



Published in final edited form as:

Gastroenterology. 2018 April ; 154(5): 1320–1333.e10. doi:10.1053/j.gastro.2018.01.002.

Glycosylation of Immunoglobulin G Associates With Clinical Features of Inflammatory Bowel Diseases

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Abstract

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Conflict of Interest:

GL is founder and owner of Genos LTD, company that specializes in high-throughput glycomics and has several patents in this field. FV, JS, ITA and GR are employees of Genos. GL is also founder and shareholder of GlycanAge LTD, company that markets GlycanAge test as a biomarker of healthy ageing. DPBM has consulted for Janssen, Cidara, Q Biologics, and Pfizer. Other authors have no conflict of interest to declare.

Author Contributions: Dermot P.B. McGovern, Vito Annese, Manfred Wuhrer and Gordan Lauc designed the study. Dermot P.B. McGovern, Vito Annese, Anna Khon, Anna Latiano, Renata D'Incà, Silvio Danese, Stephan Targan, Carol Landers and Marla Dubinsky collected patient samples and interpreted clinical data. Mirna Šimurina and Noortje de Haan performed experimental analysis. Jerko Štambuk, Genadij Razdorov, David Falck and Florent Clerc contributed to experimental analysis. Frano Vučković performed statistical analysis. Mirna Šimurina, Noortje de Haan and Nicholas A Kennedy drafted the manuscript. Nicholas A Kennedy, Irena Trbojević-Akmačić contributed to critical revision of the manuscript and provided technical support.

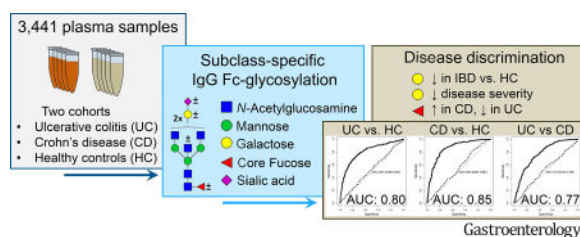
Background and aims—Causes of inflammatory bowel diseases are not well understood and the most prominent forms Crohn’s disease (CD) and ulcerative colitis (UC) are sometimes hard to distinguish. Glycosylation of immunoglobulin G (IgG) has been associated with CD and UC. IgG Fc-glycosylation affects IgG effector functions. We evaluated changes in IgG Fc-glycosylation associated with UC and CD, as well as with disease characteristics in different patient groups.

Methods—We analyzed 3441 plasma samples, obtained from 2 independent cohorts of patients with CD (874 patients in Italy and 391 in the United States [US]) or UC (1056 in Italy and 253 in the US) and healthy individuals (controls) (427 in Italy and 440 in the US). IgG Fc-glycosylation (tryptic glycopeptides) was analyzed by liquid chromatography coupled to mass spectrometry. We analyzed associations between disease status (UC vs controls, CD vs controls, and UC vs CD) and glycopeptide traits, and associations between clinical characteristics and glycopeptide traits, using a logistic regression model with age and sex included as covariates.

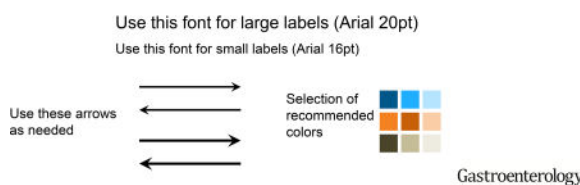
Results—Patients with CD or UC had lower levels of IgG galactosylation than controls. For example, the odds ratio (OR) for IgG1 galactosylation in patients with CD was 0.59 (95% CI, 0.51–0.69) and for patients with UC was 0.81 (95% CI, 0.71–0.92). Fucosylation of IgG was increased in patients with CD vs controls (for IgG1: OR, 1.27; 95% CI, 1.12–1.44) but decreased in patients with UC vs controls (for IgG23: OR, 0.72; 95% CI, 0.63–0.82). Decreased galactosylation associated with more severe CD or UC, including the need for surgery in patients with UC vs controls (for IgG1: OR, 0.69; 95% CI, 0.54–0.89) and in patients with CD vs controls (for IgG23: OR, 0.78; 95% CI, 0.66–0.91).

Conclusion—In a retrospective analysis of plasma samples from patients with CD or UC, we associated levels of IgG Fc-glycosylation with disease (compared to controls) and its clinical features. These findings could increase our understanding of mechanisms of CD and UC pathogenesis and be used to develop diagnostics or guide treatment.

Graphical abstract



Fonts, Arrows and Color Palette for Use



Keywords

IBD; glycans; glycopeptides; biomarker

Introduction

The incidence of inflammatory bowel disease (IBD) is increasing¹, and currently affects approximately 1 in 200 people in developed countries². In Europe, IBD has a prevalence of 2.5 to 3 million people^{3,4} with healthcare costs of €4.6 to 5.2 billion per year³ while in the USA the costs for IBD are estimated at US\$ 11–28 billion per year⁵. The two main types of IBD are Crohn's disease (CD) and ulcerative colitis (UC) which are further sub-categorized by the Montreal classification, based on age of onset, disease location and behavior (CD), and on disease extent and severity (UC)⁶.

IBD results from an aberrant host immune response to luminal gut microbiota occurring in genetically susceptible individuals⁷. However, genetic variants associated with IBD explain only 7.5 % and 13.6 % of UC and CD susceptibility⁸, indicating the importance of studying other factors that contribute to the course of IBD. One of these factors is the regulation of innate and adaptive immunity.

The majority of extracellular and membrane proteins are glycosylated, and glycans are directly involved in the pathophysiology of every major disease^{9,10}. Alternative glycosylation affects the protein structure and its function in a similar manner to mutations in the amino acid sequence¹¹. Protein glycosylation has been reported to change significantly in various diseases^{12–15}, including cancer¹⁶ and IBD^{17–19}. Current strategies for diagnosis, prognosis and monitoring of IBD are often invasive and/or lack adequate sensitivity^{20,21}, therefore the measurement of protein glycosylation in serum could be an attractive, minimally invasive biomarker and assist patient stratification for precision medicine.

Recent studies have shown that immunoglobulin G (IgG), which is a key effector of the humoral immune system and has multiple roles in balancing inflammation on the systemic level²², has altered glycosylation in a number of different diseases²³, including IBD^{17,18,24}. Additionally, genome-wide association studies of IgG glycosylation have shown pleiotropy with IBD susceptibility loci, suggesting a role for IgG glycosylation in the onset and progression of IBD^{8,25,26}. However, none of these studies have elucidated the mechanisms behind the observed changes, or their clinical relevance.

IgG molecules contain two diantennary *N*-glycans covalently attached to conserved *N*-glycosylation sites at Asn-297 on each of its heavy chains. The most complex Fc-glycan FA2BG2S2 is a diantennary (A2) digalactosylated (G2) and disialylated (S2) structure with a bisecting β (1,4) *N*-acetylglucosamine (GlcNAc) (B) and an α (1,6) fucose (F) attached to core GlcNAc (Fig. 1.)^{27,28}. Other IgG glycans correspond to this complex structure with the lack of one or more sugar units. Fc-glycosylation of IgG is complex and affected by multiple genetic²⁶, epigenetic²⁹ and environmental factors³⁰, resulting in a glycome composition which is very variable between individuals, but stable within an individual in homeostatic conditions³¹. Age is a notable exception that strongly affects the composition of the IgG glycome of an individual³². IgG in mice can have pro- and anti-inflammatory activity, depending on its glycosylation status. Sialylation of murine IgG is associated with anti-inflammatory activity³³, while IgG core fucosylation limits IgG-mediated antibody-

dependent cellular cytotoxicity (ADCC)^{30,34} and activates complement³⁵. Decreased galactosylation of IgG is reported in inflammatory diseases, suggesting an anti-inflammatory role of the attached galactoses²⁴. However, pro-inflammatory effects of IgG galactosylation have also been observed^{36,37}, hinting at alternative interpretations. The composition of the *N*-glycan attached to the Fc-region of IgG affects binding of IgG to both high- and low-affinity Fc-gamma receptors^{11,38}. Although the exact molecular mechanism is still elusive³⁹, the glycans attached to IgG strongly affect immunosuppressive properties of IgG, as exemplified in therapeutic function of intravenously administered immunoglobulins (IVIg)⁴⁰. Inter-individual variability in IgG glycome composition and its changes in disease thus have profound effects on the immune system.

