Original research

Specific gut pathobionts escape antibody coating and are enriched during flares in patients with severe Crohn's disease

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2023-330677).

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Received 12 July 2023
Accepted 4 December 2023
Published Online First 16 December 2023

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To cite: Eriksen C, Danneskiold-Samsøe NB, Moll JM, *et al*. *Gut* 2024;**73**:448–458.

ABSTRACT

Objective Patients with Crohn's disease (CD) exhibit great heterogeneity in disease presentation and treatment responses, where distinct gut bacteria and immune interactions may play part in the yet unresolved disease aetiology. Given the role of antibodies in the barrier defence against microbes, we hypothesised that gut bacterial antibody-coating patterns may influence underlying disease-mediated processes.

Design Absolute and relative single and multicoating of gut bacteria with IgA, IgG1, IgG2, IgG3 and IgG4 in patients with CD and healthy controls were characterised and compared with disease activity. IgG2-coated and non-coated taxa from patients with severe CD were identified, profiled for pathogenic characteristics and monitored for enrichment during active disease across cohorts.

Results Patients with severe CD exhibited higher gut bacterial IgG2-coating. Supervised clustering identified 25 bacteria to be enriched in CD patients with high IgG2-coating. Sorting, sequencing and *in silico*-based assessments of the virulent potential of IgG2-coated and bulk stool bacteria were performed to evaluate the nature and pathogenicity of IgG2-coated and non-coated bacteria. The analyses demonstrated IgG2-coating of both known pathogenic and non-pathogenic bacteria that co-occurred with two non-coated pathobionts, *Campylobacter* and *Mannheimia*. The two non-coated pathobionts exhibited low prevalence, rarely coincided and were strongly enriched during disease flares in patients with CD across independent and geographically distant cohorts.

Conclusion Distinct gut bacterial IgG2-coating was demonstrated in patients with severe CD and during disease flares. Co-occurrence of non-coated pathobionts with IgG2-coated bacteria points to an uncontrolled inflammatory condition in severe CD mediated via escape from antibody coating by two gut pathobionts.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous findings reported enhanced IgA coating of gut bacteria in inflammatory bowel disease.

⇒ IgA-coating of microbes is acknowledged as an important barrier defence mechanism, but little is known of the role of IgG isotype-coating of bacteria in the human gut and in relation to disease.

WHAT THIS STUDY ADDS

⇒ The study identifies distinctly elevated gut bacterial IgG2-coating in patients with severe Crohn's disease, and finds that two non-antibody-coated immune evasive pathobionts associate with high IgG2-coating levels of other co-occurring bacteria and disease activity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings point to selective IgG2-coating of gut bacteria as a marker for an exaggerated proinflammatory gut microbial environment.

⇒ Targeting of the identified non-antibody-coated disease flare-associated gut bacteria may be a means for disease control in patients with severe disease.

INTRODUCTION

Crohn's disease (CD) is a chronic and relapsing inflammatory bowel disease (IBD) with great heterogeneity in disease presentation, progression and treatment responses.[1] There is currently no curative treatment for CD, making monitoring of mucosal inflammation crucial in order to limit disease progression and complications. It is well substantiated that gut bacteria may induce immune activation during the course of CD as demonstrated by the consistent associations between certain gut bacteria and CD disease aetiology.[2] Still, we have limited evidence that specific bacteria are consistent disease drivers among patients. Classically, the immunopathogenesis of CD has been described to be driven by uncontrolled type 1 and type 17/3 immune reactions with IL-12p70 and IFN-ɣ,[3] and IL-17A, IL-21, IL-22 and IL-23[4] as the major disease-associated cytokines, respectively. These

immune reactions may result in distinct effector responses in the form of distinct Ig production, but still the involvement of different IgG isotypes in disease protection or exacerbation in CD remains unresolved. Cytokines associated with type 1 immunity have previously been described to prime the production of the IgG2 subtype,[5 6] while the influence of type 17/3 immune-related cytokines on class-switching is less well documented. There are presently few reports on a role for IgG in the antimicrobial barrier defence in the intestine, although IgG is recognised as the major antibody class of circulating blood in bacterial infections.[7] A recent study identified very limited fractions (0.16%) of gut bacteria to be coated with IgG,[8] despite serum-derived IgG has been shown to hold the capacity to bind various gut bacteria.[8] The latter emphasises that IgG indeed may be induced in the gut mucosa, from where they may be transported to the gut lumen via binding to the neonatal Fc receptor.[9] IgG responses are less promiscuous than those associated with IgA[10] due to their requirement for T-cell help in isotype switching to IgG, resulting in increased antibody affinity towards targeted antigens.[11]

We here hypothesise that gut bacterial Ig-coating patterns, including the IgG isotypes IgG1, IgG2, IgG3 and IgG4 as well as the notoriously present mucosal IgA, may be used to define underlying immune-mediated processes in CD, thereby helping in differentiating disease endophenotypes. We first characterised single coating and multicoating of gut bacteria with IgA and the four IgG isotypes (in quantitative and relative numbers) in 20 healthy controls and 60 patients with CD from the IBD South Limburg (IBDSL) cohort, a population-based inception cohort from the South Limburg area of the Netherlands (NL).[12] We identified IgG2-coating in patients with severe CD (defined as gastrointestinal (GI) surgery, higher faecal calprotectin, more often having a B3 disease behaviour compared with B1 or B2 (Montreal classification) and Harvey-Bradshaw Index (HBI)), as well as increased gut bacterial IgG2-coating during active disease, defined as faecal calprotectin >250 µg/g or faecal calprotectin >100 µg/g and at least a fivefold increase from baseline. Taxa comparisons of sorted and sequenced IgG2-coated bacteria versus bulk stool sequenced bacteria led to identification of IgG2-coated bacteria that co-occur with two non-coated gut pathobionts in patients with severe CD during disease flares. The two non-coated gut pathobionts were also enriched during active disease flares in a non-related American cohort of 297 patients with CD. Thus, IgG2-coated gut bacteria were identified in patients with severe CD, where they co-occur with distinct non-coated gut pathobionts that appeared during flares, hence pointing to an immunologically uncontrolled presence of certain gut pathobionts.

RESULTS

IgG2 gut bacterial coating associates with CD severity

Previous studies have reported on the binding of IgA[13 14] and total IgG[15] to gut bacteria in humans. However, bacterial coating with the four IgG isotypes has not been thoroughly studied, although they may be involved in barrier protection in the intestine. To examine the dynamics of IgA and IgG1-4 bacterial coating in healthy individuals and patients with varying severity of CD, we determined the levels of coated gut bacteria in 60 patients with CD and 20 healthy individuals (online supplemental table 1, for cohort statistics), as relative and actual numbers of coated bacteria per gram of stool, using multiparametric flow cytometry (figure 1A, online supplemental figure1).

The median relative abundance of 3.75% IgA-coated gut bacteria in healthy individuals (figure 1B, table 1) was consistent with the average relative abundance reported by Fadlallah *et al*[16] in healthy Europeans. An average of 1.7-fold more IgA-coated bacteria were identified in patients with CD compared with healthy individuals (figure 1B, table 1, p=0.024), which is in line with findings in Palm *et al*.[14]

The relative bacterial coating with total IgG was only 2.2-fold lower than that with IgA, and did not differ between patients with CD and healthy individuals (figure 1B, table 1), thus pointing to substantial gut bacterial IgG-coating independent on disease. The IgG isotypes coated gut bacteria with varying frequency, where consistently high coating levels were seen for IgG1 and IgG4, while coating with IgG2 and IgG3 was relatively rare, and no significant differences were identified in the overall IgG-coating between healthy individuals and patients with CD (figure 1C, table 1).

Flow cytometry-based counting of bacteria was used to determine the total bacterial load per gram of stool thereby enabling calculation of the actual number of coated bacteria per gram of stool. The bacterial load between healthy individuals and patients with CD was not significantly different in this cohort (figure 1D, table 1). Likewise, when examining single and multi-Ig coated gut bacteria frequencies, we found no differences in the actual number of gut bacteria with single or double Ig-coating between healthy individuals and patients with CD (figure 1E). More than 96% of the coated gut bacteria were found to be single-coated with IgA and IgG1-IgG4 in both healthy individuals and patients with CD (online supplemental table 2). For IgG2, we identified only minute levels of single IgG2-coating of bacteria in the healthy and CD gut; rather, most IgG2 coating co-occurred with IgA (figure 1E, online supplemental table 2).

The percentage of IgA-coated bacteria was found to correlate positively with an increasing Bristol Stool Scale value (figure 1F, $r=0.43$, $p=7.13\times10^{-5}$), a qualitative measure for fewer gut bacteria, reduced stool consistency and faster stool transit time,[17] and also correlated inversely with increasing bacterial load (bacteria/g stool (online supplemental figure 2) $r=-0.46$, $p=2.48\times10^{-5}$). Hence, illustrating that individuals with fewer gut bacteria displayed a higher relative IgA-coating of bacteria.

To investigate the dynamics between the different gut bacterial Ig-coating patterns, the single-coating and multicoating patterns of all individuals were visualised in a PCA. Some patients were identified to separate from the rest due to their IgG2 single-coating and multicoating patterns (figure 1G; arrows to the right). When we correlated clinical parameters with Ig-coating patterns in patients with CD, we found single-coating and multicoating with IgG2 to relate with features related to disease activity (faecal calprotectin) and disease severity (surgery, more often having a B3 disease behaviour compared with B1 or B2 (Montreal classification) and HBI) (figure 1H). Generalised linear models revealed that the load of single-IgG2 coated bacteria displayed strong association with active disease (faecal calprotectin), while the load of multi-IgG2 coated bacteria linked strongly to the disease severity markers (figure 1I). The load of IgA coated bacteria or single-IgA coated bacteria did not show significant relations to active disease (faecal calprotectin), and showed weaker relations to HBI and disease behaviour than seen for single-IgG2 and double-IgG2 coated bacteria.

Several of the patients with CD were undergoing treatments with different drugs (mesalazines, thiopurines, biologicals, prednison or proton pump inhibitors (PPI)) at time of sampling (online supplemental table 1). Treatment with these drugs did

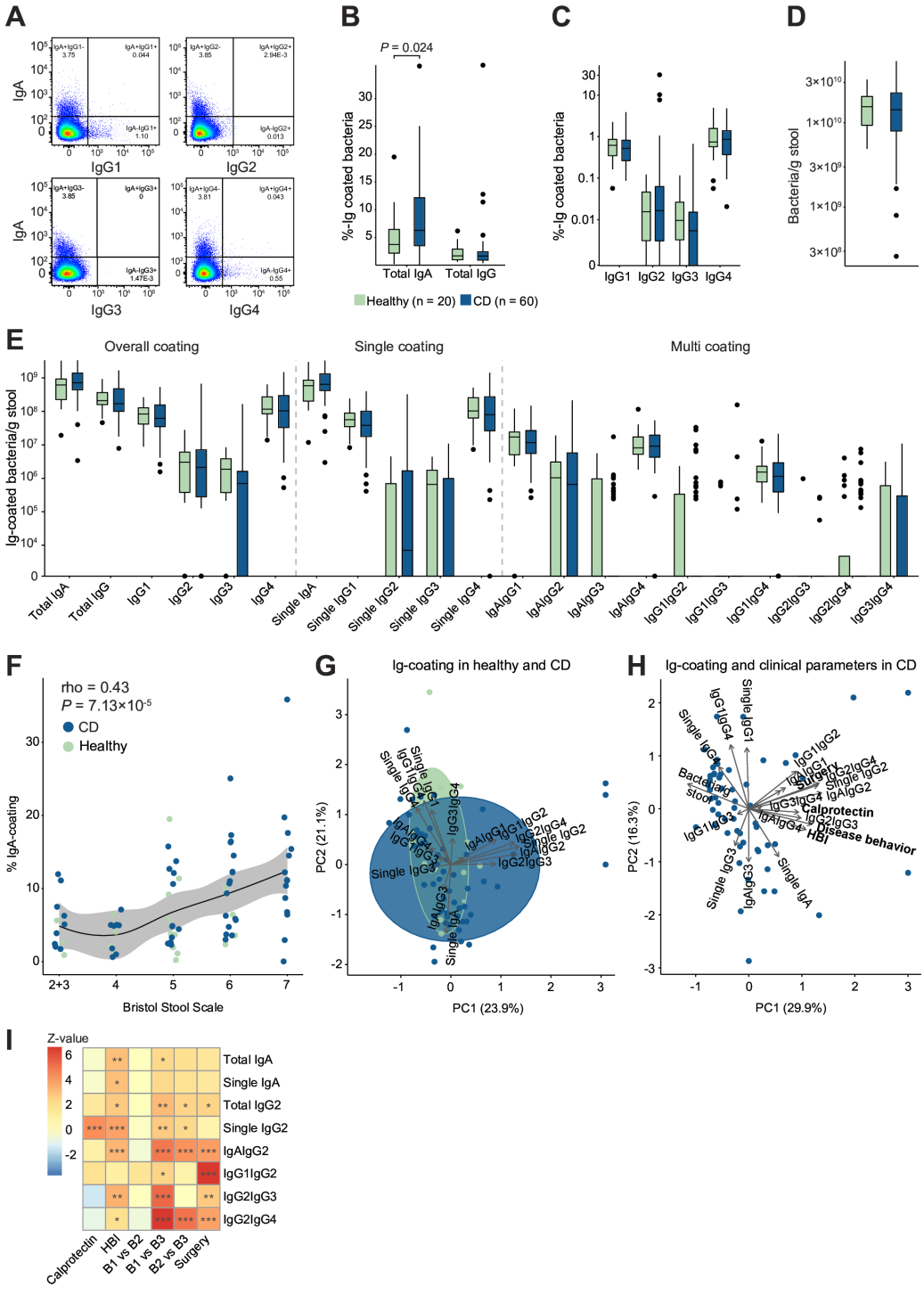

Figure 1 Immunoglobulin-coated bacteria in patients with CD and healthy individuals. (A) Representative plots of the multiparametric flow cytometry-based analysis of IgA, IgG1, IgG2, IgG3 and IgG4 coating of gut bacteria in healthy individuals and patients with CD to determine: (B) The relative Ig-coating of total IgA and total IgG (sum of IgG1–4), and (C) Individual IgG's. (D) Total number of bacteria/g stool based on flow cytometry analysis. (E) Quantity of gut bacteria/g stool coated with the different antibodies alone (single) or in combination (multi). The overall coating is the sum of single and multicoating for the respective antibodies. (F) Bristol stool scale versus % IgA coating of gut bacteria. Spearman's r statistics was used for correlations. The line depicts a local polynomial regression fit, and the shaded area is the 95% CI. (G) Principal component analysis of Ig-coating frequencies in healthy individuals and patients with CD. Dots and the confidence ellipses of the variance within each group are represented by the green colour for healthy individuals and blue for patients with CD. (H) Gut bacterial Ig-coating versus clinical parameters (calprotectin, HBI, disease behaviour and GI surgery) in patients with CD. (I) Heat map displaying the outcome of generalised linear models between IgG2, single IgG2, multi-IgG2, total IgA and single IgA coated bacteria/g stool versus disease parameters. Z-values in red represent positive association, while Z-values in blue represent inverse association. Asterisks represent p values, for significant associations. In all plots: healthy individuals, n=20; patients with CD, n=60. Statistical analyses were based on Wilcoxon rank-sum test (B–E) for group comparisons shown as boxplots where centre lines indicate the median and the box limits indicate the quartiles. Whiskers extend to the data points within 1.58×the IQR, and outliers are shown as individual dots where centre lines indicate the median and the box limits indicate the quartiles. CD, Crohn's disease; HBI, Harvey-Bradshaw Index.

