patients. No association between the O-glycans of the HYT peptide and age was found significant after correction for multiple testing.

The N-glycosylation of IgA was associated with sex, where a decrease of antennarity was observed on the TPL peptide in CD and UC males compared to the females **Supplementary table 4**. UC males had lower bisection levels than the females in all the N-glycopeptides clusters.

IgA glycosylation is associated with inflammatory bowel disease

Compared to HC, CD patients were found to have lower galactosylation and lower sialylation on LSL (Asn₁₄₄) and TPL (Asn₂₀₅) (Figure 5 and Supplementary table 5). CD patients also showed lower levels of antennarity (lower CA3) and higher bisection on Asn₃₄₀ and its truncated versions compared to HC and UC. CD and UC patients had a smaller amount of GalNAc on the O-glycans of the Ser₈₉₋₁₂₆-containing peptide than HC. In addition, the CD group was found to have a lower relative abundance of SA and lower ratio of SA per galactose in the O-linked glycans than in UC.

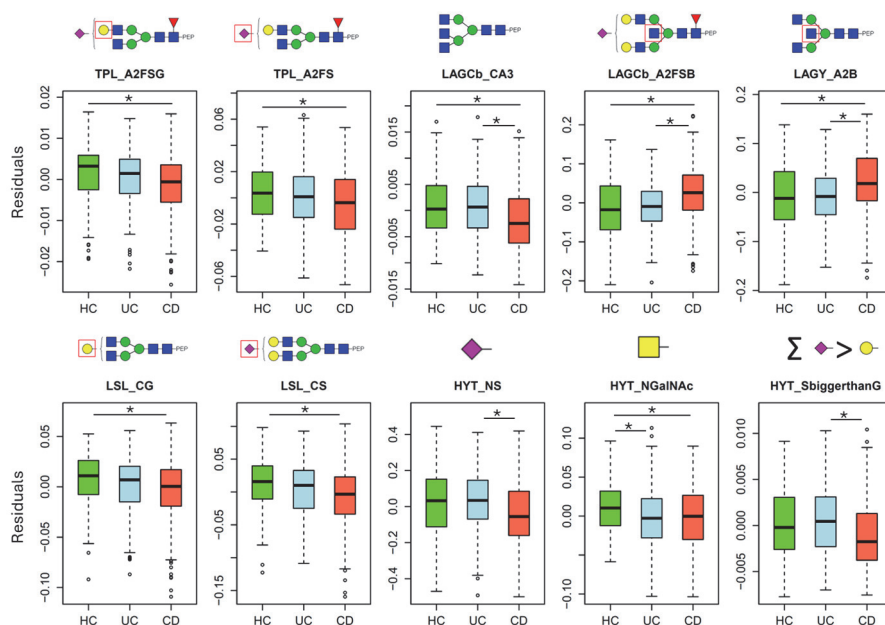


Figure 5: Main associations between IgA glycosylation derived traits and IBD per patient groups (HC = healthy controls, UC = ulcerative colitis, CD = Crohn's disease). The derived traits were corrected age, sex and age*sex and are shown in boxplots. The 25th, 50th and 75th percentiles are represented in the boxes and the whiskers are at the 1st quartile – 1.5 * IQR (inter-quartile range) and at the 3rd quartile + 1.5 * IQR. P-values, ORs and confidence intervals are reported in **Supplementary Table 4**. Statistically significant associations (multiple testing corrected threshold $3.70 \cdot 10^{-4}$ as described in the supplementary tables) are marked with an asterisk.

The ROC curves (Figure 6) that were calculated with a linear model based on the glycopeptides most significantly associated with IBD included age, sex and their interaction with the bisection and triantennarity of LAGC (LAGCb_CB, LAGCb_CA3),

the bisection of sialylated diantennary LAGY glycopeptides (LAGY_A2SB) and the sum of O-glycopeptides featuring higher number of SAs than galactoses (HYT_S>G). The prediction power for CD vs HC was good with an AUC of 0.790 ± 0.045 . The power of the model was fair for predicting UC vs HC with an area under the curve (AUC) of 0.641 ± 0.052 SE and also fair for UC vs CD with an AUC of 0.674 ± 0.035 .