The understanding of functional significance of glycosylation changes in disease are complicated by subclass-specific effects, as demonstrated in different models⁴¹. Until recently, the IgG glycome in IBD was analyzed on the level of total released glycans²⁴ by ultra-performance liquid chromatography (UPLC), which does not discriminate between individual IgG subclasses nor glycan location. Fc- and Fab-glycosylation of IgG differ significantly and we also demonstrated that disease course can specifically associate with Fc-glycosylation⁴². In this study we used liquid chromatography coupled to mass spectrometry (LC-MS), that enables high-throughput analysis of IgG Fc-glycans in a subclass-specific manner^{43,44} and provides a more detailed insight into IgG glycosylation changes in IBD. By measuring subclass-specific IgG Fc *N*-glycosylation in 3,441 IBD patients and controls from two independent cohorts participating in the IBD-BIOM project, we demonstrated a clear difference in IgG Fc-glycosylation between diseased and healthy individuals, but also between the different forms of IBD, and associations with disease severity.

Materials and methods

Clinical samples and ethical considerations

Samples were collected from two case-control populations, the Italian cohort (ITA) from Italy (N = 2,357) and the American cohort (US) from the USA (N = 1,084) each including CD (ITA: 874, US: 391) and UC (ITA: 1056, US: 253) patients as well as healthy controls (HC; ITA: 427, US: 440). Both cohorts were collected with the approval of the local ethics committees and informed consent was obtained from all participants. Phenotype was defined using the Montreal classification at the last follow up⁶. Clinical characteristics were obtained by chart review according to criteria agreed by the clinicians and as previously described^{45–47} (Table 1.).

Sample preparation and data pre-processing

Sample preparation and glycopeptide analysis (IgG purification by Protein G affinity chromatography, tryptic digestion, nanoLC-MS analysis and data pre-processing) were performed separately for the ITA and US cohort^{30,43,44,48,49} (details in the Supp. Materials and Methods). The tryptic Fc-glycopeptides for IgG2 and 3 have identical peptide moieties in the Caucasian population and are therefore not distinguishable with our methods^{50,51}. Annotation of the spectra was done based on accurate mass and literature^{28,30}. Using the

directly measured glycopeptides, derived glycosylation traits were calculated per IgG subclass (Supp. Table 3., and 4.) which average particular glycosylation features like galactosylation, fucosylation, sialylation and the presence of a bisecting GlcNAc (bisection).

Statistical analysis

Data analysis and visualization was performed with 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria.). Association analyses between disease status (UC patients and HC, CD patients and HC and UC and CD patients) and glycopeptide traits as well as within-disease analyses of associations between clinical characteristics and glycopeptide traits were performed using a logistic regression model with age and sex included as additional covariates. For UC we assessed disease location, duration of the disease and the need for a colectomy. Regarding disease location, we analyzed the differences between Montreal E1 (proctitis) and E2 (left-sided UC) against E3 (extensive UC). For CD we assessed disease location and behavior, duration of disease, and the need for surgery. For CD behavior, we compared Montreal B2 (stricturing) and B3 (internal penetrating) with B1 (inflammatory disease). For location, we compared Montreal L1 (ileal) against L2 (colonic disease) and L1 against L3 (ileocolonic disease). For both diseases, we used a cut-off of 5 years since diagnosis to stratify disease duration into two groups. In the ITA cohort, for CD as well as for UC, patients treated with the third most potent medication (steroids) were compared to patients only treated with mesalazine, and in addition patients treated with the first most potent medication (anti-TNF) were compared to patients treated with the second most potent medication (azathioprine and 6-mercaptopurine, AZA/6MP). These latter tests were also done in the US cohort. Both case/control and within-disease analyses were first performed for each cohort separately and then combined using an inverse variance-weighted meta-analysis approach (R package “metafor”⁵²). The false discovery rate (FDR) was controlled for each analysis using the Benjamini-Hochberg method with FDR set to 0.05. All the *p*-values were corrected for multiple testing.

For prediction of disease status, a regularized logistic (elastic net) regression model was applied (R package “glmnet”⁵³) using direct glycosylation traits as predictors (Supp. Materials and Methods). Three models were built for each cohort (UC vs. HC, CD vs. HC, and UC vs. CD), using age, sex and glycopeptide measurements as predictors. To evaluate the performance of the predictive models based on the individual glycoforms, a 10-cross-validation procedure was used. The predictions from each validation round were merged into one validation set on which the performance of each model was evaluated based on the area under the curve (AUC) criteria.

Results

IgG Fc-glycosylation differences between IBD patients and HC

For both CD and UC, we observed an increase in agalactosylated IgG glycopeptides in both cohorts as compared to HC and a corresponding decrease of monogalactosylated (not significant for IgG1 in CD patients of the US cohort, but significant in meta-analysis of both cohorts) and digalactosylated IgG glycopeptides (not significant for IgG1 and IgG4 in UC patients of the ITA cohort, but significant in meta-analysis of both cohorts; Table 2., Supp.

Table 6., 8., and 10., Fig. 2.). In both cohorts, all IgG subclasses showed lower overall galactosylation (A2G) for CD patients as compared to UC patients. A decrease in sialylation was also associated with CD in both the US and the ITA cohort, but for UC this effect was only seen in the US cohort. Sialylation per galactose of diantennary glycans (A2GS) increased with disease, or did not change at all. We observed a subclass-and disease-specific association for fucosylated IgG glycopeptides (A2F). Increased A2F was associated with CD in the US cohort for both IgG1 and IgG23 and for IgG1 in ITA cohort. Conversely, there was a negative association between IgG23 A2F and UC in both cohorts. Furthermore, both cohorts showed IgG1 and IgG23 A2F to be high in CD patients as compared to UC patients. IgG4 bisection (A2FB) was low for CD and UC as compared to HC in both cohorts. A decrease in bisection (A2B) was also observed for the other IgG subclasses in UC patients of the ITA cohort, on the other hand IgG1 and IgG23 A2B was increased for CD as compared to HC in the US cohort. For both cohorts IgG1 and IgG23 A2B was higher in CD as compared to UC. Results for the individual IgG glycoforms are shown in Supp. Table 7., 9., 11., and Supp. Fig. 2.