Table 1 Relative and quantitative Ig-coating of gut bacteria

	Healthy (n=20)		CD (n=60)		
% Ig-coating	Median (quartiles)	Mean±SD	Median (quartiles)	Mean±SD	P value
% IgA	3.75 (2.19; 6.45)	5.10±4.62	6.28 (3.52; 12.14)	8.42±6.61	**0.024**
% IgG	1.69 (0.99; 2.9)	2.05±1.51	1.67 (0.94; 2.41%)	2.66±4.87	0.93
% IgG1	0.62 (0.35; 0.85)	0.68±0.48	0.52 (0.27; 0.82%)	0.71±0.70	0.56
% IgG2	0.02 (3.11×10^{-3}; 0.05)	0.03±0.04	0.02 (3.09×10^{-3}; 0.06)	0.89±4.25	0.89
% IgG3	0.01 (3.27×10^{-3}; 0.03)	0.02±0.03	0.01 (0.00; 0.02)	0.03±0.09	0.39
% IgG4	0.74 (0.57; 1.58)	1.32±1.27	0.86 (0.37; 1.39)	1.04±0.89	0.48
Bacteria/g stool					
Total load	1.54×10^{10} (9.33×10^{9}; 2.04×10^{10})	$1.59\times10^{10}\pm8.00\times10^{9}$	1.42×10^{10} (7.99×10^{9}; 2.25×10^{10})	$1.57\times10^{10}\pm1.03\times10^{10}$	0.73
IgA	6.17×10^{8} (2.27×10^{8}; 9.30×10^{8})	$7.29\times10^{8}\pm6.99\times10^{8}$	7.26×10^{8} (4.42×10^{8}; 1.39×10^{9})	$9.83\times10^{8}\pm7.56\times10^{8}$	0.15
IgG	2.10×10^{8} (1.60×10^{8}; 3.64×10^{8})	$2.87\times10^{8}\pm2.02\times10^{8}$	1.70×10^{8} (1.01×10^{8}; 4.75×10^{8})	$3.16\times10^{8}\pm3.13\times10^{8}$	0.68
IgG1	8.40×10^{7} (4.18×10^{7}; 1.29×10^{8})	$1.00\times10^{8}\pm7.60\times10^{7}$	6.25×10^{7} (3.53×10^{7}; 1.55×10^{8})	$9.91\times10^{7}\pm1.03\times10^{8}$	0.48
IgG2	3.04×10^{6} (3.87×10^{5}; 6.07×10^{6})	$5.31\times10^{6}\pm7.07\times10^{6}$	2.12×10^{6} (2.80×10^{5}; 7.08×10^{6})	$2.81\times10^{7}\pm1.08\times10^{8}$	0.83
IgG3	1.89×10^{5} (3.93×10^{5}; 3.86×10^{6})	$2.65\times10^{6}\pm2.71\times10^{6}$	6.99×10^{5} (1.80×10^{3}; 1.61×10^{6})	$5.11\times10^{6}\pm2.15\times10^{7}$	0.11
IgG4	1.17×10^{8} (8.57×10^{7}; 2.73×10^{8})	$1.79\times10^{8}\pm1.60\times10^{8}$	1.04×10^{8} (3.29×10^{7}; 2.98×10^{8})	$1.84\times10^{8}\pm2.30\times10^{8}$	0.60

Bold font: statistically significant difference between CD patients and healthy controls.
CD, Crohn's disease.

not significantly change bacterial coating with IgG2 (online supplemental table 3).

Gut bacterial IgG2-coating is enhanced in patients with CD with active disease and high IgA-coating

Based on the relations between bacterial IgG2-coating and disease activity and severity, we next focused on the varying levels of IgG2-coating across patients with CD, and stratified the cohort based on IgG2-coating tertiles. This resulted in three IgG2-coating phenotypes (IgG2-low (IgG2-lo; 0.0% (0.00%; 0.003%) (median (25th; 75th quartile))), IgG2-intermediate (IgG2-int; 0.02% (0.007%; 0.03%)) and IgG2-hi (IgG2-hi; 0.24% (0.09%; 0.58%)) (figure 2A). Healthy individuals were only represented within the IgG2-lo group, while patients with CD in IgG2-hi displayed higher numbers of single-IgG2 coated bacteria/g stool during active disease versus remission (figure 2B, p=0.028). The number of double-IgG2IgA coated bacteria did not differ significantly with disease activity (online supplemental figure 3). It is noteworthy that CD patients with active disease can hold any of the three IgG2-coating levels (IgG2-lo, N=8; IgG2-int, N=11; IgG2-hi, N=8), stressing that total bacterial IgG2-coating is not a generic marker of active disease. However, we find the likelihood of having severe disease (Montreal disease behaviour B3 compared with B1 or B2) is increased in CD patients with an IgG2-hi versus IgG2-lo and IgG2-int phenotype (OR: B1 vs B3: 7.52, p=0.017; B2 vs B3 25.14, p=0.004, (online supplemental table 4).

IgA-coating in IgG2-hi (11.68% (10.43%; 15.66%); median (25th; 75th Quartile)) was significantly enhanced compared with IgG2-lo (4.89% (2.72%; 6.7%), $p=3.6\times10^{-4}$), IgG2-int (4.82% (3.16%; 10.23%), $p=2.5\times10^{-3}$) and healthy controls (3.75% (2.19%; 6.45%), $p=7.1\times10^{-5}$), while concurrent IgG1-coating, IgG3-coating and IgG4-coating did not differ significantly between healthy and IgG2 subgroups (figure 2C). This finding is not unexpected given our identified association between higher relative IgA-coating versus higher Bristol stool scale (figure 1F), or inverse correlation to bacterial load per g faeces (online supplemental figure 2), and a higher Bristol stool scale found in IgG2-hi patients with CD (online supplemental table 5).

Twenty-five indicator taxa characterise the gut microbiota in IgG2-hi patients with CD

The load of gut bacteria in patients with CD, defined as bacteria per gram of stool, was found to strongly associate with the bacterial α-diversity calculated using the Shannon index (online supplemental figure 4A), r=0.747, $p<2.2\times10^{-16}$), indicating that individuals with a low Shannon index have a lower bacterial load. Patients with IgG2-hi bacterial coating versus IgG2-lo and IgG2-int displayed a significantly lower Shannon index (figure 3A, IgG2-hi vs IgG2-lo, p=0.015 and IgG2-hi vs IgG2-int, p=0.010), as well as reduced bacterial richness (figure 3A, IgG2-hi vs IgG2-lo, p=0.013 and IgG2-hi vs IgG2-int, p=0.013).

The composition of the overall gut microbiota in patients with IgG2-hi gut bacterial coating differed significantly from patients with IgG2-lo and IgG2-int as visualised using non-metric multidimensional scaling (NMDS) of the Bray-Curtis dissimilarity (figure 3B, PERMANOVA: IgG2-hi vs IgG2-lo: $r^2=0.094$, p=0.007; IgG2-hi vs IgG2-int: $r^2=0.085$, p=0.003 (online supplemental table 6). Among the bacteria associated with IgG2-hi coating, we found several bacteria previously reported to be associated with CD, such as *Escherichia/Shigella*, *Veillonella*, *Morganella*, *Proteus*, *Campylobacter*, *Haemophilus* and *Mannheimia*.[18–20] By correlating Ig-coating and clinical parameters for patients with CD with bacterial β-diversity patterns, we identified several bacterial taxa associated with IgG2-hi coating to follow a similar direction as several disease severity parameters (GI surgery, HBI and disease behaviour)

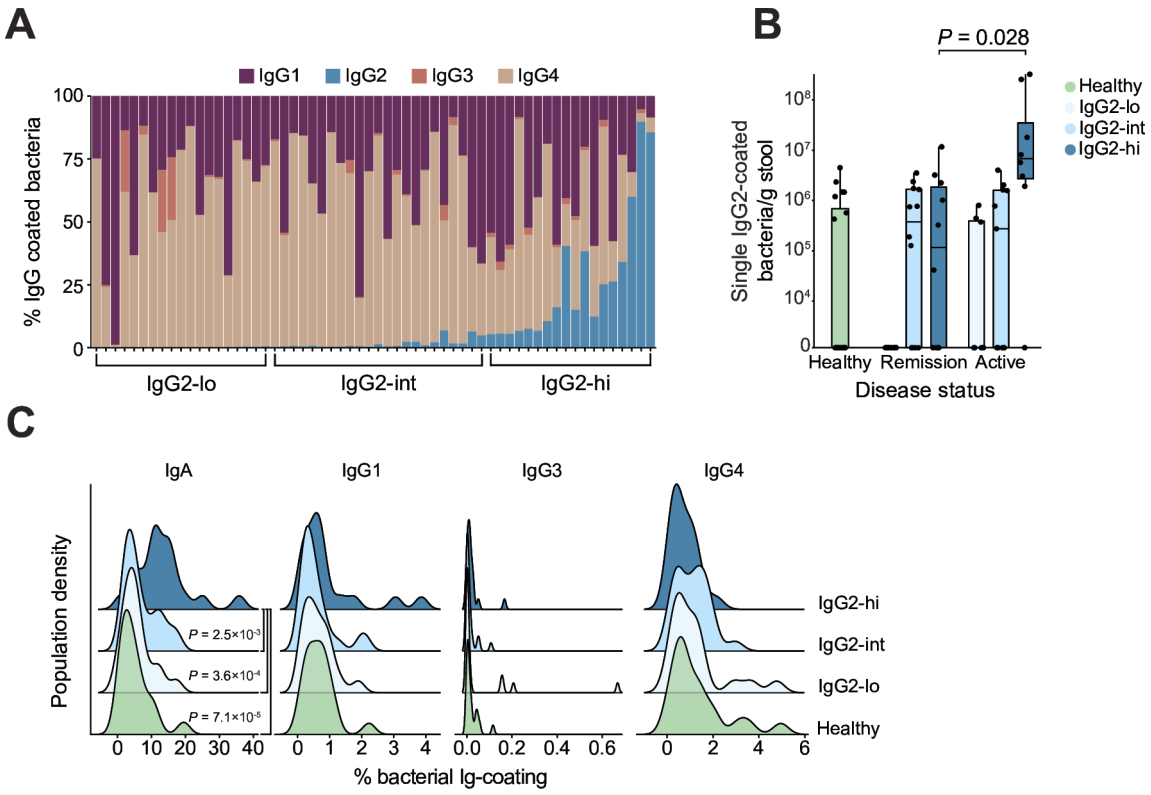

Figure 2 Patients with CD exhibit differential gut bacterial IgG2-coating during active disease. (A) Subgrouping of patients with CD based on tertiles of gut bacterial IgG2-coating levels. (B) Load of single IgG2-coated bacteria/g stool in healthy controls (n=20) and patients with CD in remission (n=33) or with active disease (n=27) for each IgG2-coating subgroup. Centre lines of box plots indicate the median and the box limits indicate the quartiles. Whiskers extend to the data points within 1.58×the IQR. Dots represent the level within each individual. (C) Joyplot illustrating population densities of IgA and IgG1, IgG3 and IgG4 in healthy individuals and patients with CD stratified on the IgG2-coating subgroup. Statistical analyses were based on Wilcoxon rank-sum test (B, C) for group comparisons. CD, Crohn's disease.

and enhanced IgA-coating, and to inversely relate to gut bacterial coating with single IgG1 and IgG4, and IgG1IgG4 double-coating (figure 3C).

We confirmed by a Procrustes analysis that the distribution of all Ig-coating data and the distribution of the total bacterial community showed comparable patterns (Ig-coating (figure 1H) vs total community β-diversity (figure 3B,2=0.386, p=0.001). Since single-coating and double-IgG2-coating were main drivers of these patterns, and overlapped with disease severity parameters, these findings implied that IgG2-coating is the main driver of gut community patterns distinguishing severity of disease in patients with CD.

We next performed a sparse partial least squared discriminant analysis (sPLS-DA)[21] to identify bacterial indicator taxa within the overall gut microbiota that were enriched in IgG2-hi versus IgG2-lo and IgG2-int. The sPLS-DA represents a cross-validated supervised clustering algorithm capable of identifying features important for separation of these groups. The model showed good predictive power (online supplemental figure 4B) (area under the curve (AUC): 0.827)) and resulted in the identification of 25 bacterial indicator taxa that characterised the gut microbiota of IgG2-hi patients (figure 3D, (online supplemental table 7), q<0.1). The sPLS-DA-identified IgG2-hi indicator taxa strongly overlapped with the disease severity-associated bacteria identified using the above NMDS, including *Escherichia/Shigella, Veillonella, Morganella*, *Proteus*, *Campylobacter*, *Haemophilus* and *Mannheimia*.

Identifying the nature of IgG2-coated gut bacteria in patients with CD

FACS was next used to sort out IgG2-hi gut bacteria followed by sequencing of the bacterial V3-V4 16S rRNA gene region. This resulted in identification of 84 uniquely IgG2-coated taxa out of 153 taxa found in bulk stool from IgG2-hi patients with CD (figure 4A (inner circle vs outer circle) online supplemental tables 8 and 9). When comparing the nature of the IgG2-coated bacteria with the identified IgG2-high indicator taxa, it appeared that only 48% of the IgG2-hi indicator taxa were IgG2-coated, meaning that some of the IgG2-hi indicator taxa were non-coated. The non-coated bacteria in IgG2-hi patients with CD included the Proteobacteria *Mannheimia*, *Morganella*, *Proteus*, *Campylobacter*, *Alcaligenes* and members of the *Enterobacteriaceae*, while *Veillonella*, *Escherichia/Shigella*, *Klebsiella* and *Haemophilus* were IgG2-coated.

To improve our understanding of functional differences and pathogenic potential between IgG2-hi coated and non-coated taxa, we performed *in silico* genome-based functional assessments using Picrust2[22] for functional imputation based on taxonomy and the virulence factor database[23] for identification of virulence factors important for invasion, immune evasion and adherence (figure 4B). Among the bacteria in bulk stool from patients with CD, we identified 38 bacterial taxa harbouring at least one relevant virulence factor, among which 12 were IgG2-hi indicator taxa, 17 were IgG2-coated and 21 non-coated. Notably, the non-coated Proteobacteria *Morganella*, *Proteus*, *Campylobacter* and *Enterobacteriaceae* contained most virulence factors. We earlier

