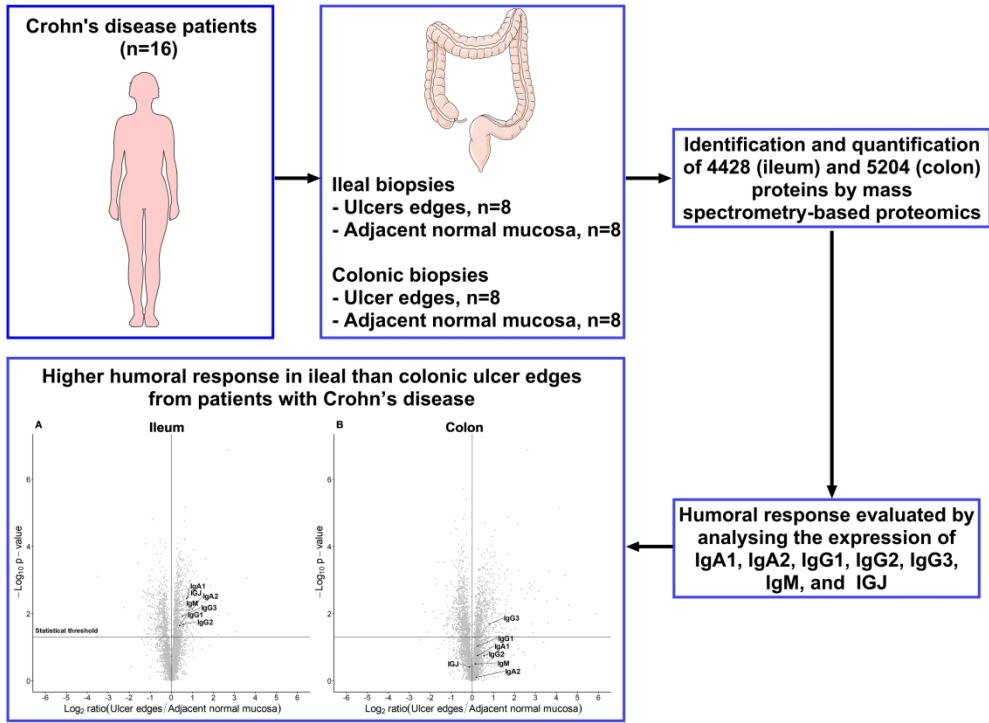




Higher humoral response in ileal versus colonic Crohn's disease

Journal:	<i>Clinical and Experimental Immunology</i>
Manuscript ID	CEI-2025-0125.R2
Manuscript Type:	Short Report
Date Submitted by the Author:	n/a
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Key Words:	Inflammation, Mucosa, B Cells, Immunoglobulins, Proteomics

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286x210mm (300 x 300 DPI)

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3 **1 Higher humoral response in ileal versus colonic Crohn's disease**

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5 **2 Short title: Crohn's disease location and humoral response**

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Abstract

Despite its interest for the development of personalised medicine, the immunological differences between ileal and colonic Crohn's disease (CD) have been understudied. For unknown reasons, some circulating antibodies are associated with CD location (ileal CD: anti-*Saccharomyces cerevisiae* antibodies, anti-flagellins antibodies, anti-granulocyte macrophage-colony stimulating factor autoantibodies, and some pancreatic autoantibodies; colonic CD: perinuclear antineutrophil cytoplasmic autoantibodies). Based on these observations, we hypothesised that, in tissues, the humoral response differs between ileal and colonic CD. This hypothesis was tested by analysing the expression of IgA1, IgA2, IgG1, IgG2, IgG3, IgM and immunoglobulin J chain (IGJ) in our previous dataset comparing the proteome of ulcer edges and adjacent normal mucosa (paired design) in the ileum (4 428 proteins screened in 16 biopsies) and colon (5 204 proteins screened in 16 biopsies) of 16 patients with CD. All these proteins were increased in ileal ulcer edges compared with adjacent normal mucosa, whereas only IgG3 was increased in colonic ulcer edges compared with adjacent normal mucosa. These data highlight the distinct role of humoral immunity in ileal and colonic CD, thereby opening a new avenue of research for developing therapies tailored to CD location.