Discrimination of disease status

The discriminatory performance of individual glycoforms per IgG subclass in distinguishing UC from HC, CD from HC and UC from CD, was evaluated for ITA and US cohorts separately, using a regularized logistic regression model. The ROC curves for the ITA cohort showed a good performance in discriminating UC from HC (AUC = 0.801; Fig. 3. A) and CD from HC (AUC = 0.854; Fig. 3. B), and fair performance in discriminating UC from CD (AUC = 0.770; Fig. 3. C). This was replicated in the US cohort, showing a good performance in discriminating UC from HC (AUC = 0.814; Fig. 3. D) and CD from HC (AUC = 0.849; Fig. 3. E) and fair performance in discriminating UC from CD (AUC = 0.746; Fig. 3. F). To assess which glycoforms were most important in these models, individual ROC analyses were performed per glycoform per IgG subclass, revealing for example G1 on IgG23 to be in the top 5 of most important glycoforms discriminating UC from HC in both cohorts (Supp. Fig. 3. A, and 4. A). In addition, IgG23 and IgG4 G0F were in the top 5 between CD and HC in both cohorts (Supp. Fig. 5. A, and 6. A) and IgG23 G0F, G0FN, G2 and G2F showed in both cohorts to be in the top 5 of most discriminating glycans between UC and CD (Supp. Fig. 7. A, and 8. A). These findings were also reflected in the predictive values of the individual derived traits (Supp. Fig. 3.–8., panels B).

Disease behavior, location and classification

In the ITA cohort (and the meta-analysis of both cohorts) an increase in agalactosylated IgG glycopeptides and a decrease in digalactosylated IgG glycopeptides were associated with extensive UC and the need for surgery in UC. In addition, the need for surgery was associated with less sialylated glycans on all IgG subclasses. In both cohorts, agalactosylated IgG1 glycopeptides decreased and monogalactosylated IgG1 glycopeptides increased with the duration of UC (Supp. Table 12., and Fig. 4.).

In addition, for surgery in CD patients, the IgG23 agalactosylation increased in both cohorts, while IgG23 digalactosylation was decreased. Furthermore, IgG23 sialylation decreased in the ITA cohort (and the meta-analysis of both cohorts). Also a worse disease behavior

(Montreal B2 + B3 versus B1) was associated with increased IgG23 agalactosylation and decreased IgG23 digalactosylation and sialylation in the ITA cohort (and the meta-analysis of both cohorts), while a more extensive CD (ileal (L1) versus ileocolonic (L3)) was associated with increased IgG1 agalactosylation in the US cohort (and the meta-analysis of both cohorts; Supp. Tables 14. and Fig. 5.). Results for the individual IgG glycoforms are shown in Supp. Table 13. and 15.

Use of medication

For UC patients in the ITA cohort, an increase in overall agalactosylation was associated with the use of steroids, as compared to mesalazine. This difference was not observed for CD patients in the ITA cohort (Supp. Table 16 and 18).

In the US cohort, UC patients treated with anti-TNF showed a decrease in overall galactosylation, and IgG1 and IgG4 sialylation when compared to patients treated with AZA/6MP. This was not replicated in the ITA cohort. However, the same observation was made for CD patients in the US cohort (and the meta-analysis of both cohorts), where a decrease in overall galactosylation was associated with treatment with anti-TNF compared to treatment with AZA/6MP. Results for the individual IgG glycoforms are shown in Supp. Tables 17. and 19.

Discussion

In this study we analyzed subclass-specific IgG Fc-glycosylation in IBD in two independent cohorts, using a nanoLC-MS method⁴³. The importance of altered glycosylation in IBD has been reported before in different models^{19,54} and specifically IgG Fc-glycosylation showed to play an important role in a number of inflammatory processes⁵⁵, including the course of IBD²⁴.

Associations between IgG Fc-glycosylation and IBD

Associations between galactosylation as well as sialylation and disease were, although observed for both diseases, consistently more pronounced in CD than in UC. IgG Fc-galactosylation was decreased in IBD patients as compared to HC. This decrease was previously shown in the total IgG N-glycome²⁴ and we revealed that this change is not subclass-specific (Fig. 2.). Decreased IgG Fc-galactosylation has been reported in different inflammatory diseases²⁴. Since IgG galactosylation has shown to also decrease with aging²⁹, observed changes in galactosylation are most likely connected to inflammation in general and are not IBD-specific. On the other hand, decreased galactosylation on antigen-specific antibodies in rheumatoid arthritis precedes the onset of the disease^{56,57} which indicates that the individual differences in IgG galactosylation could be associated with predisposing factors for the development of inflammatory disease including IBD⁵⁸.

In addition, sialylation was decreased in IBD, which was previously observed in CD but not UC²⁴. This effect was less pronounced than the galactosylation effect and it was also not replicated in both cohorts for all IgG subclasses. Likely, the decrease in sialylation is a by-effect of the observed decrease in galactosylation, as galactosylation is required for the addition of a sialic acid to a glycan. This is supported by our observation that sialylation per

galactose (derived trait A2GS) did not show a difference between either CD or UC and the HC, or increased with disease. Various studies in humans have shown the predominant role of galactosylation rather than sialylation in the regulation of pro- and anti-inflammatory effects of IgG^{37,59,60}.

Recently it was discovered that five genetic loci associated with IgG glycosylation, showed pleiotropy with IBD, suggesting the role of IgG glycosylation in the development and course of IBD^{8,25,26}. These genetic loci associated with IgG galactosylation, fucosylation and bisection, features also significantly changing with IBD as found in the current study.

As multiple derived glycosylation traits were associated with IBD, we hypothesized that IgG Fc-glycosylation might be used as tool to discriminate between IBD patients and HC. Our prediction models were based on the individual glycoforms per IgG subclass and showed an improved discriminatory performance compared to previously published models based on individual glycoforms²⁴, this is likely due to the higher number of individual glycoforms included in our model and the subclass-specificity of our analysis. For example, in both cohorts, IgG23 and IgG4 glycoforms showed the largest effect size between HC and IBD patients. IgG23 and IgG4 are less abundant in human plasma than IgG1⁶¹, likely causing the effect of their glycoforms to be partly masked during released glycan analysis. Despite the differences between the two cohorts in terms of disease behavior in CD and disease extent in UC (both more severe in the US cohort, likely due to a longer disease duration), and collection of the samples (a single tertiary/quaternary IBD center for the US cohort and multiple primary centers for the ITA cohort), we still found equally performing models for both of them.

These findings suggest possible clinical utility of glycans as minimally invasive, diagnostic markers. However, future studies confirming these findings and contrasting/combining these markers with others minimally invasive, prognostic markers are warranted^{62–64}. Previous studies have identified IBD-associated serologies, transcriptomics, and genetics. Especially the comparative utility of IgG Fc-glycosylation as peripheral biomarker as compared to IBD-associated serologies, that measure antibodies to commensal flora (e.g. ASCA, anti-CBIR1, ANCA etc.)^{45,63,65,66} should be evaluated. The preferred study design for this would be a prospective longitudinal study that further explores the impact of changes in disease severity and progression over time. Previously it has been suggested that the IgG glycome of healthy individuals is stable over time⁶⁷, although influenced by changes in lifestyle and environmental factors⁶⁷. In the context of IBD, it is likely to change with disease activity.

Differences between CD and UC

Reliable differentiation between UC and CD is currently done by colonoscopy (invasive) or radiology (radiation exposure)⁶⁸. Current methods based on serology markers, like antibodies specific for microbial antigens, still do not reach specificity and sensitivity demanded for a diagnostic test⁶⁹. In addition, the differences in mechanisms leading to the development of these diseases remain unclear⁷⁰. Lower IgG galactosylation was more pronounced in CD than in UC, which might indicate a more severe inflammatory response in CD⁷¹. Fucosylation was decreased in UC, but increased in CD, suggesting different

regulation mechanisms. The absence of a fucose on an IgG Fc-glycan has shown to improve Fc binding to Fc γ RIII and thereby enhancing ADCC⁷². As an increased galactosylation also enhances the ADCC activity of antibodies in *in vitro* models, the combination of a lower fucosylation and higher galactosylation in UC as compared to CD might result in higher ADCC activity in UC than in CD³⁶. IgG Fc-bisection was also different between UC and CD patients, showing a higher bisection in CD than in UC. This was consistent with the previous observation that IgG bisection was higher in CD, but not in UC, as compared to HC²⁴. Although bisection has a large influence on the structure of a glycan, its effect on antibody function is largely unknown³⁰. Various studies report an increased bisection to be related to a higher antibody affinity for Fc γ RIII and therefore an associated increase of ADCC^{73,74}.