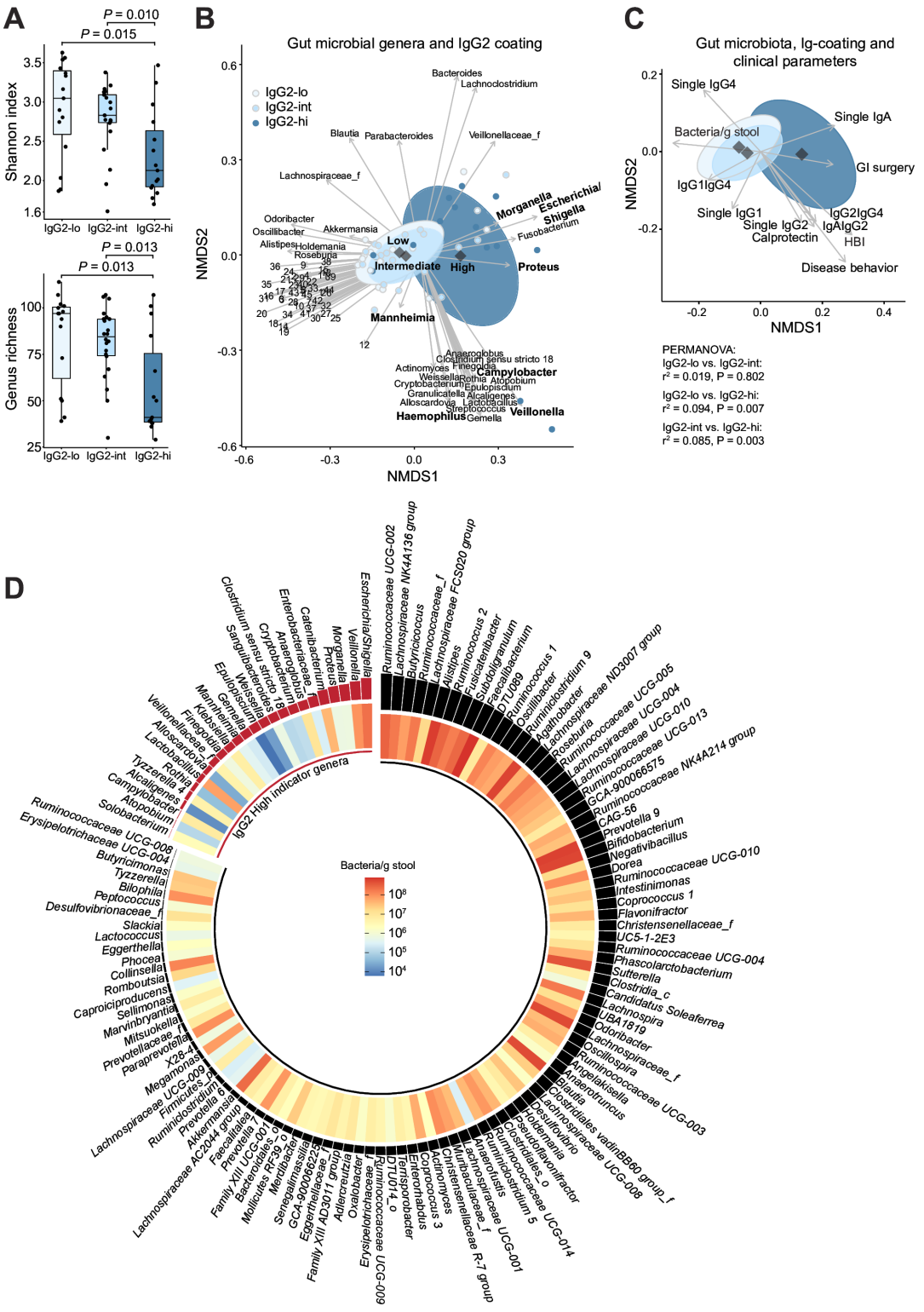

Figure 3 The gut microbiota of patients with CD with high bacterial IgG2-coating associates with severe disease. (A) Alpha-diversity of bacterial communities determined by Shannon index and genus richness within IgG2-coating subgroups. (B) Non-metric multidimensional scaling (NMDS) plot, based on Bray-Curtis distances of the gut microbiota determined by 16S rRNA gene amplicon sequencing in patients with CD. Individuals are represented as coloured dots. Confidence ellipses represent the variance of the mean within each IgG2 subgroup. Individual gut bacterial taxa are fitted onto the plots and represented by arrows (see the Methods section for details). Taxa names for the included numbers are provided in online supplemental table 6. (C) NMDS displaying Ig-coating levels and clinical parameters (instead of individual bacterial taxa). (D) Heatmap showing the bacterial taxa and their abundance (number of bacteria/g stool) identified to separate the IgG2-hi subgroup from the IgG2-lo/int subgroups using sPLS-DA. Bars on top of each tile represent how important the individual bacterium is for the separation. Red bars highlight IgG2-hi indicator taxa, while black bars highlight taxa representing the IgG2-lo/int subgroups. For analysis, (B, C) the length of arrows corresponds to r^2 values (FDR-adjusted using q<0.1) and each rhombus represent the centre of the groups. Statistical analyses were based on Wilcoxon rank-sum test. GI, gastrointestinal; HBI, Harvey-Bradshaw Index; sPLS-DA, sparse partial least squared discriminant analysis.

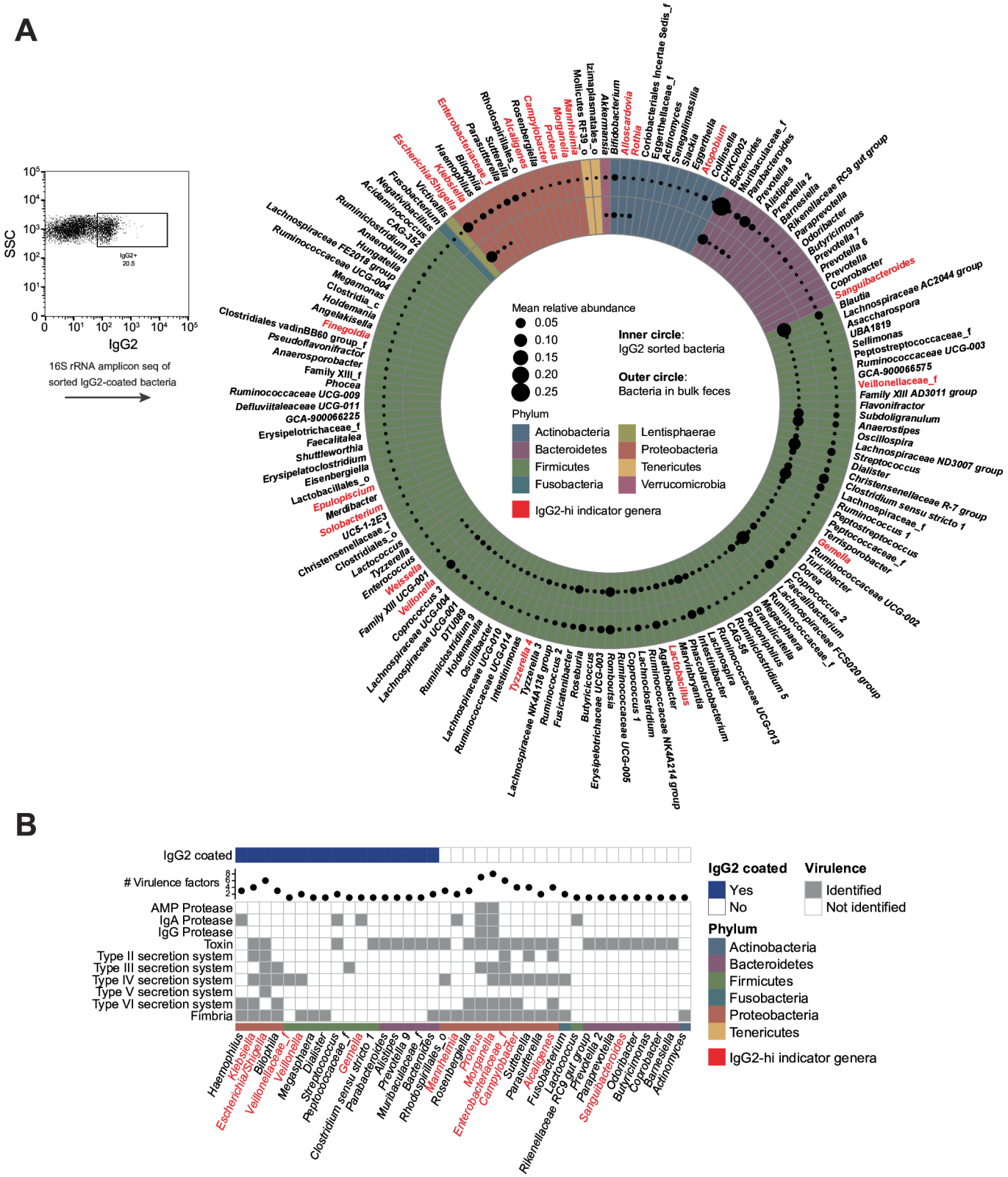

Figure 4 Identification of IgG2-coated gut bacteria in patients with CD. (A) IgG2-coated bacteria were sorted using FACS, and taxa were determined by 16S rRNA gene amplicon sequencing. Bacteria in bulk stool were sequenced from the same individuals, and the mean relative abundance of bacteria (dot size) in bulk stool (outer circle) and sorted IgG2-coated bacteria (inner circle) from patients with CD within the IgG2-hi subgroup was determined. Data are shown at family or genus level with the phylum level as tile colour. (B) *In silico* analysis of the presence of virulence factors important for invasion and immune evasion (grey square), in bacteria from A with at least one virulence factor. Bacteria identified as IgG2-coated are marked with a blue square in the upper panel and the total number of virulence factors is presented as a dot in the middle panel. (A, B) Bacteria highlighted in red represent IgG2-hi indicator taxa identified in figure 3. CD, Crohn's disease.

identified these bacteria to be associated with severe disease and to represent IgG2-hi CD patients. Based on the presence of genes encoding all enzymes required for specific microbial biosynthetic pathways, we performed *in silico* prediction of the capability for flagella, hexa-acylated and penta-acylated lipopolysaccharide (LPS) production in the bacteria, and found either flagella or hexa-acylated LPS to be present in the above non-coated bacteria identified in IgG2-hi CD patients (online supplemental figure 5). Flagellin in flagella and hexa-acylated LPS are microbial ligands known for stimulating the immune system via toll-like receptor 5 (TLR5) and TLR4 activation, respectively,[24 25] while penta-acylated LPS acts as a sequester of the TLR4 co-receptor myeloid differentiation factor 2 (MD-2) and diminishes human TLR4 activation.[26 27]

Distinctly co-occurring IgG2-coated and non-coated bacteria prevail in active disease

We next analysed for presence of IgG2-hi gut bacteria with at least one virulence factor in CD patients with active versus remissive disease status in the IBDSL cohort from The NL. *Campylobacter*, *Haemophilus*, *Mannheimia* and *Veillonella* were significantly enriched in individuals with active disease, while *Parasutterella*, *Lactococcus*, the *Muribaculaceae* family and the *Rhodospirillales* order were enriched in patients with remissive disease status (NL, figure 5A). We performed a replication of this analysis in an independent cohort from the USA (N=297),[28] and likewise found *Haemophilus*, *Campylobacter* and *Mannheimia* to associate with an active disease state (figure 5A).

Co-occurrence network analyses revealed distinct interconnections between the IgG2-hi gut bacteria with at least one virulence factor, as we discovered the existence of three different gut bacterial clusters in CD patients with active disease in the NL cohort (figure 5B). Cluster 1 consisted of several known pathogens implicated in CD, like *Klebsiella*, *Campylobacter*, *Proteus*, *Veillonella* and *Fusobacterium*,[18–20 29] all identified as IgG2-hi indicator taxa. Cluster 2 harboured several taxa normally found in the oral cavity like *Streptococcus*, *Actinomyces*, *Haemophilus* and *Prevotella*,[30] none of which were identified to be IgG2-hi indicator taxa. Cluster 3 included taxa commonly found in the intestine, and among them were three IgG2-hi indicator taxa, *Escherichia/Shigella*, *Morganella* and *Veillonellaceae_f*. All three clusters contained IgG2-coated bacteria, but in cluster 1, we also identified the active-disease associated and IgG2-hi indicator *Campylobacter*, which was found to be non-coated, and to coexist with several IgG2-coated bacteria. This coexistence between IgG2-coated and non-coated bacteria may explain why *Campylobacter* was identified as an IgG2-hi indicator genus, despite being non-coated. The analogous co-occurrence network analysis for the US cohort revealed a similar network structure of one cluster with mostly IgG2-hi indicator taxa like *Klebsiella*, *Mannheimia, Proteus*, *Veillonella* and *Fusobacterium* (cluster 1), one cluster with taxa often found in the oral community (cluster 2), and one cluster with bacteria often found in the gut (cluster 3) (online supplemental figure 6). Interestingly, *Campylobacter* was part of the main network in CD patients with active disease from NL, but not in the US cohort, while the opposite was the case for *Mannheimia*, despite both being significantly associated to disease activity in both cohorts. These findings point to IgG2-hi indicator taxa like the non-coated *Campylobacter* and *Mannheimia* as important, and apparently mutually exclusive drivers of active disease in patients with CD, as we do not find them to exist together.

DISCUSSION

Understanding the natural dynamics of microbe–host interactions at mucosal surfaces might help in identifying new means of treatment for individuals with microbe-driven inflammatory diseases, including IBD. We here demonstrated that on average 1.7% of gut bacteria, corresponding to ca. 1.9×10^8 bacteria/g stool, are coated with IgG in healthy individuals and patients with CD. This fraction is only 2–3 fold lower than the number of IgA-coated gut bacteria in healthy individuals and patients with CD, indicating that IgG may hold a yet unrecognised role in regulating microbial dynamics in the gut. Although we found no overall significant differences in gut bacterial IgG-coating between healthy individuals and patients with CD, we identified a subgroup of patients with severe CD and enhanced IgG2-coating that harboured a distinct microbiota with several gut pathobionts as indicator taxa. Amplicon sequencing of the sorted IgG2-coated bacteria showed that IgG2-coating was quite promiscuous in nature, and coated 84 out of the 153 taxa found in CD patients with IgG2-coating of gut bacteria. Notably, we found less than 40% of IgG2-hi indicator taxa to be IgG2-coated and that the IgG2-hi indicator taxa with increased virulence potential were identified as non-coated. The two non-coated IgG2-hi indicator species *Campylobacter* and *Mannheimia* associated strongly with active disease in the NL cohort, which replicated in a US cohort. *Campylobacter* and *Mannheimia* were identified to co-cluster with bacteria in microbial cluster 1, in either the NL or the US cohort, where they coexisted with genera like *Veillonella* that represent a top-tier IgG2-coated IgG2-hi indicator taxa. *Veillonella*, together with the other IgG2-hi indicator taxa, *Klebsiella*, *Proteus* and *Escherichia/Shigella* have previously been associated with CD.[30–33] We speculate that IgG2-coating of gut bacteria could be a means for the host to delimit specific bacterial growth and invasion in individuals harbouring highly virulent bacteria, while the lack of IgG2-coating of certain gut pathobionts might be due to specific yet uncovered immune evasion mechanisms existing in these non-coated bacteria, like IgA-degrading and IgG-degrading proteases, which we identified in some non-coated IgG2-hi indicator taxa, or the ability to change the type of flagellin in the flagella via phase variation. The latter may leave a way for propagating proinflammatory responses, and thus disease flares in CD, as our findings imply. Targeted treatments with, for example, antibodies that bind to adhesion molecules on the two non-coated IgG2-hi indicator genera *Campylobacter* or *Mannheimia* may result in reduced mucosal invasion and inflammation, and thereby lessen disease activity by diminishing their interaction with the intestinal epithelium.