51 **Introduction**

52 In Crohn's disease (CD), a therapeutic ceiling is observed with a response rate limited to ~30%¹.
53 CD has not yet entered the era of personalised medicine, leading to suboptimal treatments.
54 Currently, treatments do not target particular CD phenotypes and the pipeline of novel therapies
55 is not designed to change this situation². Breaking the therapeutic ceiling in CD will require a
56 better understanding of the mechanisms behind the disease heterogeneity.

57 CD is mostly located in the terminal ileum and colon. The distribution of CD location is
58 approximately 30% ileal, 40% ileocolonic, and 30% colonic³. Constitutively, the small and the
59 large intestines are distinct immunological sites⁴. It is therefore not surprising that CD location
60 is a major source of heterogeneity. Ileal and colonic CD can be considered as distinct entities,
61 as supported by epidemiological, clinical, and biological data^{4,5}. At a therapeutic level, the
62 current biologics used in CD (anti-TNF α , anti- $\alpha_4\beta_7$ integrin, anti-IL12/IL23, anti-IL23, and
63 anti-JAK inhibitor) have shown less efficacy in ileal than colonic CD⁶⁻¹¹. In this context,
64 uncovering the immunological differences between ileal and colonic CD is critical for
65 developing personalised medicine. However, this research topic has been overlooked^{4,12}.

66 Although humoral immunity is fundamental to maintaining gut homeostasis, its role in CD
67 have been underappreciated¹³. In 1975, a pioneer work of Brandtzaeg's group supported a
68 pathogenic role of humoral immunity in CD. In colonic CD, they reported a disproportionate
69 increase of gut IgG plasma cells compared with IgA and IgM plasma cells¹⁴. This IgG response
70 is suspected to promote gut inflammation. Indeed, IgG1 and IgG3 activate the complement
71 system, while binding of IgG to their receptors, the Fc γ receptors (Fc γ Rs), is known to promote
72 an inflammatory response in myeloid cells¹⁵. Recently, data from humans and mice supported
73 the pathogenic role of IgG-Fc γ Rs signaling in CD¹⁶.

74 The importance of humoral immunity in CD has also been highlighted by recent studies
75 showing increases in several circulating antibodies years before diagnosis¹⁶⁻¹⁹. These

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3 76 observations concern anti-*Saccharomyces cerevisiae* antibodies (ASCAs), anti-*Escherichia*
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5 77 *coli* outer membrane porin C (OmpC) antibodies, anti-flagellins antibodies (anti-CBir1 and
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7 78 anti-flagellin X), perinuclear antineutrophil cytoplasmic autoantibodies (pANCA), and anti-
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10 79 granulocyte macrophage-colony stimulating factor (GM-CSF) autoantibodies. For unknown
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12 80 reasons, ileal CD is associated with ASCAs²⁰, anti-flagellins antibodies^{21,22}, and anti-GM-CSF
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14 81 autoantibodies²³, and some pancreatic autoantibodies²⁴; whereas colonic CD is associated with
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17 82 pANCA²⁵. It has also been reported that IgA^{-/-} mice develop spontaneous ileitis but not
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19 83 colitis²⁶. Based on these observations, we hypothesised that the humoral response differs
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21 84 between ileal and colonic CD. To test this hypothesis, we compared the humoral response in
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24 85 ileal and colonic biopsies from patients with CD.
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28 87 **Materials and methods**