Associations between IgG Fc-glycosylation and disease status

The potential role of IgG Fc-glycosylation in the course and development of IBD has been consolidated through confirming, for the first time, the changes in IgG Fc-glycosylation with clinical subphenotypes of UC and CD. The increase of aglycosylated glycoforms with more severe disease and the need for surgery in both UC and CD might suggest that when the disease involves more of the colon (more extensive (E3) UC or ileocolonic (L3) CD) and is more severe (there is a need for surgery), IgG has less possibilities to suppress the inflammation⁷⁵. With longer duration of UC, on the other hand, a decrease in agalactosylation was observed, which was not detected with duration in CD. This can be connected to the different disease behaviors, as for UC it is known that disease activity can decrease over time, while CD usually has a worsening pattern of activity⁷⁶.

The treatment exposures in the two cohorts were different, IBD patients in the US cohort had all been exposed to corticosteroids, which was not the case in the ITA cohort, where the cases on and off corticosteroids were compared. In both cohorts patients on anti-TNF therapy (more severe cases) were compared to the ones exposed to AZA/6MP (less severe cases)^{77,78}. In the US cohort we observed increased agalactosylation for all subclasses in IBD patients on anti-TNF therapy compared to patients on AZA/6MP. This was not replicated in the ITA cohort which likely reflects the heterogeneity of the two cohorts in terms of treatment guidelines. However, in the ITA cohort an increase in agalactosylated structures was found with the use of corticosteroids as most potent treatment, compared to the less potent one with mesalazine. Steroids compared to mesalazine and anti-TNF compared to AZA/6MP might be considered surrogate markers for disease severity as they are used when the disease progresses^{77,78}. This corresponds to our findings that more severe disease was also associated with a decrease in IgG Fc-galactosylation. Although corticosteroids have an anti-inflammatory effect⁷⁹, our findings suggest that the observed glycosidic changes are not an effect of therapy, but are rather connected to a more severe disease.

Conclusion

In this study, we confirmed previous associations of reduced galactosylation in IBD compared to HC. In addition, it was demonstrated that this same glycosylation trait was associated with more extensive and progressive disease, suggesting a potential role of IgG

Fc-glycosylation as diagnostic and/or prognostic tool. Furthermore, we found the IgG glycosylation features: fucosylation, galactosylation and bisection to be different between UC and CD patients. Individual glycoforms showed good performance for distinguishing both UC and CD from healthy controls and fair performance for distinguishing UC from CD, which gave an insight into the difference in mechanisms behind the two diseases. The reported differences in IgG Fc-glycans might influence the development and behavior of IBD through affecting binding of IgG to Fc γ Rs^{11,38}. Furthermore, individual differences in IgG glycosylation might affect efficacy of therapeutic monoclonal antibodies, which have to compete with circulating IgG to activate effector functions⁸⁰. The reported changes in IgG Fc-glycosylation in the current study give guidelines for future, prospective studies that should elucidate the longitudinal relationship between changes in IgG Fc-glycans and development of disease, and disease progression, as well as their role in predicting treatment response. Clinical exploitation of these glycan markers will be facilitated by the existing broad application in clinical laboratories of mass spectrometry or capillary electrophoresis which show great potential for glycomics assays^{81,82}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant support: This work was supported by the European Union Seventh Framework Programmes IBD-BIOM (contract #305479), H2020 grants SYSCID (contract #733100), GlySign (contract #722095), and IMForFuture (contract #721815); by the European Structural and Investment Funds IRI (grant #KK.01.2.1.01.0003) and Croatian National Centre of Research Excellence in Personalized Healthcare (grant #KK.01.1.1.01.0010); as well as by The Netherlands Genomic Initiative Horizon Programme Zenith project (Grant No. 93511033). Research at the IBD Center at Cedars-Sinai is supported by NIH grants including U01DK062413 (DPBM), P01DK046763 (SRT, DPBM), R01HS021747 (DPBM) and U01AI067068 (DPBM).

Abbreviations

A2	diantennary
A2B	structures with bisecting $\beta(1,4)$ <i>N</i> -acetylglucosamine
A2F	fucosylated structures
A2FB	structures with bisecting $\beta(1,4)$ <i>N</i> -acetylglucosamine on IgG4
ADCC	antibody-dependent cellular cytotoxicity
Agal	agalactosylated
Anti TNF	inhibitor of tumor necrosis factor
AUC	area under the curve
AZA	azathioprine
B	bisecting $\beta(1,4)$ <i>N</i> -acetylglucosamine
B1	inflammatory Crohn's disease

B2	stricturing Crohn's disease
B3	internal penetrating Crohn's disease
CD	Crohn's disease
CI	confidence intervals
Digal	digalactosylated
E1	proctitis
E2	left-sided ulcerative colitis
E3	extensive ulcerative colitis
F	$\alpha(1,6)$ fucose
Fab	antigen-binding fragment
Fc	fragment crystallizable
FcγRIII	Fc-gamma receptor III
G	galactose
G2	digalactosylated
GlcNAc	<i>N</i> acetylglucosamine
GWAS	genome-wide association study
HC	healthy controls
IBD	inflammatory bowel disease
IgG	immunoglobulin G
ITA	Italian cohort
IVIG	intravenously administered immunoglobulins
L1	ileal Crohn's disease
L2	colonic Crohn's disease
L3	ileocolonic Crohn's disease
LC-MS	liquid chromatography coupled to mass spectrometry
M	mannose
Mono	monogalactosylated
N	<i>N</i> -acetylhexosamine
OR	odds ratio

RP	reverse-phase
S	<i>N</i> -acetylneuraminic acid/ sialic acid
S2	disialylated
Sial	sialylated
UC	ulcerative colitis
UPLC	ultra-performance liquid chromatography
US	American cohort
USA	United States of America
6MP	6-mercaptopurine

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10 IBD-BIOM consortium

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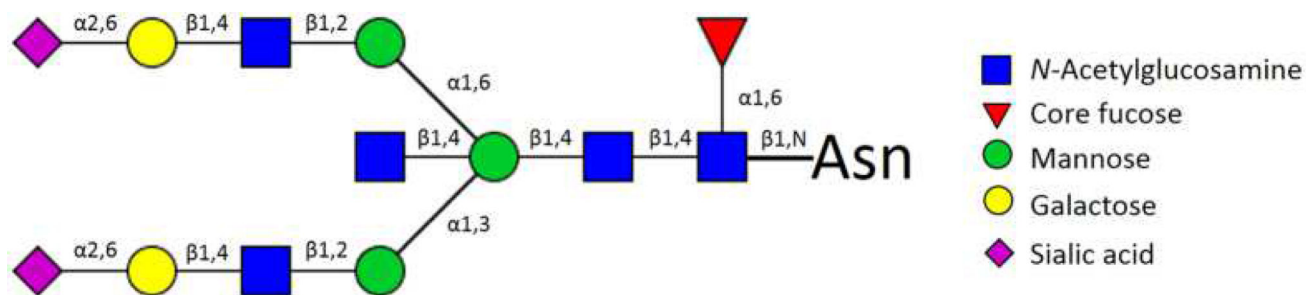


Figure 1. The most complex IgG Fc-glycan found in our samples: FA2BG2S2

A diantennary (A2) digalactosylated (G2) and disialylated (S2) glycan with a bisecting $\beta(1,4)$ GlcNAc (B) and an $\alpha(1,6)$ fucose (F) attached to core *N*-acetylglucosamine (GlcNAc). Linkages and anomeric configurations are shown^{27,28}. Blue square: *N*-acetylglucosamine, red triangle: fucose, green circle: mannose, yellow circle: galactose, pink diamond: *N*-acetylneuraminic acid.