A few previous studies have reported that IgG's are generated against gut bacteria by demonstrating the binding of serum-derived IgGs to a panel of gut bacteria, and it has also been reported that IgG responses are generated against microbes in both healthy individuals[8] and in patients suffering from autoimmune disorders and CD.[31] These previous studies demonstrated a profound overlap between the gut bacterial taxa that are recognised by serum-derived IgA and IgG, as we do in this study by identification of double-coated bacteria. Moreover, it was earlier demonstrated that serum-derived IgG2 can bind to gut-derived bacteria, which was further supported by the presence of 35.9% of IgG2+plasmablasts in terminal ileum mucosa.[34] These previous data support our findings that IgG2 can bind to both enteropathogenic and commensal bacteria in the human gut. One recent study that profiled the coating of gut bacteria with IgA, IgM and IgG, identified a significant increase

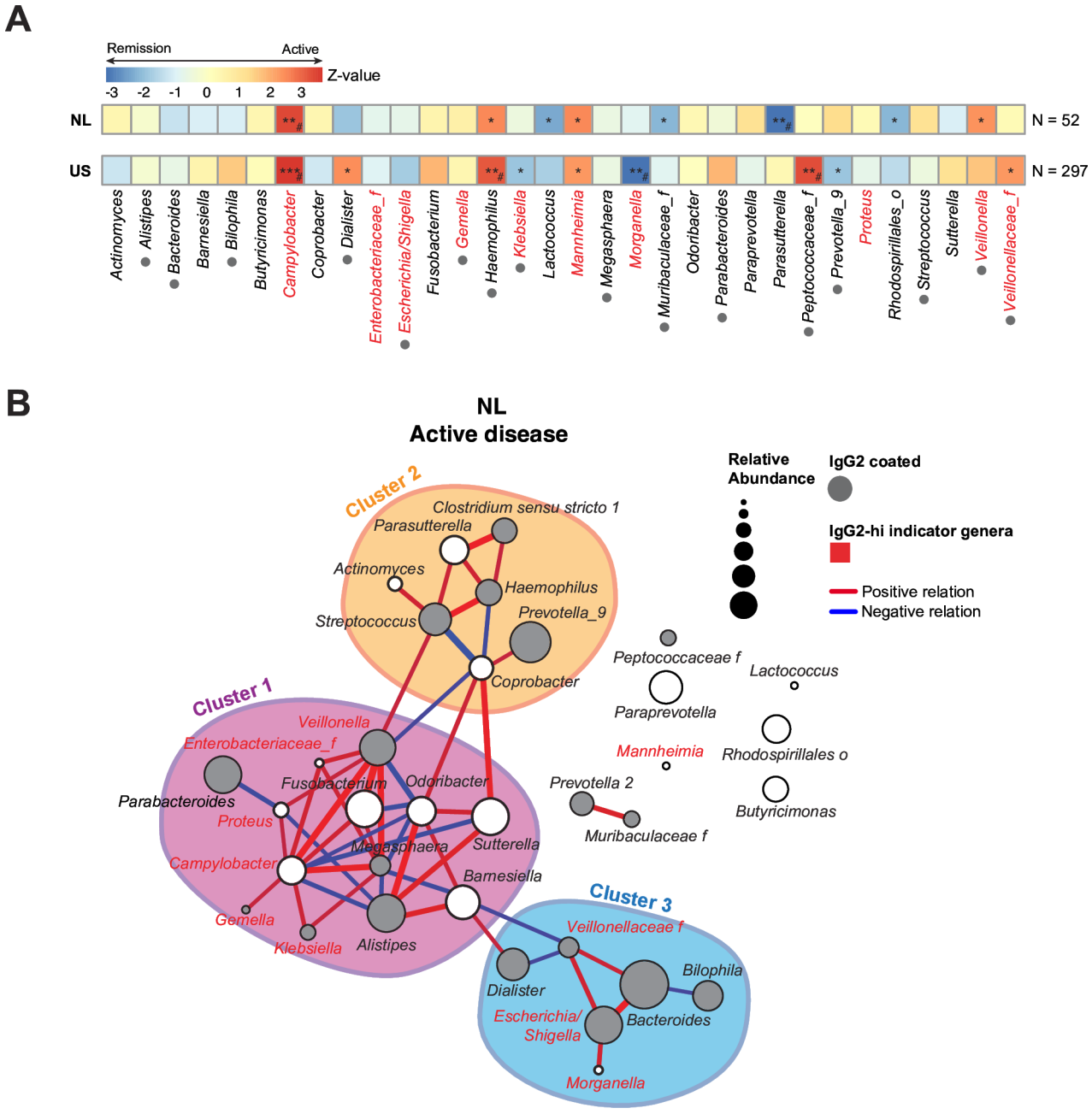

Figure 5 Distinct IgG2-coated gut bacteria and non-coated gut pathobionts coexists in CD patients with active disease. (A) Heatmap representing enrichment of identified IgG2-hi related bacteria harbouring at least one virulence factor during active or remissive disease in patients with CD from the IBDSL cohort from the Netherlands (NL) and an independent cohort from the USA (US). Z-values represent test statistics of coefficients for generalised linear models modelled over a negative binomial distribution. P values are marked with stars: *p<0.05, **p<0.01, ***p<0.001, while # highlights taxa significant after FDR-adjustment, q<0.05. (B) Co-occurrence network of bacteria identified in CD patients from A with active disease (n=19) in the NL cohort, where the taxonomy of IgG2-coated bacteria was determined. Clusters were identified using the walktrap algorithm and are indicated by an orange, blue or purple circle. Red and blue edges represent positive and negative relations, respectively. Node size is scaled by the relative abundance, and grey dots indicate that the bacterium is IgG2-coated. Bacteria highlighted in red represent IgG2-hi indicator taxa identified in figure 3. CD, Crohn's disease; NL, The Netherlands.

in the percentage of IgG2-coated bacteria in patients with CD compared with healthy controls.[35] Although their study did not link findings to disease severity parameters nor identified the nature of IgG2-coated bacteria, our combined findings point to a role of IgG2-coating as a biomarker for CD patients with severe disease in which specific bacterial targeting with antibodies may be a way forward to relieve disease symptoms.

We identified two major non-coinciding gut pathobionts (*Mannheimia* and *Campylobacter*) as IgG2-hi indicator taxa enriched in patients with active CD that remained non-coated despite high IgG2-coating of other coexisting bacteria, like *Veillonella*. *Mannheimia* has mostly been described as an animal pathogen infecting the airways, but cases of humans being infected by *Mannheimia* have been reported.[36] Our *in*

silico functional analysis of *Mannheimia*'s virulence potential showed that it may have the capacity to produce IgA-specific proteases, hence escaping IgA coating. *Campylobacter* is a well-known human pathogen and is often the causative agent of food poisoning,[37] but has also been identified in the gut of patients with CD.[38 39] Because of the enteroinvasive nature and complex outer membrane of *Campylobacter*, they are often found to infect intestinal epithelial cells as a mean to evade humoral responses.[37 40]

We speculate that the particular functional differences between bacteria that become IgG2-coated, and those that are present in an IgG2 rich environment while avoiding Ig-coating, might be of importance for driving inflammatory responses, since IgG2 reactions are otherwise known for their effectiveness in clearing invasive pathogens, for example, in patients with aggressive periodontitis.[6] It may be that invasive and/or toxin-producing bacteria, like *Campylobacter*, can initiate the breakdown of the intestinal integrity. This would increase influx of luminal antigens and coexisting gut bacteria into the underlying tissue and initiate a type 1-immune reaction dominated by IL-12p70 and IFN-γ (due to immunostimulating ligands in *Campylobacter*), and thus subsequently, promote IgG2-production.[18 41 42] When the barrier is impaired, bacteria like *Escherichia*[32] or *Klebsiella*,[33] both expressing the Type 1-immune activating ligand hexa-acylated LPS, may get in contact with immune cells in the lamina propria and prime a type 1 immune response, thereby stimulating processes that may result in antigen-specific IgG2 production against *Escherichia* or *Klebsiella*, as well as other coexisting microorganisms. Since IgG2-coating was found to be more predominant in CD patients with severe disease, these patients will likely undergo more frequent treatments with drugs. Although we did not find drug use at time of sampling to influence bacterial IgG2-coating, future studies of CD patients undergoing treatment with drugs that specifically target type 1 immune reactions, such as JAK inhibitors, should take into consideration that these drugs might interfere with IgG production, which could possibly affect bacterial coating. JAK inhibitors were not used as treatment for CD in the NL at time of sampling, and were, therefore, not part of the present analysis.

This study has several strengths and limitations. One major strength is that study materials derived from a well-established patient cohort with longitudinal follow-up and were analysed using state-of-the-art laboratory and *in silico* prediction pipelines, combined with multivariate statistics and mathematical modelling, which allowed for rigorous analyses of Ig-coating profiles in relation to disease parameters. This enabled us to provide insights into the so far poorly recognised role of IgG subtypes in coating of gut bacteria, and to identify varying IgG2-coating of bacteria dependent on CD disease severity. Although the main findings were replicated in an independent cohort, it is a limitation that the study includes a relative low number of participants with Ig-coating information (N=80).

Combined, we here demonstrated that gut bacterial IgG2-coating characterises individuals with severe CD and is enhanced during disease flares. In this patient group we identified the distinct presence of two non-coated gut pathobionts, *Campylobacter* or *Mannheimia*, that we speculate may drive the inflammatory processes, and thus enhance disease severity, as they may linger in an uncontrolled manner when non-coated. The current findings point to new ways for subgrouping of patients with CD by using the host immune system in identifying disease-propagating bacteria and in pinpointing the underlying immune reactions directed against these bacteria. In terms of future perspectives, specific therapeutic elimination of *Campylobacter* or *Mannheimia* may, therefore, be a strategy to relieve disease symptoms in IgG2-hi CD patients with severe disease burden.

Materials and methods

Materials and methods are available in online supplemental materials.

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Acknowledgements The authors thank the participants of the IBDSL and the Maastricht IBS cohorts, Maastricht, The Netherlands, for providing samples.

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Funding This research was supported by a scholarship from the Technical University of Denmark (DTU) to CE under supervision of SB, and the Danish National Research Foundation (grant number DNRF148) to TJ, SB is the incumbent of the FII institute Research Chair at DTU in Immune-based Prediction of Disease.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Patients in the original cohort provided written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Sequencing data are available in a public, open access repository. Personal individual-level data are not available according to the Danish Data Protection Act and European Regulation 2016/679 of the European Parliament and of the Council (GDPR). Sequencing data are publicly available in NCBI Sequence Read Archive under BioProject: PRJNA418765. Analysis software including quality control, taxonomic and functional inference tools are publicly available and referenced as appropriate. Individual-level personally identifiable data from the subjects participating in the cohort cannot be made freely available, to protect the privacy of the participants, in accordance with the Danish Data Protection Act and European Regulation 2016/679 of the European Parliament and of the Council (GDPR) that prohibit distribution even in pseudoanonymised form.

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REFERENCES

1 Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012;380:1590–605.
2 Vich Vila A, Imhann F, Collij V, *et al*. Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. *Sci Transl Med* 2018;10.
3 Fuss IJ, Neurath M, Boirivant M, *et al*. Disparate Cd4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996;157:1261–70.
4 Monteleone I, Pallone F, Monteleone G. Th17-related cytokines: new players in the control of chronic intestinal inflammation. *BMC Med* 2011;9:122.
5 Kawano Y, Noma T, Yata J. Regulation of human IgG subclass production by cytokines. IFN-gamma and IL-6 act antagonistically in the induction of human Igg1 but additively in the induction of Igg2. *J Immunol* 1994;153:4948–58.
6 Schenkein HA, Barbour SE, Tew JG. Cytokines and inflammatory factors regulating immunoglobulin production in aggressive periodontitis. *Periodontol 2000* 2007;45:113–27.
7 Ferrante A, Beard LJ, Feldman RG. Igg Subclass distribution of antibodies to bacterial and viral antigens. *Pediatr Infect Dis J* 1990;9(8):S16–24.
8 Fadlallah J, Sterlin D, Fieschi C, *et al*. Synergistic convergence of microbiota-specific systemic IgG and secretory IgA. *J Allergy Clin Immunol* 2019;143:1575–85.
9 Yoshida M, Kobayashi K, Kuo TT, *et al*. Neonatal FC receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest* 2006;116:2142–51.
10 Bunker JJ, Erickson SA, Flynn TM, *et al*. Natural polyreactive IgA antibodies coat the intestinal microbiota. *Science* 2017;358.
11 Zeng MY, Cisalpino D, Varadarajan S, *et al*. Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens. *Immunity* 2016;44:647–58.
12 van den Heuvel TR, Jonkers DM, Jeuring SF, *et al*. Cohort profile: the inflammatory bowel disease South Limburg cohort (IBDSL). *Int J Epidemiol* 2017;46:e7.
13 Bunker JJ, Flynn TM, Koval JC, *et al*. Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity* 2015;43:541–53.
14 Palm NW, de Zoete MR, Cullen TW, *et al*. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 2014;158:1000–10.
15 van der Waaij LA, Kroese FGM, Visser A, *et al*. Immunoglobulin coating of Faecal bacteria in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2004;16:669–74.
16 Fadlallah J, El Kafsi H, Sterlin D, *et al*. Microbial ecology perturbation in human IgA deficiency. *Sci Transl Med* 2018;10.
17 Choung RS, Locke GR, Zinsmeister AR, *et al*. Epidemiology of slow and fast colonic transit using a scale of stool form in a community. *Aliment Pharmacol Ther* 2007;26:1043–50.
18 Hamilton AL, Kamm MA, Ng SC, *et al*. Proteus Spp. As putative gastrointestinal pathogens. *Clin Microbiol Rev* 2018;31.
19 Lloyd-Price J, Arze C, Ananthakrishnan AN, *et al*. Multi-Omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019;569:655–62.
20 Pittayanon R, Lau JT, Leontiadis GI, *et al*. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. *Gastroenterology* 2020;158:930–46.
21 Rohart F, Gautier B, Singh A, *et al*. mixOmics: an R package for 'Omics feature selection and multiple data integration. *PLoS Comput Biol* 2017;13.
22 Douglas GM, Maffei VJ, Zaneveld JR, *et al*. Picrust2 for prediction of metagenome functions. *Nat Biotechnol* 2020;38:685–8.
23 Liu B, Zheng D, Zhou S, *et al*. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res* 2022;50:D912–7.
24 Brix S, Eriksen C, Larsen JM, *et al*. Metagenomic heterogeneity explains dual immune effects of Endotoxins. *J Allergy Clin Immunol* 2015;135:277–80.
25 Gewirtz AT, Navas TA, Lyons S, *et al*. Cutting edge: bacterial flagellin activates basolaterally expressed Tlr5 to induce epithelial proinflammatory gene expression. *J Immunol* 2001;167:1882–5.
26 Bäckhed F, Normark S, Schweda EKH, *et al*. Structural requirements for Tlr4-mediated LPS signalling: a biological role for LPS modifications. *Microbes and Infection* 2003;5:1057–63.
27 d'Hennezel E, Abubucker S, Murphy LO, *et al*. Total Lipopolysaccharide from the human gut Microbiome silences toll-like receptor signaling. *mSystems* 2017;2.
28 Doherty MK, Ding T, Koumpouras C, *et al*. [dataset] 28]. Data from: Fecal Microbiota Signatures Are Associated with Response to Ustekinumab Therapy among Crohn's Disease Patients, Available: https://www.ncbi.nlm.nih.gov/bioproject/?term=(PRJNA418765)%20AND%20bioproject_sra[filter]%20NOT%20bioproject_gap[filter]
29 Nakatsu G, Li X, Zhou H, *et al*. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun* 2015;6:8727.
30 Lin D, Yang L, Wen L, *et al*. Crosstalk between the oral microbiota, mucosal immunity, and the epithelial barrier regulates oral mucosal disease pathogenesis. *Mucosal Immunol* 2021;14:1247–58.
31 Paun A, Yau C, Meshkibaf S, *et al*. Association of HLA-dependent islet autoimmunity with systemic antibody responses to intestinal Commensal bacteria in children. *Sci Immunol* 2019;4.
32 Viladomiu M, Kivolowitz C, Abdulhamid A, *et al*. Iga coated E. coli enriched in crohn's disease spondyloarthritis promote Th17-dependent inflammation. *Sci Transl Med* 2017.
33 Atarashi K, Suda W, Luo C, *et al*. E$_c$topic colonization of oral bacteria in the intestine drives Th1 cell induction and inflammation. *Science* 2017;358:359–65.
34 Benckert J, Schmolka N, Kreschel C, *et al*. The majority of intestinal Iga+ and Igg+ plasmablasts in the human gut are antigen-specific. *J Clin Invest* 2011;121:1946–55.
35 Masu Y, Kanazawa Y, Kakuta Y, *et al*. Immunoglobulin subtype-coated bacteria are correlated with the disease activity of inflammatory bowel disease. *Sci Rep* 2021;11:16672.
36 Punpanich W, Srijuntongsiri S. Pasteurella (mannheimia) haemolytica septicemia in an infant: a case report. *J Infect Dev Ctries* 2012;6:584–7.
37 Moore JE, Corcoran D, Dooley JSG, *et al*. Campylobacter. *Vet Res* 2005;36:351–82.
38 Mahendran V, Riordan SM, Grimm MC, *et al*. Prevalence of campylobacter species in adult crohn's disease and the preferential colonization sites of campylobacter species in the human intestine. *PLoS One* 2011;6.
39 Zhang L, Man SM, Day AS, *et al*. Detection and isolation of campylobacter species other than C. Jejuni from children with crohn's disease. *J Clin Microbiol* 2009;47:453–5.
40 Thompson SA. Campylobacter surface-layers (S-layers) and immune evasion. *Ann Periodontol* 2002;7:43–53.
41 Garrett WS, Gallini CA, Yatsunenko T, *et al*. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 2010;8:292–300.
42 Rees LEN, Cogan TA, Dodson AL, *et al*. Campylobacter and Ifngamma interact to cause a rapid loss of epithelial barrier integrity. *Inflamm Bowel Dis* 2008;14:303–9.