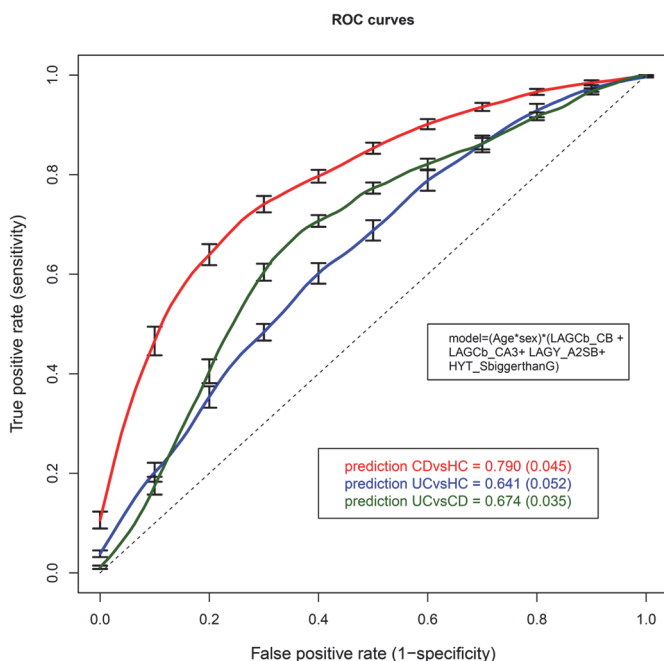


Figure 6: Receiver operating characteristic curves showing the power of selected glycans traits to predict CD and UC. The mean and SE of 20 predictions are reported for the area under the curve. The prediction model included the interactions of $age*sex*(LAGCb_CB + LAGCb_CA3 + LAGY_A2SB + HYT_S>G)$.

Associations of IgA glycopeptides with CD and UC disease location and behavior were tested as previously described^{3,4} but no significant results were found in this study (**Supplementary table 6, Supplementary table 7 Supplementary table 8**). No significant associations were found between patients who received surgery vs non-surgery patients, and no associations were found in IgA glycosylation profiles of patients before and after surgery (**Supplementary table 9**).

When searching for associations of IgA glycosylation with the most potent drug administered to the patients, UC patients using AZT/6-MP were found to have lower galactosylation and lower sialylation of TPL and LSL peptides compared to UC mesalazine users (**Figure 7**).

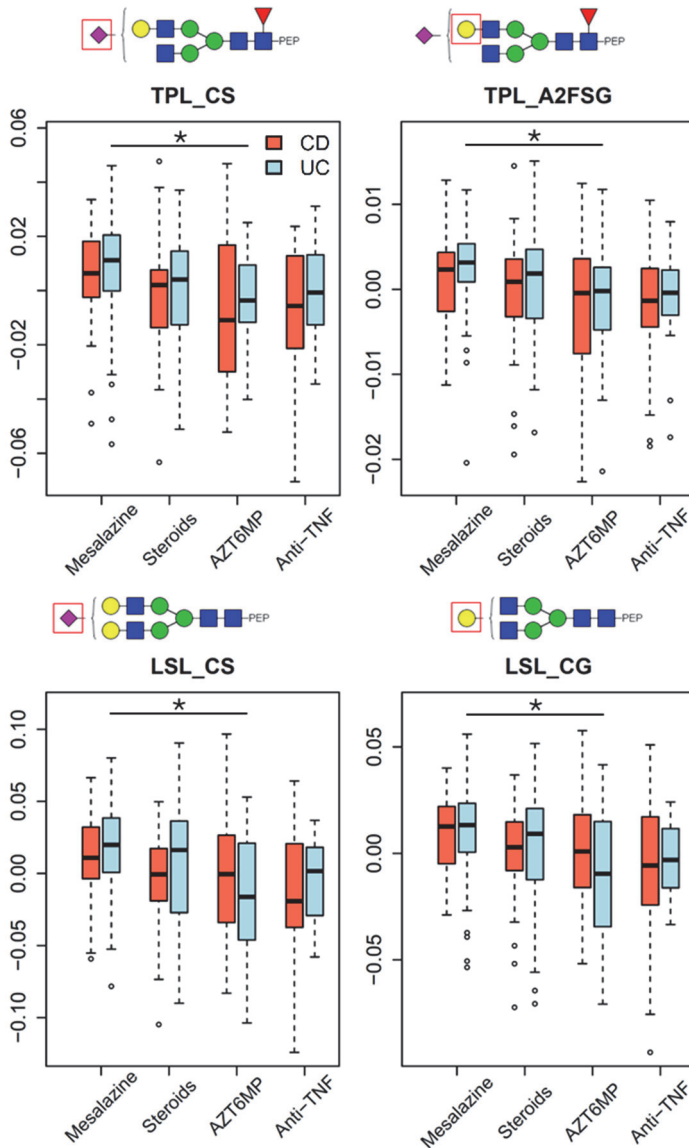


Figure 7: Main associations between IgA glycosylation derived traits and medication in CD and UC. The derived traits were corrected age, sex and age*sex and are shown in boxplots. The 25th, 50th and 75th percentiles are represented in the boxes and the whiskers are at the 1st quartile – 1.5 * IQR (inter-quartile range) and at the 3rd quartile + 1.5 * IQR. P-values, ORs and confidence intervals are reported in **Supplementary Table 9**. Statistically significant associations (multiple testing corrected threshold $1.85 \cdot 10^{-4}$ as described in the supplementary tables) are marked with an asterisk.