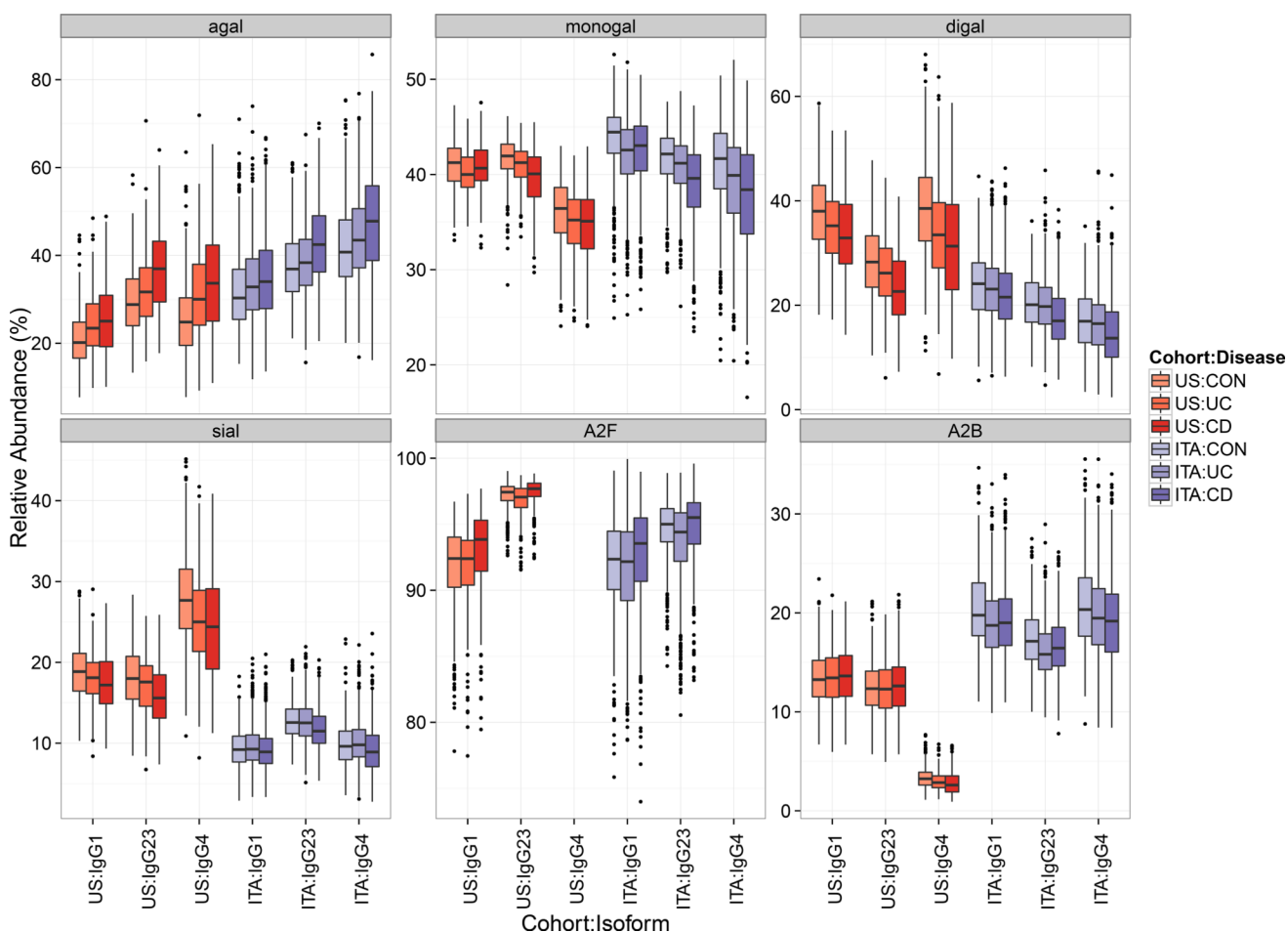


Figure 2. Differences in derived IgG Fc-glycan traits between HC, UC and CD for all IgG subclasses in both cohorts

Differences in derived glycan traits for all IgG subclasses between HC, UC and CD are shown separately for the US (red) and ITA (blue) cohort. Data are shown as box and whiskers plots. Each box represents the 25th to 75th percentiles (IQR). Lines inside the boxes represent the median. The whiskers represent the lowest and highest values within the boxes $\pm 1.5x$ the IQR. Derived glycan traits are listed in Supp. Table 3.-4. and their glycoforms in Supp. Table 2. Analysis of the differences in glycan traits between UC and HC, CD and HC and UC and CD, were performed using a logistic regression model with age and sex included as additional covariates (Table 2., Supp. Table 6., 8., and 10.).