29 88 *Data source*

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31 89 Data were obtained from our previous proteomic study comparing the proteome of ulcer edges
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33 90 and adjacent normal mucosa (paired design) in the ileum (n=16 biopsies) and colon (n=16
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35 91 biopsies) of 16 patients with CD (Table 1)²⁷. In this work, we screened 4 428 proteins in the
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37 92 ileum and 5 204 proteins in the colon using mass spectrometry-based proteomics. The
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39 93 methodology used to generate this dataset has been described in detail previously²⁷. Briefly,
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41 94 intestinal biopsies (~5 mg, ~4 mm³) were lysed using RIPA buffer and sonication. In these
42
43 95 homogenates, protein concentration was measured using the RCDC Protein Assay Kit (BioRad,
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45 96 USA). Proteins (20 µg) were purified using 2D clean-up assay (GE Healthcare, USA) and then
46
47 97 digested using Trypsin/Lys-C Mix Mass Spec Grade (Promega, USA). The resulting peptides
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49 98 were purified on ZipTip C18 (Thermo Fisher Scientific, USA) and analysed (2 µg) by ultra-
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51 99 performance liquid chromatography/electrospray ionization tandem mass spectrometry
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54 100 (UPLC-ESI-MS/MS). This system consisted of a 2D nanoAcquity chromatography (Waters)
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3 101 coupled online with a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer
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5 102 (Thermo Fisher Scientific). Proteins were identified and quantified using MaxQuant Version
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7 103 1.5.5.1²⁸. Proteins were searched in the Uniprot Human database (20 237 reviewed entries,
8
9 104 release 2017_09) with the following parameters: trypsin as enzyme, carbamidomethylation (C)
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11 105 as fixed modification, oxidation (M) and phosphorylation (S, T, Y) as variable modifications,
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13 106 a maximum of 2 miscleavages, a minimal peptide length of 7 amino acids, a minimum of 2
14
15 107 peptides per protein, at least 1 unique peptide per protein, match-between runs allowed, and a
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17 108 false discovery rate of 1% for peptide and protein identification. Protein quantification was
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19 109 performed using the MaxQuant label-free quantification (LFQ) algorithm²⁹.

23 110 *Evaluation of the humoral response*

25
26 111 The humoral response was evaluated by analysing the expression of IgA1, IgA2, IgG1, IgG2,
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28 112 IgG3, IgM, and the immunoglobulin J chain (IGJ). Of note, the IgE, IgD and IgG4 were not
29
30 113 detected in our proteomic dataset.

32 114 *Statistical analysis*

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34 115 The two-tailed paired t-test was used for the differential protein expression analysis: ileal ulcer
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36 116 edges versus adjacent normal mucosa; colonic ulcer edges versus adjacent normal mucosa. The
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38 117 significance threshold was set at a p-value <0.05. The statistical analysis was performed using
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40 118 Perseus version 1.6.0.7³⁰. The plot was generated using ggplot2 R package³¹ (R V.3.3.1).

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46 120 **Results**

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48 121 IgA1, IgA2, IgG1, IgG2, IgG3, IgM, and IGJ were increased in ileal ulcer edges compared with
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50 122 adjacent normal mucosa, whereas only IgG3 was increased in colonic ulcer edges compared
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52 123 with adjacent normal mucosa (Figures 1 and 2).

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58 125 **Discussion**

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3 126 In this study, we highlighted that the humoral response is higher in ileal than in colonic ulcer
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5 127 edges from patients with CD. This result echoes other observations. In a study comparing
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7 128 inflamed and non-inflamed mucosa from CD patients using single-cell RNA sequencing,
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9 129 plasma cell counts were increased in the ileum but not in the colon³². In 1976, Brandtzaeg's
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11 130 group reported a similar observation in patients with CD³³: "Despite similar absolute numbers
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13 131 of Ig-containing cells in the inflamed ileal and colonic mucosal units, the absolute numerical
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15 132 increase compared with normal was much larger for the former than for the latter." Finally, the
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17 133 majority of circulating antibodies associated with CD location are specifically associated with
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19 134 ileal involvement²⁴. These observations and our data support the idea that the humoral response
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24 135 is higher in ileal than in colonic CD.