Discussion

With our newly developed LC-MS/MS method, we could show that CD differed from HC in terms of IgA1 *O*-glycosylation as well as IgA1 and IgA2 *N*-glycosylation with regard to antennarity, bisection, galactosylation, and sialylation. In UC, mostly the *O*-glycosylation was altered compared to HC. Overall, for all associations of IgA glycosylation we found between IBD and HC, CD patients seemed to have the more extreme profile of inflammation-associated IgA glycosylation and UC is showing less extreme profiles, similarly to what has been found for the total plasma *N*-glycome and in an IgG-specific study^{3,4}. In the following, IgA glycosylation signatures of CD and UC will be discussed on the background of other cohort studies covering IgA glycosylation (**Figure 8**).

It is possible that with our analytical approach the changes in glycosylation observed in the IgA captured from serum reflects more global changes in glycosylation of immune system than what could be observed in localized tissue samples of the inflamed areas. However, sampling serum from patients is far less invasive than sampling tissue and is thus a more easily accessible biomarker specimen.

Antennarity and bisection

On the LAGY peptide comprising Asn₃₄₀, the glycosylation showed lower antennarity and elevated bisection in CD patients as compared to controls, parallel to what has been observed in other diseases such as RA and IgA nephropathy^{14, 15}. In contrast, pregnancy has been found to be associated with lower bisection as well as with a concomitant improvement of the inflammation status in pregnant RA patients³⁴. In other studies, a decrease of antennarity has been observed after delivery for both IgA and IgG^{14, 34}. These two glycosylation traits were found to differ significantly between CD and UC, however, the functional role of these differences is not clear. These differences are also mainly IgA1 specific as IgA2 does not normally contain the LAGC/LAGY glycopeptide, but rather has a slightly different protein backbone MAGC/MAGY (in IgA2 isoform #P01877-1) that was not detected in our study. There could be an exception to this in the case of a rare mutation of the IgA2 Met₃₁₉ which causes it to have the same glycopeptide LAG as IgA1.

Galactosylation

In this study, a lower galactosylation in CD is reported on two glycosylation sites (TPL and LSL, **Supplementary table 1**), while a previous study reported no difference in IgA galactosylation in IBD vs HC²². This might be due to the increased performance of our newly developed analytical workflow enabling us to handle the larger number of cases analyzed here. Multiple studies have reported lower IgG galactosylation in

IBD patients and other diseases as non-specific inflammation marker and this appears to be in line with our findings for IgA, although with a less prominent effect size^{3, 22, 35}. It is known that the clearance of IgA can be affected by its terminal *N*-glycosylation motifs and could thus be a factor of inflammation in IBD as well^{36, 37}. The changes in galactosylation on TPL are IgA2-specific, while the changes observed on LSL are shared between IgA1 and IgA2 which could indicate a similar glycosylation mechanism for both sites and a more pronounced pro-inflammatory profile of IgA2²³.

Sialylation

The lower sialylation of *N*-glycans that was found on LSL and TPL glycopeptides of CD patients has also been found to be associated with other diseases such as IgA nephropathy and RA, and could be promoting the inflammation and, as discussed for galactosylation, could expose more galactose-terminated glycans and impact IgA clearance from the circulation^{14, 15, 23}. The opposite effect has been observed during the late stage of pregnancy where elevated sialylation of LSL and LAGY/C glycopeptides were found before a return to normal levels post-delivery, suggesting an anti-inflammatory effect of higher sialylated *N*-glycans of IgA^{13, 14}.

O-Glycosylation

Overall, the *O*-glycopeptides covered a wide range of compositions ($H_{3-5}N_{3-6}S_{1-5}$) containing up to 6N e.g. H4N6S2 and H4N6S2, which is in accordance with literature data that indicates a variation in site occupancy from 3 to 6 sites with predominantly core 1 structures³¹. Our findings of lower number of GalNAcs on the IgA1 hinge-region *O*-glycopeptide of IBD patients are linked to increased disease activity and are in accordance with previous findings in IBD patients²². These results are also comparable to those found in IgA nephropathy and in RA (**Figure 8**)^{22, 38-40}. The similarities of deficient *O*-glycosylation in IBD and other inflammatory diseases indicate a possible role in modulating IgA function which is however largely unexplored⁴¹⁻⁴⁵. An exception to that would be the role of agalactosylated IgA1 hinge region *O*-glycans which has been reported to be a target of autoantibodies in IgA nephropathy⁴⁶.