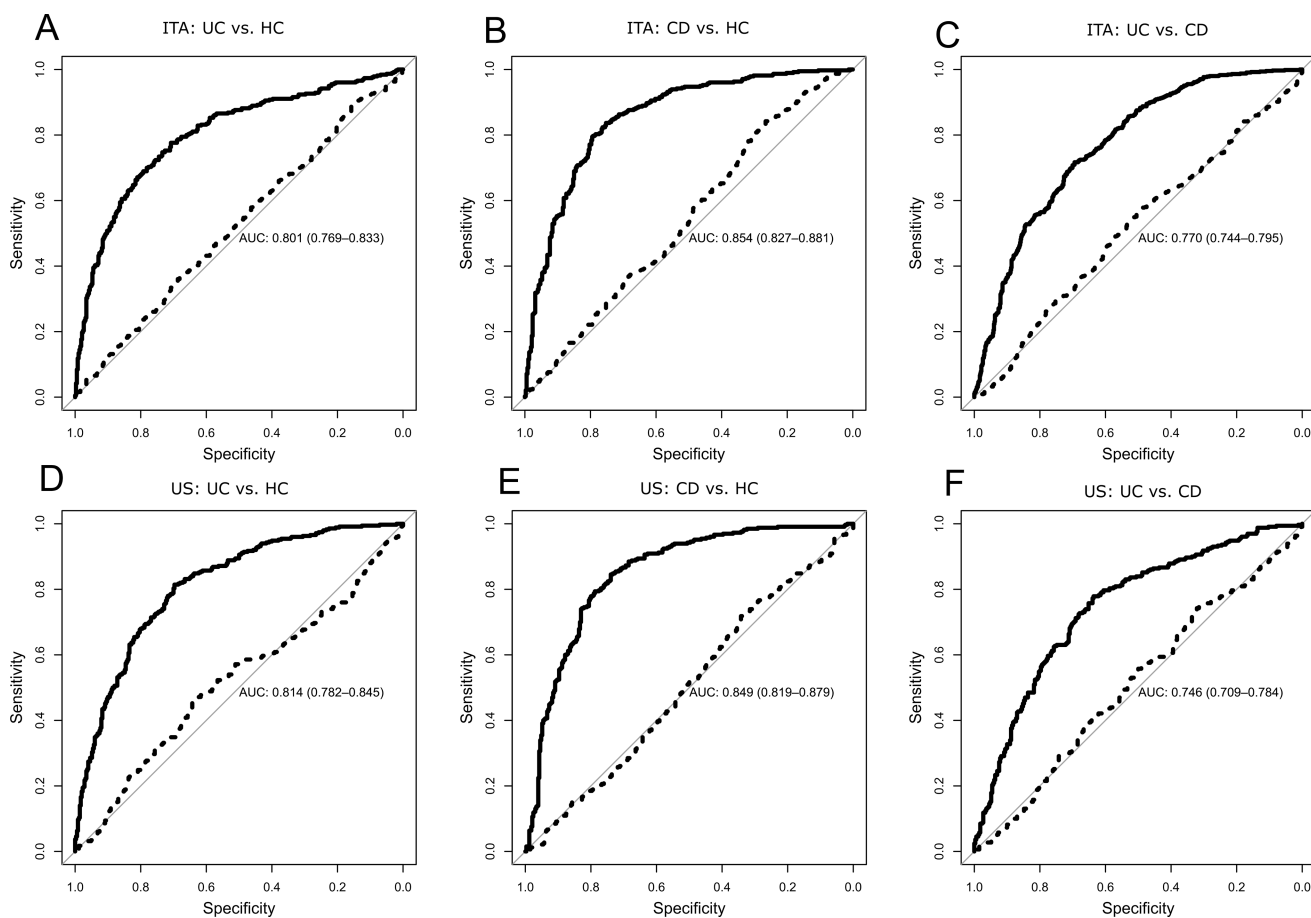


Figure 3. ROC curves illustrating the discriminative power of individual glycoforms per IgG subclass

Prediction of disease status was performed using a logistic (elastic net) regression model for the ITA cohort between UC and HC (A), CD and HC (B), UC and CD (C) and for the US cohort between UC and HC (D), CD and HC (E), and UC and CD (F). While models based only on age and sex did not show predictive power (dotted line), addition of individual glycoforms increased the predictive power of the models (solid line).

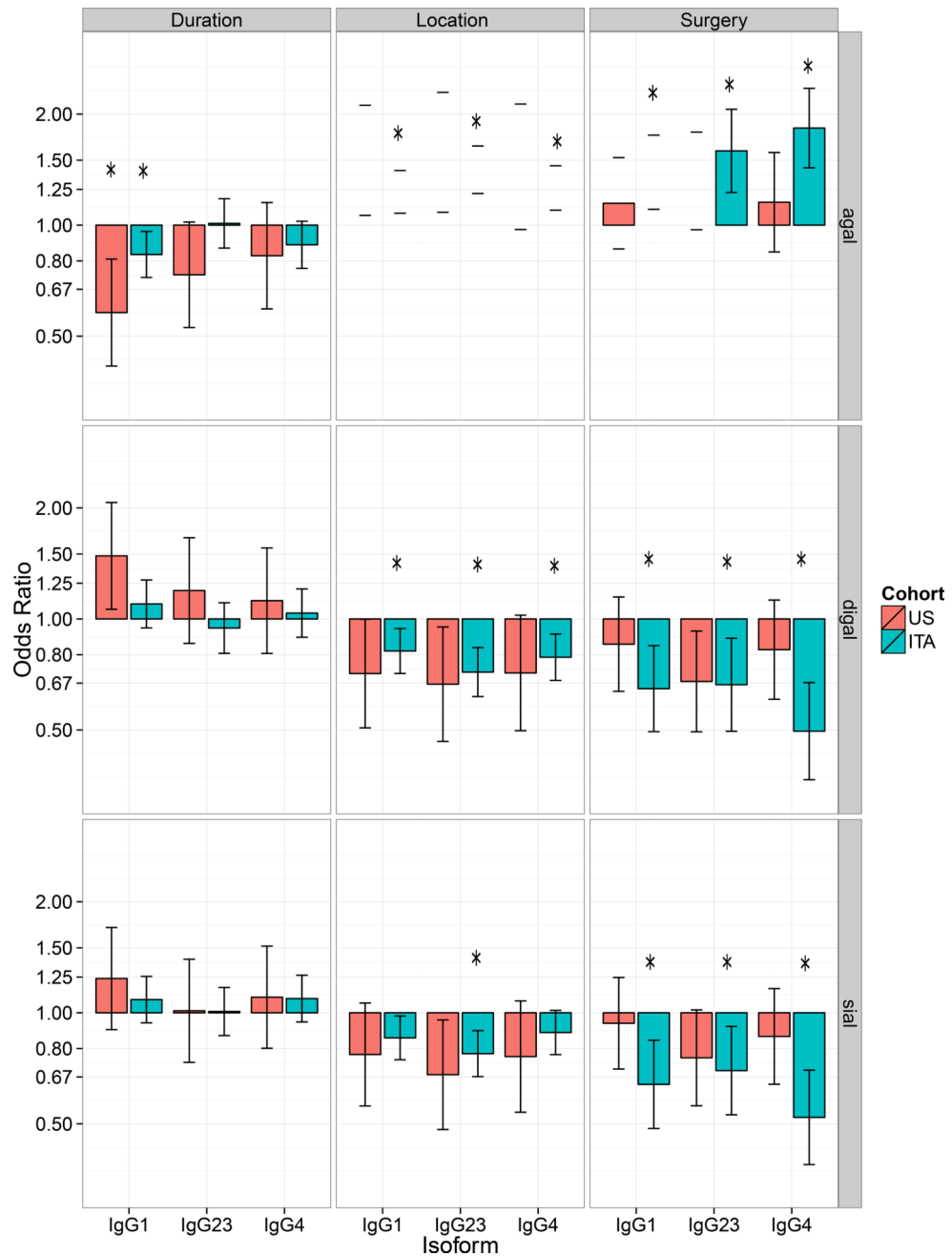


Figure 4. Associations between derived IgG Fc-glycan traits and clinical characteristics in UC (duration, location and surgery)

Odds ratios for the associations between derived glycan traits and clinical traits in UC (duration of disease: <5 years = 0, >5 years = 1, disease location: E1 (proctitis) + E2 (left-sided UC) = 0, E3 (extensive UC) = 1, and surgery: no = 0, yes = 1) for all IgG subclasses are shown for the ITA cohort (green) and the US cohort (red). Bars indicate positive/negative odds ratios. Derived glycan traits are explained in Supp. Table 3.-4. and their glycoforms in Supp. Table 2. Analysis of the association between derived glycan traits and clinical characteristics in UC were performed using a logistic regression model with age and sex

included as additional covariates, statistically significant findings are indicated with an asterisk (*) (Supp. Table 12.).