SUPPLEMENTARY MATERIAL - FIGURES

Specific gut pathobionts escape antibody coating and are enriched during flares in patients with severe Crohn's disease

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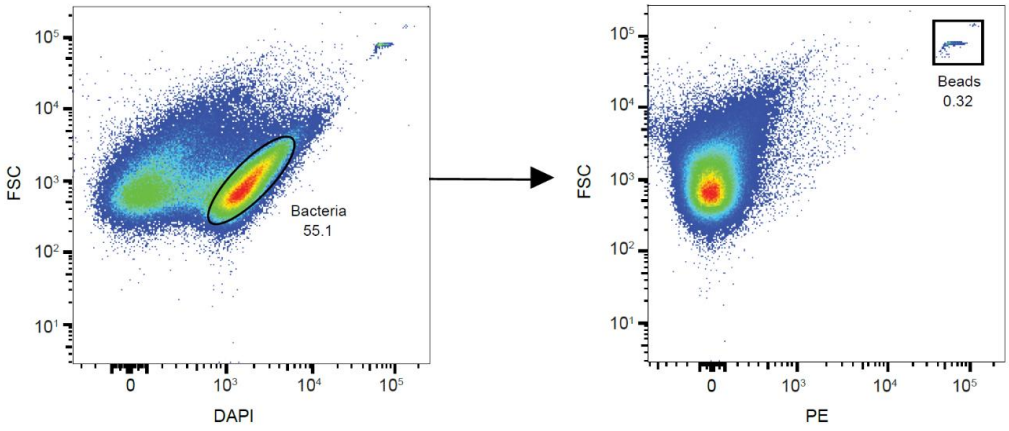
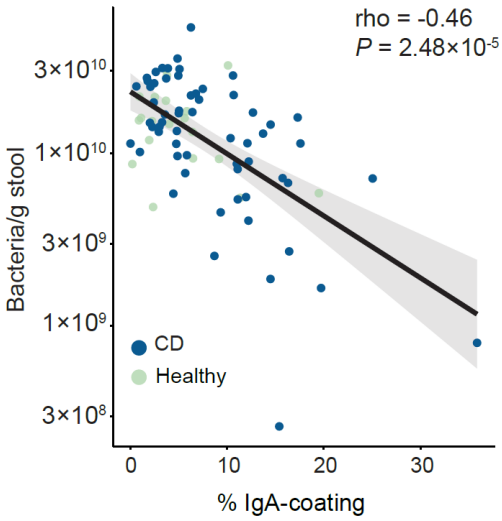
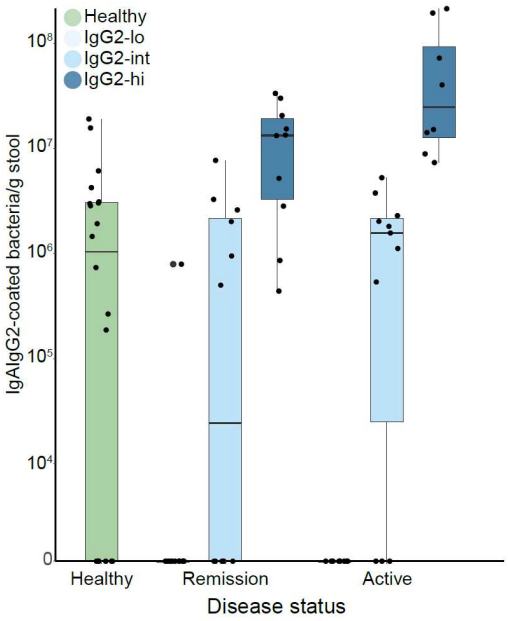
SUPPLEMENTARY FIGURES



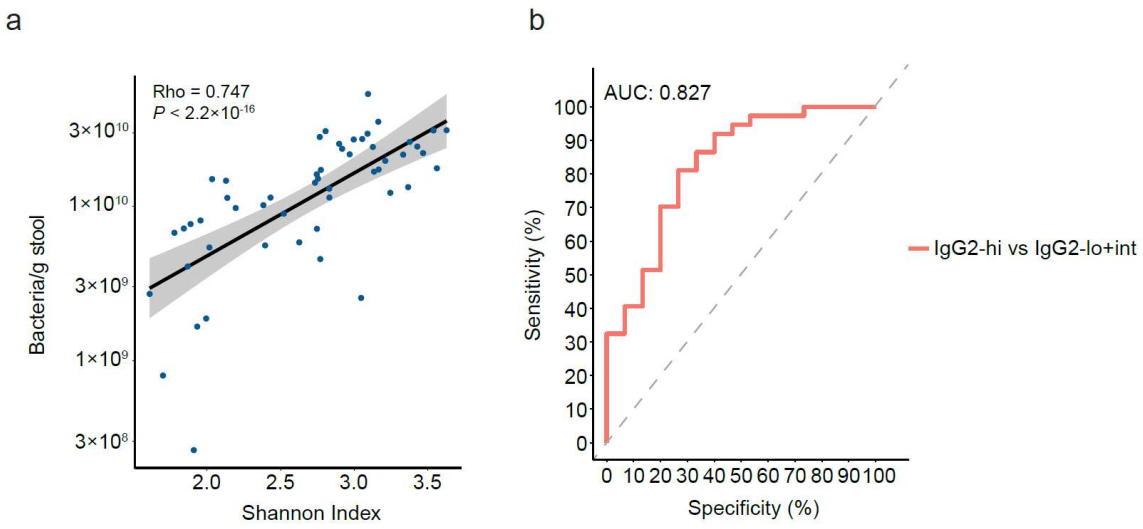
Supplementary Figure 1. Quantification of gut bacteria in stool by flow cytometry. Single cell suspensions of stool bacteria were labelled with DAPI to exclude debris (DAPI gate on the left), and then counted based on gating of count beads added to the same tube (right).



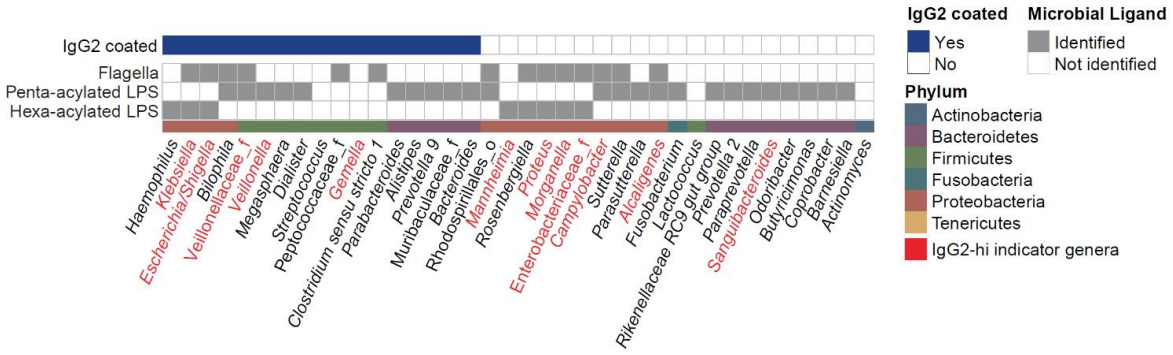
Supplementary Figure 2. % IgA-coating is inversely related to gut bacterial load. Scatter plot showing the correlation between % IgA-coated bacteria and bacteria/g stool in CD patients (n=60) and healthy controls (n=20). Spearman's rho statistics was used for correlations. The line and shaded area depict a linear fit, and the 95% confidence interval, respectively.



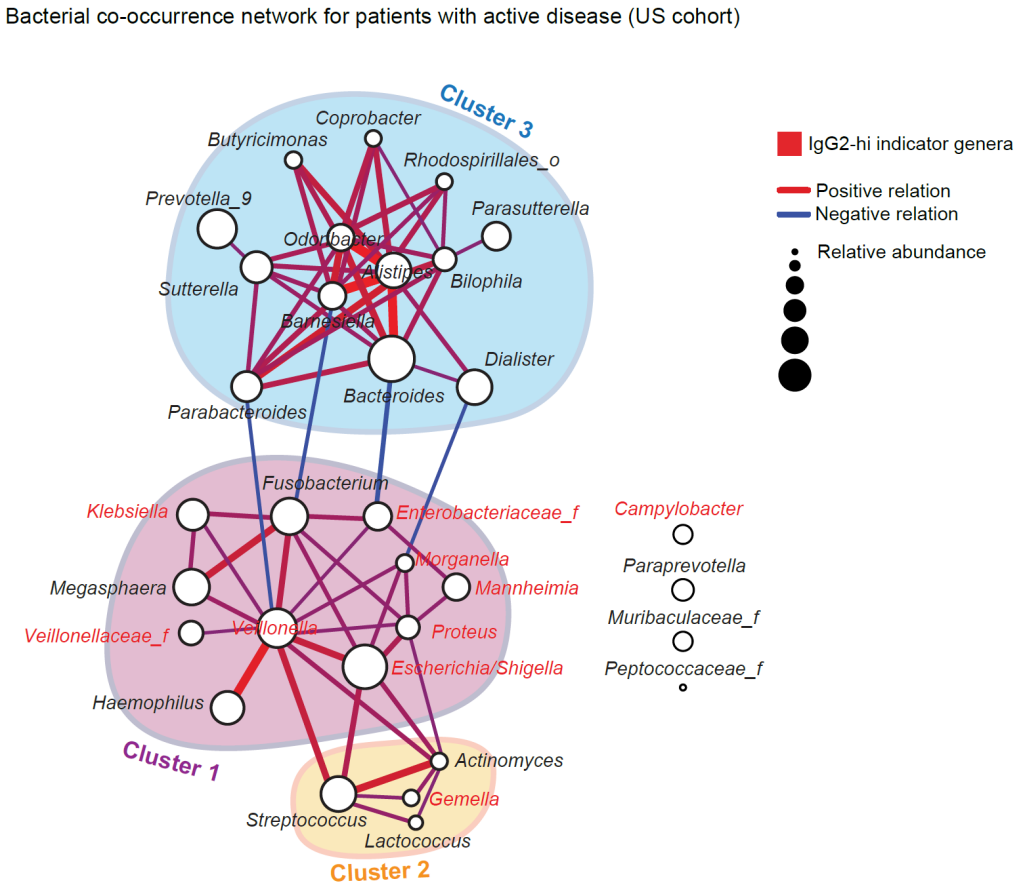
Supplementary Figure 3. Number of double-IgAIgG2 coated bacteria/g stool in healthy controls and CD patients in remission or with active disease for each IgG2-coating subgroup. Load of double-IgAIgG2 coated bacteria/g stool in healthy controls (n=20) and CD patients in remission (n=33) or with active disease (n=27) for each IgG2-coating subgroup. Center lines of box plots indicate the median and the box limits indicate the quartiles. Whiskers extend to the data points within 1.58x the interquartile range. Dots represent the level within each individual.



Supplementary Figure 4. Shannon index in relation to bacteria/g stool in CD patients and sPLS-DA ROC curve. a) Scatterplot of Shannon index in relation to number of bacteria/g stool in CD patients (n=52). Statistical test and plotting details are described in Supplementary Figure 2. b) ROC curve showing the AUC for the sPLS-DA analysis used to identify IgG2-hi indicator genera.



Supplementary Figure 5. Selected innate immune activating ligands in IgG2-coated and non-coated bacteria. Lower panel shows genome-based identification of innate immune activating ligands (grey squares) in bacteria identified in Figure 3 using PICRUST2 analysis. Bacteria found to be IgG2-coated are marked with a blue square in the upper panel. Bacteria highlighted in red represent IgG2-hi indicator genera.



Supplementary Figure 6. Bacterial co-occurrence network in CD patients with active disease from US cohort. Co-occurrence network of bacteria from Figure 5a in CD patients with active disease (n=205) from the US cohort. Clusters were identified using the walktrap algorithm and are indicated by an orange, blue or purple circle. Red and blue edges represent positive and negative relations, respectively. Node sizes represent relative bacterial abundance.

SUPPLEMENTARY MATERIAL – MATERIALS AND METHODS

Specific gut pathobionts escape antibody coating and are enriched during flares in patients with severe Crohn's disease

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MATERIALS AND METHODS

Cohort characteristics

Samples from CD patients (n=60) were collected as part of the IBD South Limburg (IBDSL) cohort, which is a population-based inception cohort from the South Limburg area of the Netherlands [1]. Since 1991, all newly diagnosed patients with IBD have been prospectively included and followed, with on-going collection of biomaterials (serum, plasma, DNA and stool), and complete data on disease phenotype, hospitalizations, surgery, (extra)intestinal complications, and diagnostic reports. We included CD patients who have not received antibiotics treatment two months before sampling. Samples were collected either during remission (n=33) or during active disease (n=27). Active disease was defined by clinicians as fecal calprotectin >250 μg/g or fecal calprotectin >100 μg/g and at least a five-fold increase from baseline. The cohort was sampled as described in the IBDSL cohort profile [1]. The control samples from healthy individuals (n=20) were collected as part of the Maastricht IBS cohort. Both the IBDSL and the Maastricht IBS cohort were approved by the local Medical Ethics Committee, registered in http://www.clinicaltrials.gov (NCT02130349 and NCT00775060), and follow the revised version of the declaration of Helsinki.

The data used for replication derive from a previous publication from a US based cohort [2]. Briefly, raw data from treatment-naïve CD patients (i.e. no treatment with Ustekinumab) were processed using the DADA2 pipeline, and samples with >5,000 reads were used in the final analysis, resulting in inclusion of 297 samples. Disease activity in patients from the replication cohort was defined as fecal calprotectin >250 μg/g.

Determination of fecal bacterial load and bacterial Ig-coating by flow cytometry

Fecal samples were incubated on ice for 1 hour in sterile PBS at 100 mg/mL, homogenized, spun down (15 min, 50g, 4°C), followed by aspiration of the supernatant. The supernatant was centrifuged (5 min, 8000g, 4°C) and washed twice in buffer 1 (PBS + 1% BSA (>98%, Sigma-Aldrich)). An aliquot was

diluted 150x in buffer 2 (PBS + 1% BSA + 0.01% Tween 20 + 1 mM EDTA) with 1 mM DAPI and 10 µL count beads (BD Biosciences) and analyzed on a FACS Canto II flow cytometer (BD Biosciences). The bacterial population was gated based on SSC-A/Pacific-Blue (**Supplementary Figure 1**), and the fecal bacterial load was determined as per instruction by the bead manufacturer. For consistent staining across samples, a total of $1.5x10^8$ bacteria per sample were transferred to a new tube. The bacteria were resuspended in buffer 1 containing 20% mouse serum and incubated for 20 min at 4°C. The percentage of bacterial coating with IgA and IgG1-4 was defined by staining of samples for 30 min at 4°C with a mixture of fluorescence-conjugated mouse anti-human Ig antibodies that each bind to the constant part of the human antibodies: Pe-Cy7-anti-IgA (Miltenyi), Biotin-anti-IgG1 + APC-Cy7-Streptavidin (SouthernBiotech), Alexa Flour 647-anti-IgG2 (SouthernBiotech), Alexa Flour 488-anti-IgG3 (SouthernBiotech), and PE-anti-IgG4 (SouthernBiotech) diluted in buffer 1. Samples were washed twice in buffer 1 and resuspended in buffer 2. An aliquot was diluted and incubated with 1 mM DAPI and 10 µL count beads (BD Biosciences) before being analyzed on a FACS Canto II flow cytometer (BD Biosciences). The analysis was based on 200,000 recorded $DAPI^+$ cells. Data were analyzed using FlowJo software (Version 10.5.0, Tree Star Inc, Ashland, OR). All gate boundaries were set using FMO controls.

Purification of IgG2-coated bacteria by FACS

Samples for sorting of IgG2-coated bacteria were prepared as described above, except for the use of PE-conjugated mouse anti-human IgG2 (0.5 mg/mL, SouthernBiotech). Samples were sorted on a MoFlo XDP Cell sorter (Beckman Coulter). The IgG2+ fraction (between $1.5x10^5$ to $3x10^6$ bacteria per sample) was collected in heat-inactivated fetal bovine serum (FBS; Gibco) coated FACS tubes, pelleted (5 min, 8000g, 4°C) and stored at -80°C until processing. Samples of sheath fluid were collected directly from the stream pre-sorting as technical controls.