Intervention and medication

The low number of significant associations found between IgA and treatments, whether surgical or medical, is most likely due to small effect size of the tested associations and the low statistical power possible in this study with the low number of patients in each category (Table 1) with the stringent Bonferroni correction with 270 tests performed (**Supplementary table 9**).

It is interesting to note that the significant traits of IgA glycosylation associated with medication found in this study are similar to what was previously reported with the analysis of the TPNG where lower galactosylation in UC patients was associated with the use of more potent drugs⁴. In a comparable study about IgAN, patients had lower levels of sialylation on the TPL and LSL glycopeptide compared to the controls¹⁵. Here, UC patients who were administered the more potent drugs similarly exhibited lower levels of sialylation on these two glycopeptide clusters.

Methodological considerations

While sialic acids usually have a strong effect on the retention time of glycopeptides in reverse-phase LC, as shown here for the MS2 identification data using a gradient containing formic acid, in our high resolution MS1 method used for the high-throughput measurements we optimized the LC conditions by adding TFA to the eluents to prevent this effect and to allow robust data processing as previously described^{15, 47}.

For the samples analyzed here, IgA concentration was not measured but it has previously been reported that IgA concentrations do not differ between IBD and HC⁴⁸. The approach used for the sample preparation included an excess of immunoaffinity beads for capturing and total area normalization of the extracted glycans during data analysis enabled us to look at changes in the glycosylation profile of IgA without correction for IgA concentration.

The LC-MS method used here is not able to provide absolute quantitative results as no internal standard were included. However, when comparing different disease groups on the same analytes, it is possible to estimate if a cluster, *e.g.*, TPL (which is specific to IgA2), is elevated compared to the other groups. In this regard, as CD had a higher relative abundance of the TPL cluster (data not shown) compared to HC and UC, this could indicate that the ratio IgA1/IgA2 is decreased in CD, and that the difference in ratio could be associated with autoimmune diseases²³. This could be further investigated by quantitative assays such as isotype-specific ELISAs or by adding isotopically labeled standards to the samples for MS analysis.

Similar studies

All the associations reported in this study were similarly reported for IgA and IgG in IBD, IgA nephropathy, and other autoimmune disease like RA, except for the sialylation of the O-glycans in RA and the bisection of IgG in CD vs HC (**Figure 8**)^{3, 13-15, 22, 34, 38, 40, 45, 49}. The changes in IgA glycosylation with IBD were mostly opposite of the associations reported during pregnancy, similar to IgG where glycosylation

changes with inflammatory conditions were opposed to those occurring with pregnancy^{13, 14, 34}.

Reference	This Study	Clerc 2021	Simurina 2018	Inoue 2012	Shinzaki 2013	Hiki 2001	Wada 2010	Bondt 2016	Bondt 2017	Bondt 2013	Field 1994
Immunoglobulin type (IgA / IgG)	IgA	IgA	IgG	IgG	IgA	IgA1	IgA	IgA	IgA	IgG	IgG IgA
IgA Type	Glycosylation site / Peptide	CD vs UC	CD vs UC	CD vs UC	CD vs UC	CD vs UC	CD vs UC	CD vs UC	CD vs UC	Preg-nancy	RA
		HC	HC	HC	HC	HC	HC	HC	HC		
IgA1 IgA2	Asn144 / Asn131 LSL	G↓ S↓	- -	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓
		G↓ S↓	- -	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓
IgA2	Asn205 TPL	G↓ S↓	- -	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓
		G↓ S↓	- -	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓	G↓ S↓
IgA1	Asn340 LAGY	A↓ B↑	- -	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑
		A↓ B↑	- -	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑
IgA1	Asn340-Y LAGC	A↓ B↑	- -	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑
		A↓ B↑	- -	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑	A↓ B↑
IgA1	Ser89-126 HVT	S↓ S/G↓ GN↓	GN↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓
		S↓ S/G↓ GN↓	GN↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓	S↓ S/G↓ GN↓

N-glycosylation

O-glycosylation

Figure 8: Comparison of associations found between IgA glycosylation and IBD in this study with IgA and IgG glycosylation associated with IBD, IgA Nephropathy, RA and pregnancy associated changes in recent literature. A= Antennarity, B = Bisection, G= Galactosylation, GN= GalNAc, S= Sialylation. ↑ = Trait elevated in cases vs HC, ↓ = Trait lower in cases vs HC. In this study, the associations found between IgA glycosylation and IBD are marked in black. In the other studies, the color green indicates a significant association in the same direction (cases vs HC) as reported here, red indicates association in the opposite direction, and blue indicates reported associations that were not found in our study. The studies used for comparison are part of the bibliography cited in this article.