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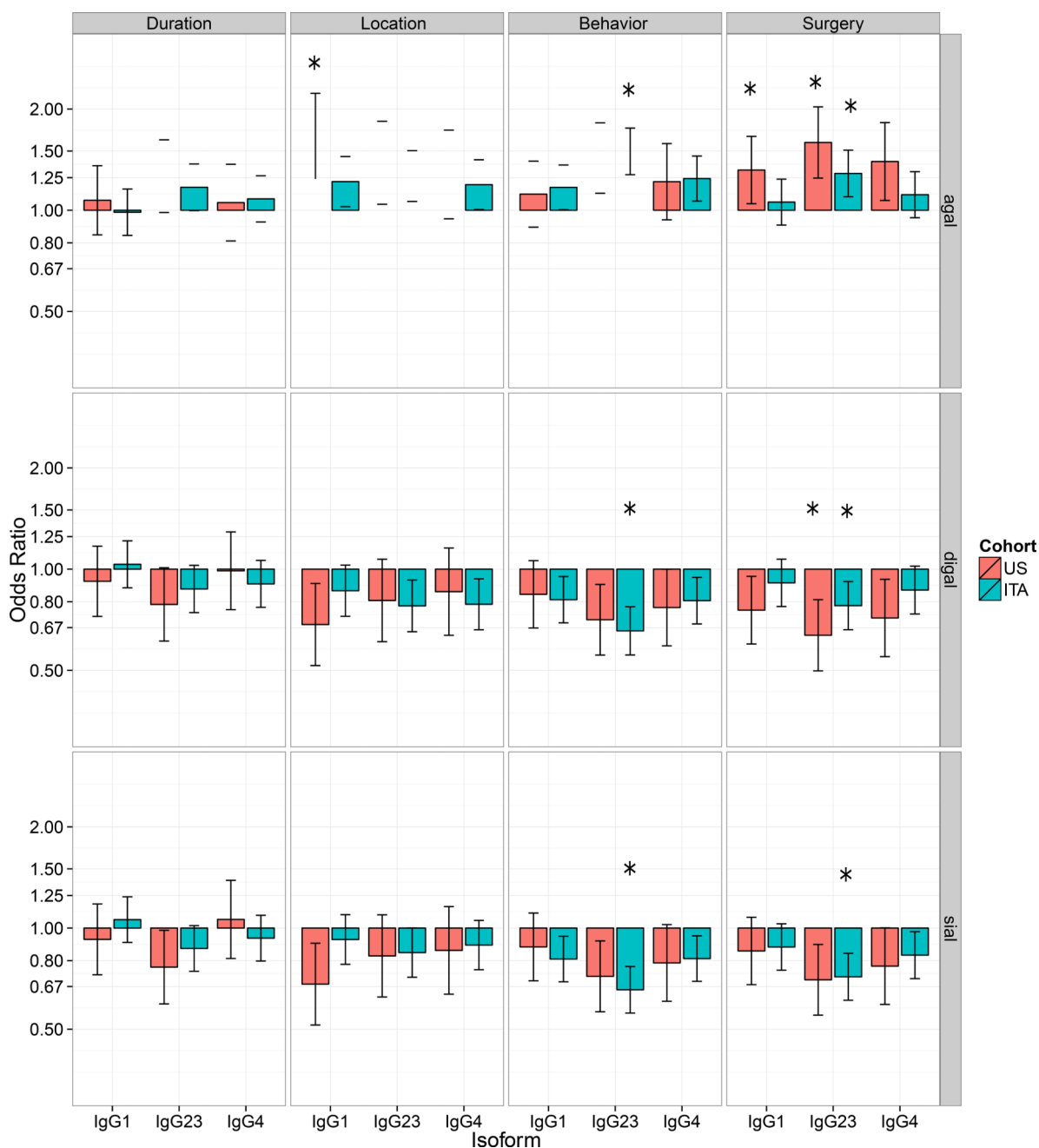


Figure 5. Associations between derived IgG Fc-glycan traits and clinical characteristics in CD (duration, location, behavior and surgery)

Odds ratios for the associations between derived glycan traits and clinical characteristics in CD (duration of disease: <5 years = 0, >5 years = 1, disease location: L1 (ileal CD)= 0, L3 (ileocolonic CD) = 1, behavior: B1 (inflammatory CD) = 0, B2 (structuring CD) + B3 (penetrating CD) = 1, and surgery: no = 0, yes = 1) for all IgG subclasses are shown for the ITA cohort (green) and the US cohort (red). Bars indicate positive/negative odds ratios. Derived glycan traits are explained in Supp. Table 3.-4. and their glycoforms in Supp. Table 2. Analysis of the association between derived glycan traits and clinical characteristics in CD were performed using a logistic regression model with age and sex included as

additional covariates, statistically significant findings are indicated with an asterisk (*) (Supp. Table 14.).

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Table 1

Demographics of American and Italian IBD cohorts.

	American cohort				Italian cohort				
	HC ^a	UC	CD	HC	UC	CD	HC	UC	CD
Sample Number	440	253	391	427	1056	874			
Age (med/IQR)	46.3 (33.5 – 56.0)	39.4 (29.2 – 54.8)	35.4 (26.0 – 48.4)	44.0 (35.0 – 56.0)	41.0 (31.0 – 52.0)	35.5 (27.0 – 46.0)			
Sex (F) (n/%)	254 (57.9%)	129 (51.0%)	169 (43.2%)	145 (34.0%)	423 (40.1%)	368 (42.1%)			
Disease Duration (med/IQR)^b		7.2 (3.3 – 15.4)	8.3 (3.0 – 14.8)		6.0 (2.0 – 13.0)	5.0 (1.0 – 11.0)			
Disease Location (CD) (n/%)^c									
Ileal (L1±L4)	-	-	91 (23.8%)	-	-	327 (38.7%)			
Colonic (L2±L4)	-	-	58 (15.2%)	-	-	161 (19.1%)			
Ileocolonic (L3±L4)	-	-	233 (61.0%)	-	-	343 (40.6%)			
Upper GI (L4 only)	-	-	0 (0%)	-	-	13 (1.5%)			
Disease Behaviour (CD) (n/%)									
Inflammatory (B1)	-	-	140 (36.3%)	-	-	504 (58.3%)			
Stricturing (B2)	-	-	109 (28.2%)	-	-	237 (27.4%)			
Penetrating (B3)	-	-	137 (35.5%)	-	-	124 (14.3%)			
Disease Extent (UC) (n/%)									
Proctitis (E1)	-	8 (3.2%)	-	-	115 (11.0%)	-			
Left-sided (E2)	-	63 (25.2%)	-	-	489 (47.0%)	-			
Extensive (E3)	-	179 (71.6%)	-	-	437 (42.0%)	-			
Medication (n/%)									
Mesalazine	-	-	-	-	320 (32.5%)	179 (22.7%)			
Prednisolone	-	all (100%)	all (100%)	-	360 (36.6%)	229 (29.0%)			
Thiopurines (AZA/6MP)	-	84 (36.5%)	71 (18.7%)	-	213 (21.6%)	174 (22.1%)			
Anti-TNF	-	146 (63.5%)	309 (81.3%)	-	90 (9.1%)	207 (26.2%)			
Surgical resection (n/%)	-	141 (55.7%)	228 (60.6%)	-	81 (8.1%)	327 (37.8%)			

^aHC: healthy controls, UC: ulcerative colitis and CD: Crohn's disease.

^bDisease duration in years

Disease location and behavior are described according to the Montreal classification.

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Table 2
Associations between derived IgG Fc-glycosylation traits and IBD in the ITA cohort.