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26 136 Depletion of CD20-expressing B-cells with rituximab is effective in several inflammatory
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28 137 diseases such as rheumatoid arthritis³⁴ and ANCA-associated vasculitis³⁵. Nevertheless, in
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30 138 ulcerative colitis (UC), a randomised controlled trial demonstrated that rituximab did not induce
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32 139 remission³⁶. Such a result could partly explain why B-cells have received little attention in
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34 140 inflammatory bowel disease (IBD)¹³, which includes CD and UC. However, CD20 expression
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36 141 is lost in plasma cells³⁷ and rituximab has been shown to deplete lamina propria CD20-
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38 142 expressing B-cells but not IgA⁺ plasmablasts³⁸. In this context, it appears crucial to identify the
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40 143 subsets of plasma cells that play a pathogenic role in CD. Our current findings suggest that this
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42 144 line of research could offer new avenues for developing treatments tailored to CD location.
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46 145 Four proteins of our dataset (IgA1, IgA2, IgM, and IGJ) are key components of the immune
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48 146 exclusion, i.e., the mechanism limiting microbial access to the intestinal epithelium through the
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50 147 luminal secretion of IgA dimers and IgM pentamers. IGJ plays a central role in immune
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52 148 exclusion. First, IGJ stabilises IgA dimers and IgM pentamers³⁹. Second, the binding of IGJ to
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54 149 the polymeric immunoglobulin receptor (expressed on the basolateral side of epithelial cells)
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56 150 enables the transport of IgA dimers and IgM pentamers across the epithelial cells (transcytosis)
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3 151 for their luminal secretion. In the present study, we showed that proteins involved in immune
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5 152 exclusion (IgA1, IgA2, IgM, and IGJ) were increased (ulcer edges versus adjacent normal
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7 153 mucosa) in the ileum, but not in the colon. This observation suggests that immune exclusion
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10 154 plays a different role in ileal and colonic CD. In 1988, Brandtzaeg's group showed that IgA
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12 155 producing cells positive for IGJ were decreased in the colonic mucosa of patients with CD
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14 156 compared to healthy controls⁴⁰. This result could support an alteration of the immune exclusion
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16 157 mechanism in colonic CD, as IGJ expression directly regulates the formation of IgA dimers⁴¹.
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19 158 In this context, the present study highlights the need to reconsider immune exclusion in CD.
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21 159 Although studies by Brandtzaeg's group were promising, their line of research has been
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23 160 neglected. Plasma cells remain understudied in CD, particularly when compared with T cells.
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25 161 It is worth noting that Brandtzaeg's group did not investigate differences between ileal and
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27 162 colonic CD, focusing instead on IBD of the large intestine (colonic CD and UC). Our results
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29 163 support the interest of studying plasma cells in ileal and colonic CD.
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33 164 We acknowledge that our study is limited by the lack of cellular resolution, as we analysed
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35 165 bulk proteomic data. Future works will be needed to identify the cell populations responsible
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37 166 for our finding. Furthermore, we acknowledge that patients' use of medications may have
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39 167 impacted the results.
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41
42 168 In summary, our data highlight that humoral response is higher in ileal than in colonic CD.
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44 169 This finding warrants further investigation and may foster research into the role of humoral
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46 170 immunity in CD.
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49 172 **Ethics and consent**

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51 173 This study complied with the Helsinki Declaration and received approval from the ethics
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53 174 reviewing board of the University Hospital of Liège, Belgium [March 26 2013] [Belgian
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55 175 reference: 707201317029]. All patients gave their written informed consent.
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177 Conflict of Interest

178 The authors declare that the research was conducted in the absence of any commercial or
179 financial relationships that could be construed as a potential conflict of interest.

180

181 Funding

182 This work was supported by the Walloon Region and the Fond européen de développement
183 régional [FEDER] [Grant portofolio 246099-510388]. Additional support was provided by
184 internal funding from the Liège University Hospital. NP is supported by a fellowship from the
185 F.R.S.-FNRS.

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187 Data Availability

188 All the raw data are available in the proteomics identifications database (PRIDE) under
189 accession number PXD012284.