Conclusion

In this study, we applied our recently developed IgA glycopeptide analytical workflow to a large number of samples and reported novel associations of IgA glycosylation with IBD. Our prediction model had a good performance to predict CD versus HC whilst the discrimination power for UC was lower. The possibility to detect IBD and discriminate CD from UC should be considered with using readily available biofluids as a first tentative approach for the early diagnostic of IBD before attempting invasive examination procedures for the comfort of the patients.

Authors

All authors had access to the study data and approved the final manuscript.

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Raw Data

Mass spectrometry proteomics data raw mzXML datafiles have been deposited to the CMMS MassIVE archive [doi:10.25345/C5W08WS34] [dataset license: CC0 1.0 Universal (CC0 1.0)] at <ftp://MSV000091731@massive.ucsd.edu> (FTP download link) and <ftp://massive.ucsd.edu/MSV000091731/> (public URL).

Supplementary Material

The supplementary material is available at <https://pubs.acs.org/doi/10.1021/acs.jproteome.3c00260>.



Supplementary table 1: Properties of analytes of IgA glycopeptides

Supplementary table 2: Byonic peptide fragmentation and identification

Supplementary table 3: Derived traits calculations

Supplementary table 4: Associations of IgA glycopeptides with age and sex

Supplementary table 5: Associations of IgA glycopeptides with IBD

Supplementary table 6: Associations of IgA glycopeptides with disease location in CD

Supplementary table 7: Associations of IgA glycopeptides with disease behavior in CD

Supplementary table 8: Associations of IgA glycopeptides with disease location in UC

Supplementary table 9: Associations of IgA glycopeptides with surgery

Supplementary table 10: Associations of IgA glycopeptides with drug strength

Supplementary Figure 1: Byonic identification of the glycopeptides TPL

Supplementary Figure 2: Byonic identification of the glycopeptides LAGC/Y

Supplementary Figure 3: Byonic identification of the glycopeptides LSL

Supplementary Figure 4: Byonic identification of the glycopeptides HYT

Supplementary Figure 5: Glycopeptide clusters and MS1 annotation of TPL

Supplementary Figure 6: Glycopeptide clusters and MS1 annotation of LAGC

Supplementary Figure 7: Glycopeptide clusters and MS1 annotation of LAGY

Supplementary Figure 8: Glycopeptide clusters and MS1 annotation of LSL

Supplementary Figure 9: Glycopeptide clusters and MS1 annotation of HYT

References

- (1) Reily, C.; Stewart, T. J.; Renfrow, M. B.; Novak, J. Glycosylation in health and disease. *Nature Reviews Nephrology* **2019**, *15* (6), 346-366
- (2) Dotz, V.; Wuhrer, M. N-glycome signatures in human plasma: associations with physiology and major diseases. *Febs Lett* **2019**, *593* (21), 2966-2976
- (3) Simurina, M.; de Haan, N.; Vuckovic, F.; Kennedy, N. A.; Stambuk, J.; Falck, D.; Trbojevic-Akmacic, I.; Clerc, F.; Razdorov, G.; Khon, A.; Latiano, A.; D'Inca, R.; Danese, S.; Targan, S.; Landers, C.; Dubinsky, M.; Inflammatory Bowel Disease Biomarkers, C.; McGovern, D. P. B.; Annese, V.; Wuhrer, M.; Lauc, G. Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases. *Gastroenterology* **2018**, *154* (5), 1320-1333 e1310
- (4) Clerc, F.; Novokmet, M.; Dotz, V.; Reiding, K. R.; de Haan, N.; Kammeijer, G. S. M.; Dalebout, H.; Bladergroen, M. R.; Vukovic, F.; Rapp, E.; Consortium, I.-B.; Targan, S. R.; Barron, G.; Manetti, N.; Latiano, A.; McGovern, D. P. B.; Annese, V.; Lauc, G.; Wuhrer, M. Plasma N-Glycan Signatures Are Associated With Features of Inflammatory Bowel Diseases. *Gastroenterology* **2018**, *155* (3), 829-843
- (5) Trbojević-Akmačić, I.; Lageveen-Kammeijer, G. S.; Heijls, B.; Petrović, T.; Deris, H.; Wuhrer, M.; Lauc, G. High-throughput glycomic methods. *Chemical reviews* **2022**, *122* (20), 15865-15913
- (6) Burisch, J.; Jess, T.; Martinato, M.; Lakatos, P. L.; EpiCom, E. The burden of inflammatory bowel disease in Europe. *Journal of Crohn's & colitis* **2013**, *7* (4), 322-337
- (7) Annese, V.; Daperno, M.; Rutter, M. D.; Amiot, A.; Bossuyt, P.; East, J.; Ferrante, M.; Götz, M.; Katsanos, K. H.; Kießlich, R. European evidence based consensus for endoscopy in inflammatory bowel disease. *Journal of Crohn's and Colitis* **2013**, *7* (12), 982-1018
- (8) Pezer, M. *Antibody Glycosylation*; Springer Cham, **2021**.
- (9) Plomp, R.; Ruhaak, L. R.; Uh, H.-W.; Reiding, K. R.; Selman, M.; Houwing-Duistermaat, J. J.; Slagboom, P. E.; Beekman, M.; Wuhrer, M. Subclass-specific IgG glycosylation is associated with markers of inflammation and metabolic health. *Scientific reports* **2017**, *7* (1), 1-10
- (10) Hansen, A. L.; Reily, C.; Novak, J.; Renfrow, M. B. Immunoglobulin A Glycosylation and Its Role in Disease. In *Antibody Glycosylation*, Pezer, M. Ed.; Springer International Publishing, **2021**; pp 433-477.
- (11) Plomp, R.; de Haan, N.; Bondt, A.; Murli, J.; Dotz, V.; Wuhrer, M. Comparative Glycomics of Immunoglobulin A and G From Saliva and Plasma Reveals Biomarker Potential. *Front Immunol* **2018**, *9*, 2436