Derived trait ^a	Description	HC vs. UC ^b			HC vs. CD			UC vs. CD		
		OR (95% CI) ^c	p-value ^d	OR (95% CI)	OR (95% CI)	p-value	OR (95% CI)	p-value		
IgG1										
IgG1_agal	Fraction of agalactosylated glycans	1.29 (1.13 – 1.47)	<u>8.9E-05</u>	1.69 (1.46 – 1.96)	<u>2.2E-13</u>	1.23 (1.11 – 1.36)	<u>3.9E-05</u>			
IgG1_digal	Fraction of digalactosylated glycans	0.88 (0.77 – 1.00)	5.7E-02	0.61 (0.52 – 0.70)	<u>2.7E-12</u>	0.71 (0.64 – 0.79)	<u>8.0E-11</u>			
IgG1_monogal	Fraction of monogalactosylated glycans	0.69 (0.61 – 0.79)	<u>5.4E-09</u>	0.69 (0.61 – 0.79)	<u>4.3E-08</u>	1.06 (0.97 – 1.17)	2.0E-01			
IgG1_sial	Fraction of sialylated glycans	1.05 (0.93 – 1.20)	4.1E-01	0.81 (0.70 – 0.92)	<u>1.4E-03</u>	0.78 (0.71 – 0.87)	<u>1.5E-06</u>			
IgG1_A2B	Bisection of diantennary glycans	0.69 (0.61 – 0.78)	<u>3.5E-09</u>	0.91 (0.79 – 1.03)	1.4E-01	1.22 (1.11 – 1.35)	<u>7.8E-05</u>			
IgG1_A2F	Fucosylation of diantennary glycans	0.93 (0.82 – 1.04)	2.1E-01	1.27 (1.12 – 1.44)	<u>2.2E-04</u>	1.36 (1.23 – 1.51)	<u>6.0E-10</u>			
IgG1_A2G	Galactosylation per antenna on diantennary glycans	0.81 (0.71 – 0.92)	<u>1.5E-03</u>	0.59 (0.51 – 0.69)	<u>3.2E-13</u>	0.77 (0.69 – 0.85)	<u>2.5E-07</u>			
IgG1_A2S	Sialylation per antenna on diantennary glycans	1.04 (0.92 – 1.18)	5.1E-01	0.78 (0.68 – 0.90)	<u>3.2E-04</u>	0.77 (0.70 – 0.85)	<u>3.7E-07</u>			
IgG1_A2GS	Sialylation per galactose on diantennary glycans	1.33 (1.18 – 1.51)	<u>3.6E-06</u>	1.15 (1.01 – 1.30)	<u>3.1E-02</u>	0.85 (0.77 – 0.94)	<u>1.1E-03</u>			
IgG23										
IgG23_agal	Fraction of agalactosylated glycans	1.30 (1.14 – 1.50)	<u>1.3E-04</u>	2.76 (2.31 – 3.29)	<u>1.7E-37</u>	2.11 (1.87 – 2.37)	<u>1.2E-40</u>			
IgG23_digal	Fraction of digalactosylated glycans	0.82 (0.71 – 0.94)	<u>3.7E-03</u>	0.41 (0.35 – 0.48)	<u>2.2E-31</u>	0.49 (0.43 – 0.55)	<u>1.0E-36</u>			
IgG23_monogal	Fraction of monogalactosylated glycans	0.77 (0.67 – 0.87)	<u>4.1E-05</u>	0.39 (0.32 – 0.46)	<u>8.5E-34</u>	0.54 (0.48 – 0.60)	<u>8.2E-32</u>			
IgG23_sial	Fraction of sialylated glycans	0.87 (0.76 – 0.99)	<u>2.9E-02</u>	0.53 (0.46 – 0.61)	<u>6.8E-20</u>	0.58 (0.52 – 0.64)	<u>1.0E-24</u>			
IgG23_A2B	Bisection of diantennary glycans	0.62 (0.55 – 0.71)	<u>3.2E-13</u>	0.91 (0.80 – 1.05)	1.9E-01	1.34 (1.21 – 1.48)	<u>8.2E-09</u>			
IgG23_A2F	Fucosylation of diantennary glycans	0.72 (0.63 – 0.82)	<u>3.2E-07</u>	1.12 (0.99 – 1.27)	7.5E-02	1.57 (1.41 – 1.74)	<u>1.6E-18</u>			
IgG23_A2G	Galactosylation per antenna on diantennary glycans	0.79 (0.68 – 0.90)	<u>5.3E-04</u>	0.38 (0.32 – 0.45)	<u>1.9E-35</u>	0.47 (0.42 – 0.53)	<u>4.1E-40</u>			
IgG23_A2S	Sialylation per antenna on diantennary glycans	0.86 (0.76 – 0.98)	<u>2.3E-02</u>	0.52 (0.45 – 0.60)	<u>5.7E-21</u>	0.57 (0.51 – 0.64)	<u>2.4E-25</u>			
IgG23_A2GS	Sialylation per galactose on diantennary glycans	1.02 (0.90 – 1.14)	8.0E-01	1.19 (1.05 – 1.35)	<u>7.6E-03</u>	1.11 (1.01 – 1.22)	<u>2.9E-02</u>			
IgG4										
IgG4_agal	Fraction of agalactosylated glycans	1.30 (1.13 – 1.48)	<u>1.2E-04</u>	2.16 (1.84 – 2.54)	<u>2.3E-24</u>	1.62 (1.46 – 1.80)	<u>3.7E-20</u>			
IgG4_digal	Fraction of digalactosylated glycans	0.89 (0.78 – 1.01)	7.9E-02	0.53 (0.46 – 0.61)	<u>2.8E-18</u>	0.60 (0.53 – 0.67)	<u>1.4E-21</u>			
IgG4_monogal	Fraction of monogalactosylated glycans	0.69 (0.60 – 0.79)	<u>2.0E-08</u>	0.45 (0.38 – 0.53)	<u>7.9E-26</u>	0.68 (0.62 – 0.76)	<u>6.9E-14</u>			
IgG4_sial	Fraction of sialylated glycans	1.01 (0.89 – 1.14)	9.2E-01	0.68 (0.59 – 0.78)	<u>2.2E-08</u>	0.66 (0.60 – 0.74)	<u>3.0E-15</u>			

Derived trait ^a	Description	HC vs. UC ^b		HC vs. CD		UC vs. CD	
		OR (95% CI) ^c	<i>p</i> -value ^d	OR (95% CI)	<i>p</i> -value	OR (95% CI)	<i>p</i> -value
IgG4_A2FB	Bisection of diantennary glycans	0.79 (0.70 – 0.90)	<u>3.5E-04</u>	0.84 (0.73 – 0.96)	<u>1.1E-02</u>	0.97 (0.88 – 1.08)	5.9E-01
IgG4_A2FG	Galactosylation per antenna on diantennary glycans	0.81 (0.71 – 0.92)	<u>1.5E-03</u>	0.48 (0.41 – 0.57)	<u>2.2E-22</u>	0.61 (0.55 – 0.68)	<u>5.4E-21</u>
IgG4_A2FS	Sialylation per antenna on diantennary glycans	1.03 (0.90 – 1.17)	6.8E-01	0.70 (0.61 – 0.80)	<u>1.3E-07</u>	0.67 (0.60 – 0.74)	<u>8.5E-15</u>
IgG4_A2FGS	Sialylation per galactose on diantennary glycans	1.38 (1.21 – 1.57)	<u>8.5E-07</u>	1.39 (1.22 – 1.60)	<u>7.2E-07</u>	1.00 (0.90 – 1.10)	9.3E-01

^aDerived glycosylation traits were calculated as described in Supp. Table 4.

^bTo assess differences between HC and UC patients (HC = 0, UC = 1), HC and CD patients (HC = 0, CD = 1) and between UC and CD patients (UC = 0, CD = 1), logistic regression was performed, using age and sex as co-variables.

^cDisplayed are the odds ratios (OR) and their 95 % confidence intervals (CI) for the ITA cohort.

^d*p*-values were indicated in bold when statistically significant after multiple testing correction (5% FDR). Underlined *p*-values indicate results that were replicated in the US cohort (Supp. Table 6., 8., and 10.).