190

191 Author contributions

192 Sophie Vieujean (Formal analysis, Investigation, Writing-Review & Editing), Nathalie Jacobs
193 (Writing-Review & Editing), Rodrigo Fernández-Verdejo (Writing-Review & Editing), Judith
194 Fraussen (Writing-Review & Editing), Dominique Baiwir (Investigation, Resources), Gabriel
195 Mazzucchelli (Investigation, Resources), Catherine Reenaers (Resources), Catherine Van
196 Kemseke (Resources), Edouard Louis (Resources, Writing-Review & Editing, Funding
197 acquisition), Nicolas Pierre (Conceptualization, Methodology, Software, Validation, Formal
198 analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review & Editing,
199 Visualization, Supervision, Project administration).

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3 201 **Acknowledgments**
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5 202 The authors are grateful to the patients who participated in the study.
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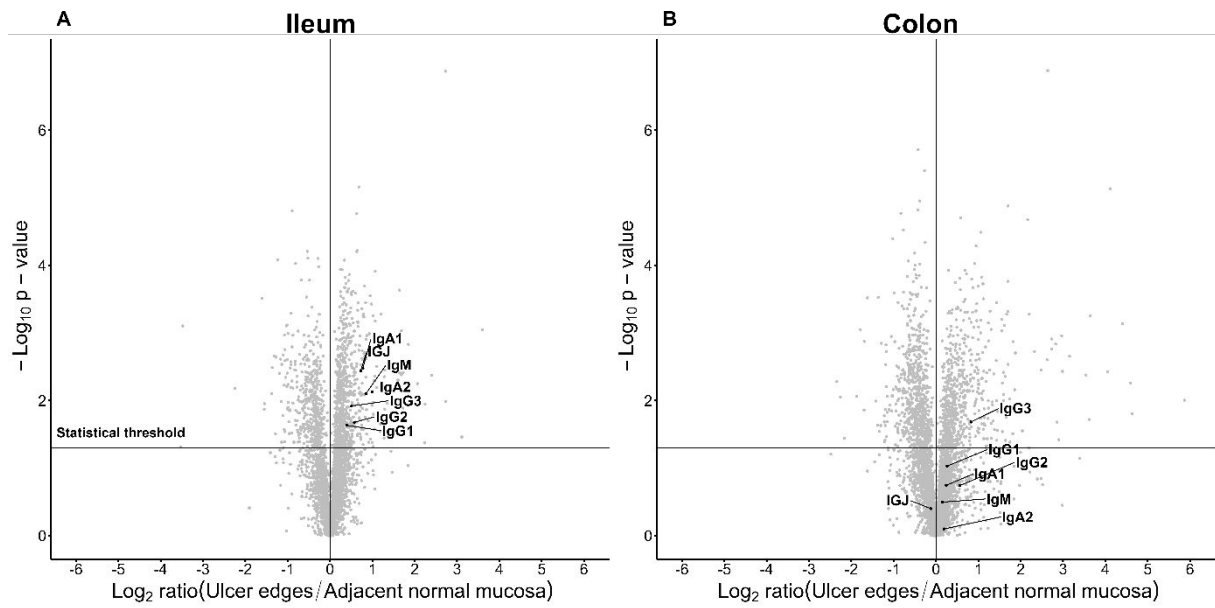
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Table 1. Patients' characteristics

	Patients with ileal ulcers	Patients with colonic ulcers
Patients, n (males/females)	8 (1/7)	8 (4/4)
Age, median years (min-max)	37.5 (30-68)	38.0 (30-43)
Disease duration, median years (min-max)	9 (1-41)	12.5 (0-34)
Smoking, n		
Yes	4	4
Former	3	2
No	1	2
Disease location at the time of the endoscopy, n		
Ileal	5	0
Colonic	0	3
Ileocolonic	3	5
Medication, n*		
None	3	4
Corticoids	2	3
Antibiotics	0	0
Antimetabolites	2	1
Anti-TNF α	3	1
Anti- $\alpha_4\beta_7$ integrin	0	2