- (12) Xue, J.; Zhu, L.-P.; Wei, Q. IgG-Fc N-glycosylation at Asn297 and IgA O-glycosylation in the hinge region in health and disease. *Glycoconjugate J* **2013**, *30* (8), 735-745
- (13) Bondt, A.; Nicolardi, S.; Jansen, B. C.; Stavenhagen, K.; Blank, D.; Kammeijer, G. S.; Kozak, R. P.; Fernandes, D. L.; Hensbergen, P. J.; Hazes, J. M.; van der Burgt, Y. E.; Dolhain, R. J.; Wuhrer, M. Longitudinal monitoring of immunoglobulin A glycosylation during pregnancy by simultaneous MALDI-FTICR-MS analysis of N- and O-glycopeptides. *Sci Rep* **2016**, *6*, 27955
- (14) Bondt, A.; Nicolardi, S.; Jansen, B. C.; Kuijper, T. M.; Hazes, J. M.; Van Der Burgt, Y. E.; Wuhrer, M.; Dolhain, R. J. IgA N-and O-glycosylation profiling reveals no association with the pregnancy-related improvement in rheumatoid arthritis. *Arthritis research & therapy* **2017**, *19* (1), 1-8
- (15) Dotz, V.; Visconti, A.; Lomax-Browne, H.; Clerc, F.; Ederveen, A. H.; Medjeral-Thomas, N.; Cook, H. T.; Pickering, M.; Wuhrer, M.; Falchi, M. O-and N-Glycosylation of Serum Immunoglobulin A is Associated with IgA Nephropathy and Glomerular Function. *Journal of the American Society of Nephrology* **2021**, *32* (10), 2455-2465
- (16) Clerc, F.; Reiding, K. R.; Jansen, B. C.; Kammeijer, G. S.; Bondt, A.; Wuhrer, M. Human plasma protein N-glycosylation. *Glycoconj J* **2016**, *33* (3), 309-343
- (17) Trimarchi, H. M.; Iotti, A.; Iotti, R.; Freixas, E. A.; Peters, R. Immunoglobulin A nephropathy and ulcerative colitis. *American journal of nephrology* **2001**, *21* (5), 400-405
- (18) Forshaw, M.; Guirguis, O.; Hennigan, T. IgA nephropathy in association with Crohn's disease. *International journal of colorectal disease* **2005**, *20* (5), 463-465
- (19) Pouria, S.; Barratt, J. Secondary IgA nephropathy. In *Seminars in nephrology*, 2008; Elsevier: Vol. 28, pp 27-37.
- (20) Wang, Y.-N.; Zhou, X.-J.; Chen, P.; Yu, G.-Z.; Zhang, X.; Hou, P.; Liu, L.-J.; Shi, S.-F.; Lv, J.-C.; Zhang, H. Interaction between GALNT12 and C1GALT1 associates with galactose-deficient IgA1 and IgA nephropathy. *Journal of the American Society of Nephrology* **2021**, *32* (3), 545-552
- (21) Shi, D.; Zhong, Z.; Wang, M.; Cai, L.; Fu, D.; Peng, Y.; Guo, L.; Mao, H.; Yu, X.; Li, M. Identification of susceptibility locus shared by IgA nephropathy and inflammatory bowel disease in a Chinese Han population. *Journal of Human Genetics* **2020**, *65* (3), 241-249
- (22) Inoue, T.; Iijima, H.; Tajiri, M.; Shinzaki, S.; Shiraishi, E.; Hiyama, S.; Mukai, A.; Nakajima, S.; Iwatani, H.; Nishida, T.; Mizushima, T.; Yasui, T.; Isaka, Y.; Kanto, T.; Tsujii, M.; Miyoshi, E.; Wada, Y.; Takehara, T. Deficiency of N-acetylgalactosamine in O-linked oligosaccharides of IgA is a novel biologic marker for Crohn's disease. *Inflamm Bowel Dis* **2012**, *18* (9), 1723-1734