DNA extraction and 16S rDNA library preparation

Bacterial DNA was extracted using the NucleoSpin Soil kit (Macherey-Nagel, Germany) based on the manufacturer's protocol. The extracted DNA was amplified using a two-step PCR reaction with the 314F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 806R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAA

TCC) targeting the hypervariable regions V3 + V4 of the 16S ribosomal RNA gene. DNA was amplified using Phusion Green High-Fidelity DNA Polymerase (Thermo Fisher) kit. For PCR, master mix was added to 20 µL extracted DNA in concentrations according to manufacturer's recommendations. PCR was performed using the following conditions: initial denaturation for 30s at 98°C followed by 28 cycles of 10s 98°C, 15s 56°C and 30s 72°C with a final elongation at 72°C for 5 min. The products were tagged with Illumina adapters (Forward: AATGATACGGCGACCACCGAGATCTACAC, Reverse: CAAGCAGAAGACGGCATACGAGAT) using 10 cycles under the same PCR conditions. Products from both PCRs were purified using Agencourt AMPure XP beads (Beckman Coulter). Similar volumes of all amplicons were pooled and the library was diluted to a total concentration of 4 nM before sequencing on the MiSeq platform (Illumina, USA) using the v3 kit (paired-end).

16S rRNA gene data processing

Sequencing adapters were removed using the BBDuk tool in the BBTools package v38.37 (BBDuk, sourceforge.net/projects/bbmap/). Reads were analyzed and denoised using DADA2 [3]. Resulting amplicon sequence variants (ASVs) were compared to the 99% identity clustered SILVA database v132 [4] using a naive bayesian classifier [5] trained on the amplified region as implemented in DADA2. Since samples and technical controls were adjusted to sample-volume rather than DNA concentration in the PCR, the false-positive count contribution originating from the technical controls, is similar between samples. This enabled us to correct for reagent and pre-sorting fluid contaminating bacterial DNA by

subtracting the read counts found in the above technical controls. Bacterial reads data are found in **Supplementary Table 5**.

In silico-based inference of virulence factors and immunostimulatory ligands production capability in gut bacteria

Bacteria were annotated for virulence factors and capacity to produce hexa- or penta-acylated LPS, and flagellin by predicting their functional capacity using PICRUST2 [6] (v. 2.4.1) to identify Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KOs) [7]. For each ASV, flagellin production was evaluated by the presence of K numbers responsible for production of flagellin as described in the flagellar assembly map (map02040). The type of LPS, or whether a bacterium could produce it at all, was evaluated by the ability of each species to convert UDP-N-acetylglucosamine to KDO_2-lipid A as described in the lipopolysaccharide biosynthesis pathway (module M00060) [8]. For virulence factors, we downloaded protein sequences from the Virulence Factors Database (VFDB) [9] and mapped the sequences to K numbers using GhostKOALA (https://www.kegg.jp/ghostkoala) [7]. Resulting KOs were integrated with the predicted functions from the PICRUST2 analysis to assign information on the virulence potential to each bacterium in our samples.

Statistical analysis

Statistical analyses were performed using R (v. 4.0.0). Associations between Ig-coated bacteria/g stool and markers of disease severity and activity were examined using generalized linear modelling modelled over a Poisson distribution with logged bacteria/g stool as offset-term. The Shannon index and species richness were calculated using the vegan package (v. 2.6-2). Bray-Curtis dissimilarity measures were used to compute differences in bacterial communities within the IgG2 subgroups and presented using an NMDS based on seven dimensions. For the latter, the metaMDS function from the vegan package was used to iteratively add dimensions until lowest level of stress was achieved; taxa or clinical variables and

Ig-coating were added to the NMDS using the envfit function. Group differences were tested for inference using a permutational multivariate analysis of variance (PERMANOVA with 999 permutations, using the Adonis2 function from the vegan package). To identify high IgG2 indicator species within bulk fecal sequences, we trained models of sPLS-DA using the mixomics package (v. 6.2) [10] on the log10-transformed relative abundances, using 1/2 of the lowest non-zero value as pseudocounts. The optimal number of species was identified by 10-fold cross-validation using AUC statistics to avoid overfitting the models. Disease activity was fitted to bacterial counts from virulent genera (defined as number of virulence factors ≥ 1) using negative binomial generalized linear models with logged sequencing depths as offset-term and using the NBZIMM package (v. 1.0) [11]. Microbiota community structures in CD patients with active disease were evaluated by building co-occurrence networks of virulent species (#virulence factors ≥ 1) using Sparse Correlations for Compositional data (SparCC) algorithm [12] from the SpiecEasi package (v. 1.1.2) [13]. Bacteria-bacteria correlation coefficients were estimated as the average of 100 inference iterations refined by 999 exclusion iterations with a strength threshold of 0.6. Correlation coefficients with an absolute value of 0.3 or above were visualized. Clustering was done using the walktrap community algorithm from the igraph package (v. 1.3.2) using default parameters.

Statistical non-parametric tests were used for all data comparisons: the Wilcoxon rank-sum test was used when comparing two groups, and Spearman rank coefficient correlations were used for association analysis. *P*-values were deemed significant using $P < 0.05$ as significance level. When indicated, *P*-values were adjusted for multiple testing using a false discovery rate (FDR) adjustment of $q < 0.10$ or < 0.05.

References for Materials and Methods

1. van den Heuvel TR, Jonkers DM, Jeuring SF et al. Cohort Profile: The Inflammatory Bowel Disease South Limburg Cohort (IBDSL). Int J Epidemiol. 2017;46:e7. DOI: 10.1093/ije/dyv088.

2. Doherty MK, Ding T, Koumpouras C et al. Data from: Fecal Microbiota Signatures Are Associated with Response to Ustekinumab Therapy among Crohn's Disease Patients. mBio. 2018;9:e02120-17. Deposited at NCBI BioProject (PRJNA418765), 16-Nov-2017. . https://www.ncbi.nlm.nih.gov/bioproject/?term=(PRJNA418765)%20AND%20bioproject_sra[filter]%20NOT%20bioproject_gap[filter].

3. Callahan BJ, McMurdie PJ, Rosen MJ et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581-583. DOI: 10.1038/nmeth.3869.

4. Quast C, Pruesse E, Yilmaz P et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590-6. DOI: 10.1093/nar/gks1219.

5. Bokulich NA, Kaehler BD, Rideout JR et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018;6:90. DOI: 10.1186/s40168-018-0470-z.

6. Douglas GM, Maffei VJ, Zaneveld JR et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38:685-688. DOI: 10.1038/s41587-020-0548-6.

7. Kanehisa M, Goto S, Sato Y et al. KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res. 2012;40:D109-14. DOI: 10.1093/nar/gkr988.

8. Brix S, Eriksen C, Larsen JM et al. Metagenomic heterogeneity explains dual immune effects of endotoxins. J Allergy Clin Immunol. 2015;135:277-280. DOI: 10.1016/j.jaci.2014.09.036.

9. Liu B, Zheng D, Zhou S et al. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res. 2022;50:D912-D917. DOI: 10.1093/nar/gkab1107.10.

10. Rohart F, Gautier B, Singh A et al. mixOmics: An R package for 'omics feature selection and multiple data integration. PLoS Comput Biol. 2017;13:e1005752. DOI: 10.1371/journal.pcbi.1005752.

11. Zhang X, Yi N. NBZIMM: negative binomial and zero-inflated mixed models, with application to microbiome/metagenomics data analysis. BMC Bioinformatics. 2020;21:488. DOI: 10.1186/s12859-020-03803-z.

12. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. PLoS Comput Biol. 2012;8:e1002687. DOI: 10.1371/journal.pcbi.1002687.

13. Kurtz ZD, Müller CL, Miraldi ER et al. Sparse and compositionally robust inference of microbial ecological networks. PLoS Comput Biol. 2015;11:e1004226. DOI: 10.1371/journal.pcbi.1004226.

Supplementary Table 1 - Cohort statistics

	Healthy	CD patients
Basic characteristics		
Sex, Female, % (N)	65% (13)	66% (40)
Age, mean (SD)	41 (17)	42 (15)
Clinical parameters		
Disease activity, Active, % (N)	-	45% (27)
Fecal calprotectin (µg/g), mean (SD)	-	220.68 (307.25)
HBI, mean (SD)	-	2.92 (3.41)
Surgery, % (Total N)	-	20% (58)
none, N	-	46
ileocoecaal, N	-	4
colon, N	-	3
hemicolectomy, N	-	3
sigmoid, N	-	1
rectum+sigmoid, N	-	1
Medication use at sampling		
Mesalazines, N	-	9
Thiopurines, N	-	17
Biologicals, N	-	30
Prednison, N	-	5
Proton Pump inhibitors (PPI), N	-	14
Montreal Classification	-	
Disease Location (Total N)	-	60
L1, % (N)	-	32% (19)
L2, % (N)	-	28% (17)
L3, % (N)	-	40% (24)
Disease behavior (Total N)	-	60
B1, % (N)	-	66.7% (40)
B2, % (N)	-	20% (12)
B3, % (N)	-	13% (8)
Age at diagnosis (Total N)	-	60
A1, % (N)	-	3% (2)
A2, % (N)	-	75% (45)
A3, % (N)	-	22% (13)

Supplementary Table 2 - Quantitative coating data

Average Ig-coated bacteria/g stool in healthy controls vs. CD (Wilcoxon Rank Sum test)

Isotype	Donor type	Data type	Median	25th quantile	75th quantile	Donor type	Data type	Median	25th quantile	75th quantile	P-value	Adjusted (FDR)	Significance
Single IgA	Control	Bacteria per g stool	5.89E+08	2.05E+08	8.60E+08	CD	Bacteria per g stool	6.45E+08	4.20E+08	1.34E+09	1.73E-01	8.00E-01	ns
Single IgG1	Control	Bacteria per g stool	5.65E+07	3.53E+07	8.96E+07	CD	Bacteria per g stool	3.85E+07	1.78E+07	1.01E+08	2.28E-01	8.00E-01	ns
Single IgG2	Control	Bacteria per g stool	0	0	7.25E+05	CD	Bacteria per g stool	2.07E+04	0	1.67E+06	2.15E-01	8.00E-01	ns
Single IgG3	Control	Bacteria per g stool	6.59E+05	0	1.77E+06	CD	Bacteria per g stool	0	0	9.82E+05	9.68E-02	8.00E-01	ns
Single IgG4	Control	Bacteria per g stool	1.03E+08	6.41E+07	2.57E+08	CD	Bacteria per g stool	8.02E+07	2.64E+07	2.74E+08	5.67E-01	8.40E-01	ns
IgAIgG1	Control	Bacteria per g stool	1.72E+07	5.07E+06	2.43E+07	CD	Bacteria per g stool	1.17E+07	5.53E+06	2.66E+07	6.85E-01	9.10E-01	ns
IgAIgG2	Control	Bacteria per g stool	1.10E+06	0	3.05E+06	CD	Bacteria per g stool	6.65E+05	0	5.78E+06	9.45E-01	1.00E+00	ns
IgAIgG3	Control	Bacteria per g stool	0	0	9.69E+05	CD	Bacteria per g stool	0	0	0.00	6.51E-02	8.00E-01	ns
IgAIgG4	Control	Bacteria per g stool	8.15E+06	5.26E+06	1.72E+07	CD	Bacteria per g stool	9.17E+06	4.30E+06	1.94E+07	9.78E-01	1.00E+00	ns
IgG1IgG2	Control	Bacteria per g stool	0	0	3.63E+05	CD	Bacteria per g stool	0	0	0.00	4.61E-01	8.40E-01	ns
IgG1IgG3	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0.00	2.62E-01	8.10E-01	ns
IgG1IgG4	Control	Bacteria per g stool	1.54E+06	7.80E+05	2.29E+06	CD	Bacteria per g stool	1.14E+06	3.92E+05	2.92E+06	4.80E-01	8.40E-01	ns
IgG2IgG3	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0.00	9.77E-01	1.00E+00	ns
IgG2IgG4	Control	Bacteria per g stool	0	0	7.10E+04	CD	Bacteria per g stool	0	0	0.00	7.94E-01	9.70E-01	ns
IgG3IgG4	Control	Bacteria per g stool	0	0	5.97E+05	CD	Bacteria per g stool	0	0	3.00E+05	5.31E-01	8.40E-01	ns
IgAIgG1IgG2	Control	Bacteria per g stool	0	0	5.13E+05	CD	Bacteria per g stool	0	0	1.45E+05	7.61E-01	9.70E-01	ns
IgAIgG1IgG4	Control	Bacteria per g stool	5.24E+05	0	1.31E+06	CD	Bacteria per g stool	3.42E+05	0	1.07E+06	5.92E-01	8.40E-01	ns
IgAIgG2IgG3	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	5.83E-01	8.40E-01	ns
IgAIgG2IgG4	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	1.00E+00	1.00E+00	ns
IgAIgG3IgG4	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	1.39E-01	8.00E-01	ns
IgG1IgG2IgG4	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	4.23E-01	8.40E-01	ns
IgG1IgG3IgG4	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	4.35E-01	8.40E-01	ns
IgAIgG1IgG2IgG4	Control	Bacteria per g stool	0	0	0	CD	Bacteria per g stool	0	0	0	3.26E-01	8.40E-01	ns

Supplementary Table 3 - Drug use at time of sampling vs. IgG2-coated bacteria

Difference in IgG2-coated bacteria/g stool in CD patients receiving medicine vs. those who did not. Data were analyzed using Wilcox test. P-values are displayed.

Medicine	**N (with/without)**	**Total IgG2 coating**	**Single IgG2-coating**
		P-value	*P*-value
Mesalazines	9/51	0.85	0.08
Thiopurines	17/43	0.18	0.37
Biologicals	30/30	0.45	0.54
Prednison	5/55	0.94	0.86
Proton Pump inhibitors (PPI)	14/46	0.62	0.83

Supplementary Table 4 - Montreal disease behavior in IgG2 phenotypes

The likelihood of having a B3 Montreal disease behavior compared to B1 or B2 in IgG2-hi vs. IgG2-lo and IgG2-int tested using Fisher's exact test

	IgG2-lo + int	IgG2-hi	Odds ratio (B1 or B2 vs B3)	P-value (B1 or B2 vs B3)
B1	29	11	7.52	0.017
B2	11	1	25.14	0.004
B3	2	6		

Supplementary Table 5 - Bristol stool scale & bacteria/g stool in healthy controls and CD patients with different IgG2 phenotypes

IgG2 phenotype	Variable	Mean	SD
Control	Bristol stool scale	4.60	1.19
CD-IgG2-lo	Bristol stool scale	4.89	1.45
CD-IgG2-int	Bristol stool scale	4.87	1.36
CD-IgG2-hi	Bristol stool scale	6.06	1.00
Control	Bacteria/g stool	1.59E+10	8.00E+09
CD-IgG2-lo	Bacteria/g stool	1.81E+10	9.63E+09
CD-IgG2-int	Bacteria/g stool	1.81E+10	1.13E+10
CD-IgG2-hi	Bacteria/g stool	1.03E+10	7.89E+09