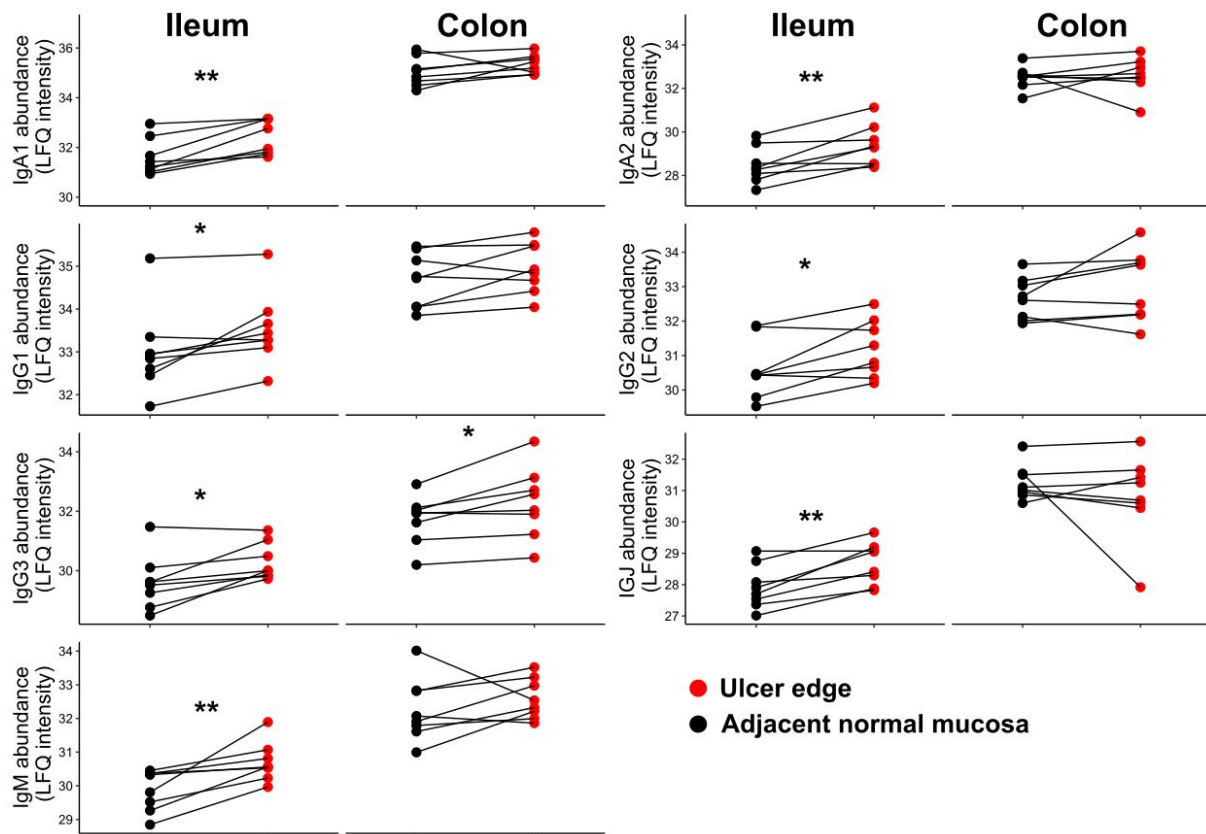
*Some patients received several medications

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333 **Figure 1. Volcano plots illustrating differential protein expression of IgA1, IgA2, IgG1,**334 **IgG2, IgG3, IgM, and IGJ. Ileal ulcer edges versus adjacent normal mucosa (A). Colonic ulcer**335 **edges versus adjacent normal mucosa (B). The statistical threshold ($p\text{-value} = 0.05$) is**336 **represented by the horizontal lines. IgA1, immunoglobulin A1; IgA2, immunoglobulin A2;**337 **IgG1, immunoglobulin G1; IgG2, immunoglobulin G2; IgG3, immunoglobulin G3; IgM,**338 **immunoglobulin M; IGJ, immunoglobulin J chain.**



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340 **Figure 2. Dot plots showing the abundance levels of IgA1, IgA2, IgG1, IgG2, IgG3, IgM,**
 341 **and IGJ. LFQ, label-free quantification. Ulcer edge versus adjacent normal mucosa: *p<0.05;**
 342 ****p<0.01.**

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