- (23) Steffen, U.; Koeleman, C. A.; Sokolova, M. V.; Bang, H.; Kleyer, A.; Rech, J.; Unterweger, H.; Schicht, M.; Garreis, F.; Hahn, J. IgA subclasses have different effector functions associated with distinct glycosylation profiles. *Nature communications* **2020**, *11* (1), 1-12
- (24) Rouwendal, G. J.; van der Lee, M. M.; Meyer, S.; Reiding, K. R.; Schouten, J.; de Roo, G.; Egging, D. F.; Leusen, J. H.; Boross, P.; Wuhrer, M.; Verheijden, G. F.; Dokter, W. H.; Timmers, M.; Ubink, R. A comparison of anti-HER2 IgA and IgG1 in vivo efficacy is facilitated by high N-glycan sialylation of the IgA. *mAbs* **2016**, *8* (1), 74-86
- (25) Ding, L.; Chen, X.; Cheng, H.; Zhang, T.; Li, Z. Advances in IgA glycosylation and its correlation with diseases. *Frontiers in Chemistry* **2022**, *10*,
- (26) Latiano, A.; Palmieri, O.; Latiano, T.; Corritore, G.; Bossa, F.; Martino, G.; Biscaglia, G.; Scimeca, D.; Valvano, M. R.; Pastore, M.; Marseglia, A.; D'Inca, R.; Andriulli, A.; Annese, V. Investigation of Multiple Susceptibility Loci for Inflammatory Bowel Disease in an Italian Cohort of Patients. *Plos One* **2011**, *6* (7), e22688
- (27) Satsangi, J.; Silverberg, M. S.; Vermeire, S.; Colombel, J. F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* **2006**, *55* (6), 749-753
- (28) Hinneburg, H.; Stavenhagen, K.; Schweiger-Hufnagel, U.; Pengelley, S.; Jabs, W.; Seeberger, P. H.; Silva, D. V.; Wuhrer, M.; Kolarich, D. The Art of Destruction: Optimizing Collision Energies in Quadrupole-Time of Flight (Q-TOF) Instruments for Glycopeptide-Based Glycoproteomics. *J Am Soc Mass Spectrom* **2016**, *27* (3), 507-519
- (29) Jansen, B. C.; Falck, D.; de Haan, N.; Hipgrave Ederveen, A. L.; Razdorov, G.; Lauc, G.; Wuhrer, M. LaCyTools: A Targeted Liquid Chromatography-Mass Spectrometry Data Processing Package for Relative Quantitation of Glycopeptides. *Journal of proteome research* **2016**, *15* (7), 2198-2210
- (30) Lippold, S.; de Ru, A. H.; Nouta, J.; van Veelen, P. A.; Palmblad, M.; Wuhrer, M.; de Haan, N. Semiautomated glycoproteomics data analysis workflow for maximized glycopeptide identification and reliable quantification. *Beilstein J Org Chem* **2020**, *16* (1), 3038-3051
- (31) Momcilovic, A.; de Haan, N.; Hipgrave Ederveen, A. L.; Bondt, A.; Koeleman, C. A. M.; Falck, D.; de Neef, L. A.; Mesker, W. E.; Tollenaar, R.; de Ru, A.; van Veelen, P.; Wuhrer, M.; Dotz, V. Simultaneous Immunoglobulin A and G Glycopeptide Profiling for High-Throughput Applications. *Anal Chem* **2020**, *92* (6), 4518-4526
- (32) Sandborn, W. J. The present and future of inflammatory bowel disease treatment. *Gastroenterology & Hepatology* **2016**, *12* (7), 438
- (33) Raine, T.; Bonovas, S.; Burisch, J.; Kucharzik, T.; Adamina, M.; Annese, V.; Bachmann, O.; Bettenworth, D.; Chaparro, M.; Czuber-Dochan, W. ECCO guidelines

on therapeutics in ulcerative colitis: medical treatment. *Journal of Crohn's and Colitis* **2022**, 16 (1), 2-17

(34) Bondt, A.; Selman, M. H.; Deelder, A. M.; Hazes, J. M.; Willemsen, S. P.; Wuhler, M.; Dolhain, R. J. Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation. *Journal of proteome research* **2013**, 12 (10), 4522-4531