Supplementary Table 6 - Taxa annotation for numbers on NMDS plot

Genus	NMDS1	NMDS2	r	pvals	pvals_adj	Numbering on plot
Clostridiales_o	-0.353	-0.117	0.283	6.00E-03	1.73E-02	1
Ruminococcaceae_UCG-003	-0.449	-0.066	0.420	1.00E-03	3.98E-03	2
GCA-900066575	-0.492	-0.139	0.533	1.00E-03	3.98E-03	3
Family_XIII_AD3011_group	-0.403	-0.064	0.340	1.00E-03	3.98E-03	4
Christensenellaceae_f	-0.419	-0.111	0.383	1.00E-03	3.98E-03	5
Subdoligranulum	-0.494	-0.138	0.536	1.00E-03	3.98E-03	6
Ruminococcaceae_UCG-010	-0.393	-0.138	0.355	1.00E-03	3.98E-03	7
Oscillospira	-0.414	-0.109	0.374	1.00E-03	3.98E-03	8
Lachnospiraceae_ND3007_group	-0.415	-0.031	0.354	1.00E-03	3.98E-03	9
Christensenellaceae_R-7_group	-0.432	-0.161	0.434	1.00E-03	3.98E-03	10
GCA-900066225	-0.345	-0.063	0.251	5.00E-03	1.51E-02	11
Bifidobacterium	-0.198	-0.282	0.243	1.00E-03	3.98E-03	12
Ruminococcaceae_UCG-009	-0.334	-0.056	0.234	1.00E-02	2.62E-02	13
Ruminococcus_1	-0.487	-0.225	0.588	1.00E-03	3.98E-03	14
Pseudoflavonifractor	-0.344	-0.046	0.246	3.00E-03	1.03E-02	15
Ruminococcaceae_UCG-002	-0.539	-0.129	0.627	1.00E-03	3.98E-03	16
Clostridiales_vadinBB60_group_f	-0.498	-0.115	0.533	1.00E-03	3.98E-03	17
Faecalibacterium	-0.523	-0.216	0.652	1.00E-03	3.98E-03	18
Lachnospiraceae_FCS020_group	-0.486	-0.242	0.602	1.00E-03	3.98E-03	19
Ruminococcaceae_f	-0.563	-0.185	0.717	1.00E-03	3.98E-03	20
Ruminiclostridium_5	-0.478	-0.076	0.478	1.00E-03	3.98E-03	21
CAG-56	-0.385	-0.084	0.316	1.00E-03	3.98E-03	22
Angelakisella	-0.450	-0.094	0.431	1.00E-03	3.98E-03	23
Ruminococcaceae_UCG-013	-0.466	-0.052	0.448	1.00E-03	3.98E-03	24
Lachnospira	-0.300	-0.199	0.265	2.00E-03	7.65E-03	25
Marvinbryantia	-0.333	-0.119	0.255	5.00E-03	1.51E-02	26
Agathobacter	-0.358	-0.167	0.319	1.00E-03	3.98E-03	27
Ruminococcaceae_NK4A214_group	-0.451	-0.148	0.460	1.00E-03	3.98E-03	28
Clostridia_c	-0.430	-0.072	0.388	1.00E-03	3.98E-03	29
Coprococcus_1	-0.372	-0.198	0.362	1.00E-03	3.98E-03	30
Ruminococcaceae_UCG-005	-0.555	-0.136	0.667	1.00E-03	3.98E-03	31
Adlercreutzia	-0.334	-0.160	0.280	1.00E-03	3.98E-03	32
Fusicatenibacter	-0.381	-0.104	0.318	1.00E-03	3.98E-03	33
Ruminococcus_2	-0.471	-0.188	0.524	1.00E-03	3.98E-03	34
Lachnospiraceae_NK4A136_group	-0.546	-0.091	0.626	1.00E-03	3.98E-03	35
Intestinimonas	-0.515	-0.036	0.544	1.00E-03	3.98E-03	36
Ruminococcaceae_UCG-014	-0.386	-0.167	0.362	1.00E-03	3.98E-03	37
Ruminiclostridium_6	-0.334	-0.020	0.228	9.00E-03	2.42E-02	38
Lachnospiraceae_UCG-008	-0.318	-0.068	0.216	5.00E-03	1.51E-02	39
Ruminiclostridium_9	-0.440	-0.092	0.412	1.00E-03	3.98E-03	40
DTU089	-0.377	-0.167	0.347	1.00E-03	3.98E-03	41
Lachnospiraceae_UCG-001	-0.372	-0.157	0.333	1.00E-03	3.98E-03	42
Negativibacillus	-0.433	-0.095	0.401	1.00E-03	3.98E-03	43
Coprococcus_3	-0.323	-0.109	0.237	3.00E-03	1.03E-02	44
Family_XIII_UCG-001	-0.402	-0.124	0.362	1.00E-03	3.98E-03	45

Supplementary Table 7 - Bacteria enriched in the IgG2 Phenotype as selected by sPLS-DA

Genus	Importance	Frequency of festure selection in 5-fold cross-validation
Escherichia.Shigella	0.119	1.00
Veillonella	0.104	1.00
Morganella	0.092	0.99
Proteus	0.090	0.97
Catenibacterium	0.081	0.92
Enterobacteriaceae_f	0.060	0.91
Anaeroglobus	0.052	0.89
Clostridium_sensu_stricto_18	0.050	0.80
Cryptobacterium	0.050	0.80
Epulopiscium	0.050	0.80
Weissella	0.050	0.80
Sanguibacteroides	0.050	0.80
Gemella	0.047	0.88
Mannheimia	0.044	0.79
Klebsiella	0.039	0.81
Finegoldia	0.039	0.83
Veillonellaceae_f	0.036	0.84
Alloscardovia	0.026	0.82
Lactobacillus	0.025	0.74
Rothia	0.021	0.82
Tyzzerella_4	0.020	0.68
Alcaligenes	0.015	0.84
Campylobacter	0.002	0.76
Atopobium	0.001	0.64
Solobacterium	0.000	0.50
Ruminococcaceae_UCG.008	-0.001	0.56
Erysipelotrichaceae_UCG.004	-0.002	0.57
Butyricimonas	-0.003	0.50
Tyzzerella	-0.003	0.46
Bilophila	-0.004	0.54
Peptococcus	-0.004	0.53
Desulfovibrionaceae_f	-0.004	0.43
Slackia	-0.006	0.50
Lactococcus	-0.008	0.55
Eggerthella	-0.008	0.57
Phocea	-0.010	0.63
Collinsella	-0.010	0.62
Romboutsia	-0.011	0.50
Caproiciproducens	-0.013	0.78
Sellimonas	-0.013	0.61
Marvinbryantia	-0.014	0.62
Mitsuokella	-0.015	0.90
Prevotellaceae_f	-0.016	0.86
Paraprevotella	-0.016	0.68

X28.4	-0.017	0.83
Megamonas	-0.017	0.78
Lachnospiraceae_UCG.009	-0.018	0.82
Firmicutes_p	-0.020	0.76
Ruminiclostridium	-0.024	0.95
Prevotella_6	-0.024	0.96
Akkermansia	-0.024	0.76
Lachnospiraceae_AC2044_group	-0.028	0.91
Faecalitalea	-0.029	0.80
Prevotella_7	-0.031	0.92
Family_XIII_UCG.001	-0.031	0.82
Bacteroidales_o	-0.031	0.96
Mollicutes_RF39_o	-0.034	0.96
Merdibacter	-0.036	0.87
Senegalimassilia	-0.038	0.93
GCA.900066225	-0.039	0.90
Eggerthellaceae_f	-0.040	0.88
Family_XIII_AD3011_group	-0.040	0.91
Adlercreutzia	-0.042	0.92
Oxalobacter	-0.043	0.99
Erysipelotrichaceae_f	-0.044	0.92
Ruminococcaceae_UCG.009	-0.048	0.96
DTU014_o	-0.049	1.00
Terrisporobacter	-0.049	0.95
Enterorhabdus	-0.051	1.00
Coprococcus_3	-0.051	0.92
Actinomyces	-0.053	0.95
Christensenellaceae_R.7_group	-0.057	0.96
Muribaculaceae_f	-0.058	1.00
Lachnospiraceae_UCG.001	-0.063	0.98
Anaerofustis	-0.065	1.00
Ruminiclostridium_5	-0.066	1.00
Ruminococcaceae_UCG.014	-0.066	1.00
Clostridiales_o	-0.068	1.00
Pseudoflavonifractor	-0.071	1.00
Holdemania	-0.073	1.00
Desulfovibrio	-0.073	1.00
Lachnospiraceae_UCG.008	-0.076	1.00
Clostridiales_vadinBB60_group_f	-0.080	1.00
Blautia	-0.080	0.97
Anaerotruncus	-0.081	1.00
Angelakisella	-0.081	1.00
Ruminococcaceae_UCG.003	-0.086	1.00
Oscillospira	-0.088	1.00
Lachnospiraceae_f	-0.088	0.97
Odoribacter	-0.089	1.00
UBA1819	-0.090	1.00
Lachnospira	-0.090	0.98

Candidatus_Soleaferrea	-0.091	1.00
Clostridia_c	-0.095	1.00
Sutterella	-0.095	1.00
Phascolarctobacterium	-0.096	1.00
Ruminococcaceae_UCG.004	-0.097	1.00
UC5.1.2E3	-0.098	1.00
Christensenellaceae_f	-0.099	1.00
Flavonifractor	-0.106	1.00
Coprococcus_1	-0.106	1.00
Intestinimonas	-0.108	1.00
Ruminococcaceae_UCG.010	-0.109	1.00
Dorea	-0.111	0.99
Negativibacillus	-0.119	1.00
Bifidobacterium	-0.124	1.00
Prevotella_9	-0.127	1.00
CAG.56	-0.127	1.00
Ruminococcaceae_NK4A214_group	-0.129	1.00
GCA.900066575	-0.129	1.00
Ruminococcaceae_UCG.013	-0.133	1.00
Lachnospiraceae_UCG.010	-0.134	1.00
Lachnospiraceae_UCG.004	-0.134	1.00
Ruminococcaceae_UCG.005	-0.135	1.00
Roseburia	-0.136	1.00
Lachnospiraceae_ND3007_group	-0.142	1.00
Agathobacter	-0.143	1.00
Ruminiclostridium_9	-0.143	1.00
Oscillibacter	-0.145	1.00
Ruminococcus_1	-0.150	1.00
DTU089	-0.161	1.00
Faecalibacterium	-0.165	1.00
Subdoligranulum	-0.165	1.00
Fusicatenibacter	-0.167	1.00
Ruminococcus_2	-0.172	1.00
Alistipes	-0.173	1.00
Lachnospiraceae_FCS020_group	-0.181	1.00
Ruminococcaceae_f	-0.184	1.00
Butyricicoccus	-0.186	1.00
Lachnospiraceae_NK4A136_group	-0.193	1.00
Ruminococcaceae_UCG.002	-0.203	1.00

Supplementary Table 8 - Bacterial counts in IgG2-sorted samples after removing reads found in technical controls

	Shared taxa		
Genus	**Technical Controls**	**Samples**	**Post-correction sample counts**
Blautia	33	6037	6004
Faecalibacterium	9	4180	4171
Pseudomonas	28	23	0
Aminobacter	841	36	0
Ruminiclostridium_5	32	613	581
Xanthobacteraceae_f	29	9	0
Bradyrhizobium	294	9	0
Coprococcus_3	1	1244	1243
Pseudomonas	95	23	0
Pseudomonas	55	39	0
Marvinbryantia	1	229	228
Blautia	3	17	14
Pseudomonas	10659	151	0
Lachnoclostridium	3	237	234
Blautia	2	45	43
Faecalibacterium	14	3549	3535
Streptococcus	4	19	15
Haemophilus	6	18	12
Faecalibacterium	3	108	105
Escherichia/Shigella	2	7259	7257
Corynebacterium_1	1	17	16
Sphingomonas	1545	8	0
Subdoligranulum	3	4456	4453
Acinetobacter	109	63	0
Micrococcus	39	100	61
Staphylococcus	62	91	29
Lachnospiraceae_f	1	78	77
Pseudomonas	30394	435	0
Anaerostipes	3	8715	8712
Blautia	2	69	67
Pseudomonas	3	50	47
Lachnospiraceae_ND3007_group	62	855	793
Mesorhizobium	2	10	8
Micrococcus	30	29	0
Achromobacter	1491	42	0
Lachnospiraceae_f	2	57	55
Coprococcus_2	1	49	48
Blautia	64	831	767
Coprococcus_2	14	1	0
Faecalibacterium	26	5497	5471
Faecalibacterium	21	6	0
Micrococcus	22	5	0
Fusicatenibacter	3	293	290
Staphylococcus	14	36	22
Blautia	26	1025	999
Ruminococcaceae_UCG-013	1	15	14
Mesorhizobium	50	4	0
Faecalibacterium	10	23	13
Faecalibacterium	17	5068	5051
Phascolarctobacterium	1	801	800
Corynebacterium_1	23	6	0
Stenotrophomonas	6969	157	0
Staphylococcus	15	4	0
Agathobacter	18	1	0
Enhydrobacter	167	38	0
Pseudomonas	375	297	0
Blautia	8	8	0
Mesorhizobium	146	118	0

Clostridium_sensu_stricto_1	71	35	0
Stenotrophomonas	3050	143	0
Pseudomonas	201	167	0
Achromobacter	1309	20	0
Ruminococcaceae_UCG-002	4	245	241
Mesorhizobium	8	11	3
Streptococcus	10	2923	2913
Blautia	24	10	0
Fusicatenibacter	4	5110	5106
Micrococcus	6	28	22
Blautia	10	213	203
Lachnospiraceae_ND3007_group	5	145	140
Mesorhizobium	1372	250	0
Lachnospiraceae_FCS020_group	2	129	127
Faecalibacterium	2	2	0
Ruminococcaceae_UCG-003	7	10	3
Lachnospiraceae_f	39	349	310
Aminobacter	251	8	0
Pseudomonas	24	6	0
Pseudomonas	133	134	1
Ruminiclostridium_5	11	10	0
Akkermansia	1	76	75
Lachnospiraceae_f	1	18	17
Pelomonas	42	66	24
Pseudomonas	46	53	7