(35) Bohm, S.; Kao, D.; Nimmerjahn, F. Sweet and sour: the role of glycosylation for the anti-inflammatory activity of immunoglobulin G. *Current topics in microbiology and immunology* **2014**, 382, 393-417

(36) Maverakis, E.; Kim, K.; Shimoda, M.; Gershwin, M. E.; Patel, F.; Wilken, R.; Raychaudhuri, S.; Ruhaak, L. R.; Lebrilla, C. B. Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: A critical review. *Journal of Autoimmunity* **2015**, 57 (0), 1-13

(37) Basset, C.; Devauchelle, V.; Durand, V.; Jamin, C.; Pennec, Y.; Youinou, P.; Dueymes, M. Glycosylation of immunoglobulin A influences its receptor binding. *Scandinavian journal of immunology* **1999**, 50 (6), 572-579

(38) Hiki, Y.; Odani, H.; Takahashi, M.; Yasuda, Y.; Nishimoto, A.; Iwase, H.; Shinzato, T.; Kobayashi, Y.; Maeda, K. Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. *Kidney international* **2001**, 59 (3), 1077-1085

(39) Ohyama, Y.; Yamaguchi, H.; Nakajima, K.; Mizuno, T.; Fukamachi, Y.; Yokoi, Y.; Tsuboi, N.; Inaguma, D.; Hasegawa, M.; Renfrow, M. B.; Novak, J.; Yuzawa, Y.; Takahashi, K. Analysis of O-glycoforms of the IgA1 hinge region by sequential deglycosylation. *Scientific Reports* **2020**, 10 (1), 671

(40) Wada, Y.; Tajiri, M.; Ohshima, S. Quantitation of saccharide compositions of O-glycans by mass spectrometry of glycopeptides and its application to rheumatoid arthritis. *Journal of proteome research* **2010**, 9 (3), 1367-1373

(41) Ding, L.; Chen, X.; Cheng, H.; Zhang, T.; Li, Z. Advances in IgA glycosylation and its correlation with diseases. *Frontiers in Chemistry* **2022**, 10, 974854

(42) Royle, L.; Roos, A.; Harvey, D. J.; Wormald, M. R.; van Gijlswijk-Janssen, D.; Redwan, E.-R. M.; Wilson, I. A.; Daha, M. R.; Dwek, R. A.; Rudd, P. M. Secretory IgA N-and O-glycans provide a link between the innate and adaptive immune systems. *J Biol Chem* **2003**, 278 (22), 20140-20153

(43) Kokubo, T.; Hiki, Y.; Iwase, H.; Tanaka, A.; Toma, K.; Hotta, K.; Kobayashi, Y. Protective role of IgA1 glycans against IgA1 self-aggregation and adhesion to extracellular matrix proteins. *Journal of the American Society of Nephrology* **1998**, 9 (11), 2048-2054

- (44) Yan, Y.; Xu, L.; Zhang, J.; Zhang, Y.; Zhao, M. Self-aggregated deglycosylated IgA1 with or without IgG were associated with the development of IgA nephropathy. *Clinical & Experimental Immunology* **2006**, *144* (1), 17-24
- (45) Field, M.; Amatayakul-Chantler, S.; Rademacher, T.; Rudd, P.; Dwek, R. Structural analysis of the N-glycans from human immunoglobulin A1: comparison of normal human serum immunoglobulin A1 with that isolated from patients with rheumatoid arthritis. *Biochem J* **1994**, *299* (1), 261-275
- (46) Rudd, P.; Fortune, F.; Patel, T.; Parekh, R.; Dwek, R.; Lehner, T. A human T-cell receptor recognizes' O'-linked sugars from the hinge region of human IgA1 and IgD. *Immunology* **1994**, *83* (1), 99
- (47) Falck, D.; Jansen, B. C.; de Haan, N.; Wuhrer, M. High-Throughput Analysis of IgG Fc Glycopeptides by LC-MS. In *High-Throughput Glycomics and Glycoproteomics: Methods and Protocols*, Lauc, G., Wuhrer, M. Eds.; Springer New York, **2017**; pp 31-47.
- (48) Macpherson, A.; Khoo, U. Y.; Forgacs, I.; Philpott-Howard, J.; Bjarnason, I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut* **1996**, *38* (3), 365-375
- (49) Shinzaki, S.; Kuroki, E.; Iijima, H.; Tatsunaka, N.; Ishii, M.; Fujii, H.; Kamada, Y.; Kobayashi, T.; Shibukawa, N.; Inoue, T. Lectin-based immunoassay for aberrant IgG glycosylation as the biomarker for Crohn's disease. *Inflammatory bowel diseases* **2013**, *19* (2), 321-331