Supplementary Table 9 - Mean relative abundance in IgG2-coated vs. bulk samples

			IgG2-coated		Bulk	
Phylum	**Family**	**Genus**	**Mean**	**SD**	**Mean**	**SD**
Firmicutes	*Acidaminococcaceae*	*Acidaminococcus*	0.00E+00	0.00E+00	9.19E-05	1.65E-04
Actinobacteria	*Actinomycetaceae*	*Actinomyces*	0.00E+00	0.00E+00	7.17E-04	1.63E-03
Firmicutes	*Lachnospiraceae*	*Agathobacter*	1.27E-03	2.84E-03	2.85E-02	3.15E-02
Verrucomicrobia	*Akkermansiaceae*	*Akkermansia*	8.26E-04	1.44E-03	1.09E-03	2.79E-03
Proteobacteria	*Burkholderiaceae*	*Alcaligenes*	0.00E+00	0.00E+00	9.92E-05	2.16E-04
Bacteroidetes	*Rikenellaceae*	*Alistipes*	4.70E-04	9.53E-04	1.26E-02	2.17E-02
Actinobacteria	*Bifidobacteriaceae*	*Alloscardovia*	2.60E-04	6.88E-04	4.96E-05	1.31E-04
Firmicutes	*Lachnospiraceae*	*Anaerobium*	0.00E+00	0.00E+00	1.47E-03	3.90E-03
Firmicutes	*Lachnospiraceae*	*Anaerosporobacter*	0.00E+00	0.00E+00	5.41E-04	1.43E-03
Firmicutes	*Lachnospiraceae*	*Anaerostipes*	6.42E-02	8.55E-02	1.14E-02	1.38E-02
Firmicutes	*Ruminococcaceae*	*Angelakisella*	0.00E+00	0.00E+00	1.70E-04	4.50E-04
Firmicutes	*Peptostreptococcaceae*	*Asaccharospora*	1.21E-04	3.21E-04	1.75E-04	3.11E-04
Actinobacteria	*Atopobiaceae*	*Atopobium*	0.00E+00	0.00E+00	2.48E-04	6.57E-04
Bacteroidetes	*Bacteroidaceae*	*Bacteroides*	5.74E-02	1.48E-01	2.46E-01	2.15E-01
Bacteroidetes	*Barnesiellaceae*	*Barnesiella*	0.00E+00	0.00E+00	5.48E-03	1.45E-02
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	7.89E-03	1.24E-02	1.30E-02	1.09E-02
Proteobacteria	*Desulfovibrionaceae*	*Bilophila*	2.14E-04	5.65E-04	5.46E-03	9.38E-03
Firmicutes	*Lachnospiraceae*	*Blautia*	1.30E-01	1.42E-01	2.91E-02	2.11E-02
Firmicutes	*Ruminococcaceae*	*Butyricicoccus*	1.09E-03	2.68E-03	1.51E-03	1.58E-03
Bacteroidetes	*Marinifilaceae*	*Butyricimonas*	0.00E+00	0.00E+00	3.61E-04	9.54E-04
Firmicutes	*Ruminococcaceae*	*CAG-352*	0.00E+00	0.00E+00	6.54E-06	1.73E-05
Firmicutes	*Lachnospiraceae*	*CAG-56*	1.18E-03	2.06E-03	8.10E-04	2.03E-03
Epsilonbacteraeota	*Campylobacteraceae*	*Campylobacter*	0.00E+00	0.00E+00	8.27E-06	2.19E-05
Actinobacteria	*Eggerthellaceae*	*CHKCI002*	0.00E+00	0.00E+00	9.44E-06	2.50E-05
Firmicutes	*Christensenellaceae*	*Christensenellaceae_f*	0.00E+00	0.00E+00	3.27E-05	8.66E-05
Firmicutes	*Christensenellaceae*	*Christensenellaceae_R-7_group*	2.51E-04	5.22E-04	6.51E-03	1.66E-02
Firmicutes	*Clostridia_c*	*Clostridia_c*	0.00E+00	0.00E+00	5.89E-05	1.56E-04
Firmicutes	*Clostridiales_o*	*Clostridiales_o*	2.89E-04	5.39E-04	8.89E-04	1.52E-03
Firmicutes	*Clostridiales_vadinBB60_group*	*Clostridiales_vadinBB60_group_f*	0.00E+00	0.00E+00	7.66E-04	2.03E-03
Firmicutes	*Clostridiaceae_1*	*Clostridium_sensu_stricto_1*	2.49E-02	5.10E-02	4.99E-02	8.48E-02
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	0.00E+00	0.00E+00	6.72E-03	6.11E-03
Bacteroidetes	*Barnesiellaceae*	*Coprobacter*	0.00E+00	0.00E+00	1.75E-03	3.84E-03
Firmicutes	*Lachnospiraceae*	*Coprococcus_1*	5.82E-04	1.28E-03	6.23E-04	9.47E-04
Firmicutes	*Lachnospiraceae*	*Coprococcus_2*	2.02E-03	5.26E-03	3.42E-03	6.02E-03
Firmicutes	*Lachnospiraceae*	*Coprococcus_3*	5.13E-03	9.23E-03	1.69E-03	2.58E-03
Actinobacteria	*Coriobacteriales_Incertae_Sedis*	*Coriobacteriales_Incertae_Sedis_f*	0.00E+00	0.00E+00	3.27E-05	8.66E-05
Firmicutes	*Defluviitaleaceae*	*Defluviitaleaceae_UCG-011*	0.00E+00	0.00E+00	1.10E-04	2.36E-04
Firmicutes	*Veillonellaceae*	*Dialister*	1.63E-02	4.12E-02	1.85E-03	3.86E-03
Firmicutes	*Lachnospiraceae*	*Dorea*	1.66E-02	1.87E-02	5.70E-03	5.44E-03
Firmicutes	*Ruminococcaceae*	*DTU089*	2.08E-04	5.49E-04	1.48E-05	2.55E-05
Actinobacteria	*Eggerthellaceae*	*Eggerthella*	0.00E+00	0.00E+00	5.14E-05	1.36E-04
Actinobacteria	*Eggerthellaceae*	*Eggerthellaceae_f*	0.00E+00	0.00E+00	1.89E-05	5.00E-05
Firmicutes	*Lachnospiraceae*	*Eisenbergiella*	0.00E+00	0.00E+00	5.89E-05	1.56E-04
Proteobacteria	*Enterobacteriaceae*	*Enterobacteriaceae_f*	0.00E+00	0.00E+00	2.60E-03	6.89E-03
Firmicutes	*Enterococcaceae*	*Enterococcus*	2.37E-04	6.27E-04	6.02E-05	1.12E-04
Firmicutes	*Lachnospiraceae*	*Epulopiscium*	0.00E+00	0.00E+00	1.74E-04	4.60E-04
Firmicutes	*Erysipelotrichaceae*	*Erysipelatoclostridium*	0.00E+00	0.00E+00	2.69E-04	4.74E-04
Firmicutes	*Erysipelotrichaceae*	*Erysipelotrichaceae_f*	0.00E+00	0.00E+00	9.26E-05	2.45E-04
Firmicutes	*Erysipelotrichaceae*	*Erysipelotrichaceae_UCG-003*	4.18E-03	8.50E-03	1.96E-02	2.99E-02
Proteobacteria	*Enterobacteriaceae*	*Escherichia/Shigella*	6.92E-02	1.20E-01	5.33E-02	8.66E-02
Firmicutes	*Ruminococcaceae*	*Faecalibacterium*	1.06E-01	1.43E-01	3.39E-02	4.54E-02
Firmicutes	*Erysipelotrichaceae*	*Faecalitalea*	0.00E+00	0.00E+00	1.67E-04	4.04E-04
Firmicutes	*Family_XIII*	*Family_XIII_AD3011_group*	1.17E-04	3.10E-04	1.40E-04	3.43E-04
Firmicutes	*Family_XIII*	*Family_XIII_f*	0.00E+00	0.00E+00	2.62E-05	6.92E-05
Firmicutes	*Family_XIII*	*Family_XIII_UCG-001*	4.48E-05	1.18E-04	8.51E-05	2.25E-04

Firmicutes	*Family_XI*	*Finegoldia*	0.00E+00	0.00E+00	7.21E-05	1.25E-04
Firmicutes	*Ruminococcaceae*	*Flavonifractor*	2.03E-04	5.38E-04	1.10E-03	2.22E-03
Firmicutes	*Lachnospiraceae*	*Fusicatenibacter*	3.14E-02	3.43E-02	8.23E-03	7.05E-03
Fusobacteria	*Fusobacteriaceae*	*Fusobacterium*	0.00E+00	0.00E+00	1.37E-04	1.85E-04
Firmicutes	*Ruminococcaceae*	*GCA-900066225*	0.00E+00	0.00E+00	1.31E-05	3.46E-05
Firmicutes	*Lachnospiraceae*	*GCA-900066575*	1.22E-04	3.24E-04	3.27E-05	8.66E-05
Firmicutes	*Family_XI*	*Gemella*	4.62E-04	1.22E-03	2.98E-04	7.88E-04
Firmicutes	*Carnobacteriaceae*	*Granulicatella*	3.25E-04	8.60E-04	5.30E-04	1.38E-03
Proteobacteria	*Pasteurellaceae*	*Haemophilus*	9.65E-04	1.72E-03	1.17E-02	2.27E-02
Firmicutes	*Erysipelotrichaceae*	*Holdemanella*	5.47E-04	1.45E-03	5.67E-04	1.50E-03
Firmicutes	*Erysipelotrichaceae*	*Holdemania*	0.00E+00	0.00E+00	3.27E-05	8.66E-05
Firmicutes	*Lachnospiraceae*	*Hungatella*	0.00E+00	0.00E+00	2.03E-04	3.43E-04
Firmicutes	*Peptostreptococcaceae*	*Intestinibacter*	3.39E-02	7.04E-02	3.51E-02	8.02E-02
Firmicutes	*Ruminococcaceae*	*Intestinimonas*	1.63E-04	2.43E-04	1.42E-04	2.49E-04
Tenericutes	*Izimaplasmatales_o*	*Izimaplasmatales_o*	0.00E+00	0.00E+00	5.23E-05	1.38E-04
Proteobacteria	*Enterobacteriaceae*	*Klebsiella*	5.86E-04	1.55E-03	3.88E-04	1.03E-03
Firmicutes	*Lachnospiraceae*	*Lachnoclostridium*	1.56E-02	1.84E-02	1.60E-02	1.51E-02
Firmicutes	*Lachnospiraceae*	*Lachnospira*	1.08E-02	1.56E-02	2.26E-02	1.96E-02
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_AC2044_group*	3.00E-04	7.94E-04	6.28E-05	1.07E-04
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_f*	7.90E-02	5.64E-02	2.38E-02	1.60E-02
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_FCS020_group*	2.87E-03	6.87E-03	3.69E-04	8.91E-04
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_FE2018_group*	0.00E+00	0.00E+00	3.91E-04	1.03E-03
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_ND3007_group*	7.24E-03	1.56E-02	2.56E-03	3.04E-03
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_NK4A136_group*	4.84E-03	7.26E-03	5.01E-03	5.98E-03
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_UCG-001*	6.93E-04	1.21E-03	5.78E-04	1.33E-03
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_UCG-004*	3.37E-03	4.55E-03	4.19E-03	3.25E-03
Firmicutes	*Lachnospiraceae*	*Lachnospiraceae_UCG-010*	2.15E-03	5.53E-03	7.80E-04	1.01E-03
Firmicutes	*Lactobacillales_o*	*Lactobacillales_o*	0.00E+00	0.00E+00	2.57E-04	6.80E-04
Firmicutes	*Lactobacillaceae*	*Lactobacillus*	9.57E-03	1.61E-02	5.58E-03	8.51E-03
Firmicutes	*Streptococcaceae*	*Lactococcus*	0.00E+00	0.00E+00	2.06E-05	5.44E-05
Proteobacteria	*Pasteurellaceae*	*Mannheimia*	0.00E+00	0.00E+00	4.10E-05	8.57E-05
Firmicutes	*Lachnospiraceae*	*Marvinbryantia*	3.42E-03	3.63E-03	2.60E-04	5.80E-04
Firmicutes	*Veillonellaceae*	*Megamonas*	0.00E+00	0.00E+00	4.24E-05	7.38E-05
Firmicutes	*Veillonellaceae*	*Megasphaera*	2.59E-04	4.03E-04	6.62E-05	1.75E-04
Firmicutes	*Erysipelotrichaceae*	*Merdibacter*	0.00E+00	0.00E+00	1.31E-05	3.46E-05
Tenericutes	*Mollicutes_RF39_o*	*Mollicutes_RF39_o*	0.00E+00	0.00E+00	8.25E-06	2.18E-05
Proteobacteria	*Enterobacteriaceae*	*Morganella*	0.00E+00	0.00E+00	9.32E-04	2.47E-03
Bacteroidetes	*Muribaculaceae*	*Muribaculaceae_f*	3.91E-04	1.03E-03	1.95E-03	5.17E-03
Firmicutes	*Ruminococcaceae*	*Negativibacillus*	0.00E+00	0.00E+00	2.22E-04	5.89E-04
Bacteroidetes	*Marinifilaceae*	*Odoribacter*	0.00E+00	0.00E+00	3.23E-03	4.10E-03
Firmicutes	*Ruminococcaceae*	*Oscillibacter*	4.17E-04	9.59E-04	1.36E-03	1.34E-03
Firmicutes	*Ruminococcaceae*	*Oscillospira*	5.18E-04	1.37E-03	3.14E-04	8.31E-04
Bacteroidetes	*Tannerellaceae*	*Parabacteroides*	7.06E-04	1.21E-03	1.33E-02	1.85E-02
Bacteroidetes	*Prevotellaceae*	*Paraprevotella*	0.00E+00	0.00E+00	2.64E-04	7.00E-04
Proteobacteria	*Burkholderiaceae*	*Parasutterella*	0.00E+00	0.00E+00	1.49E-02	2.74E-02
Firmicutes	*Peptococcaceae*	*Peptococcaceae_f*	3.41E-05	9.02E-05	1.24E-04	3.29E-04
Firmicutes	*Family_XI*	*Peptoniphilus*	6.25E-05	1.65E-04	2.71E-04	7.16E-04
Firmicutes	*Peptostreptococcaceae*	*Peptostreptococcaceae_f*	3.62E-05	9.59E-05	3.01E-05	7.95E-05
Firmicutes	*Peptostreptococcaceae*	*Peptostreptococcus*	6.99E-04	1.69E-03	1.80E-04	4.77E-04
Firmicutes	*Acidaminococcaceae*	*Phascolarctobacterium*	8.79E-03	2.31E-02	2.93E-03	7.72E-03
Firmicutes	*Ruminococcaceae*	*Phocea*	0.00E+00	0.00E+00	5.14E-05	1.36E-04
Bacteroidetes	*Prevotellaceae*	*Prevotella*	0.00E+00	0.00E+00	1.65E-05	4.37E-05
Bacteroidetes	*Prevotellaceae*	*Prevotella_2*	0.00E+00	0.00E+00	1.96E-05	5.19E-05
Bacteroidetes	*Prevotellaceae*	*Prevotella_6*	0.00E+00	0.00E+00	2.48E-05	6.55E-05
Bacteroidetes	*Prevotellaceae*	*Prevotella_7*	0.00E+00	0.00E+00	1.65E-05	4.37E-05
Bacteroidetes	*Prevotellaceae*	*Prevotella_9*	5.38E-03	1.42E-02	6.82E-02	1.78E-01
Proteobacteria	*Enterobacteriaceae*	*Proteus*	0.00E+00	0.00E+00	4.55E-04	1.06E-03
Firmicutes	*Ruminococcaceae*	*Pseudoflavonifractor*	0.00E+00	0.00E+00	1.96E-05	5.19E-05
Proteobacteria	*Rhodospirillales_o*	*Rhodospirillales_o*	0.00E+00	0.00E+00	2.29E-03	5.98E-03

Bacteroidetes	*Rikenellaceae*	*Rikenellaceae_RC9_gut_group*	0.00E+00	0.00E+00	7.83E-05	1.53E-04
Firmicutes	*Peptostreptococcaceae*	*Romboutsia*	4.64E-02	1.17E-01	3.94E-02	1.03E-01
Firmicutes	*Lachnospiraceae*	*Roseburia*	8.55E-04	1.25E-03	2.43E-02	3.74E-02
Proteobacteria	*Enterobacteriaceae*	*Rosenbergiella*	0.00E+00	0.00E+00	2.06E-05	5.44E-05
Actinobacteria	*Micrococcaceae*	*Rothia*	2.82E-03	4.94E-03	1.16E-04	3.06E-04
Firmicutes	*Ruminococcaceae*	*Ruminiclostridium_5*	1.94E-03	4.14E-03	1.36E-03	1.85E-03
Firmicutes	*Ruminococcaceae*	*Ruminiclostridium_6*	0.00E+00	0.00E+00	6.01E-04	1.51E-03
Firmicutes	*Ruminococcaceae*	*Ruminiclostridium_9*	5.24E-04	1.06E-03	2.14E-03	2.75E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_f*	2.01E-02	3.34E-02	2.37E-03	3.41E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_NK4A214_group*	4.48E-03	9.09E-03	8.03E-04	1.47E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-002*	7.75E-03	1.35E-02	8.25E-03	1.42E-02
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-003*	1.15E-04	3.03E-04	3.75E-03	7.40E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-004*	0.00E+00	0.00E+00	9.16E-05	2.42E-04
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-005*	1.02E-03	1.69E-03	1.27E-03	3.32E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-009*	0.00E+00	0.00E+00	7.20E-05	1.90E-04
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-013*	1.75E-03	2.44E-03	6.69E-04	1.59E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae_UCG-014*	4.89E-04	1.29E-03	6.71E-04	1.65E-03
Firmicutes	*Ruminococcaceae*	*Ruminococcus_1*	1.07E-02	2.83E-02	6.76E-03	1.44E-02
Firmicutes	*Ruminococcaceae*	*Ruminococcus_2*	1.61E-03	3.02E-03	6.72E-03	9.63E-03
Bacteroidetes	*Marinifilaceae*	*Sanguibacteroides*	0.00E+00	0.00E+00	3.73E-04	9.87E-04
Firmicutes	*Lachnospiraceae*	*Sellimonas*	1.58E-04	4.17E-04	3.93E-05	1.04E-04
Actinobacteria	*Eggerthellaceae*	*Senegalimassilia*	0.00E+00	0.00E+00	1.80E-04	4.77E-04
Firmicutes	*Lachnospiraceae*	*Shuttleworthia*	0.00E+00	0.00E+00	7.98E-05	1.59E-04
Actinobacteria	*Eggerthellaceae*	*Slackia*	0.00E+00	0.00E+00	1.65E-04	4.35E-04
Firmicutes	*Erysipelotrichaceae*	*Solobacterium*	0.00E+00	0.00E+00	8.27E-05	2.19E-04
Firmicutes	*Streptococcaceae*	*Streptococcus*	8.72E-02	1.66E-01	2.70E-02	5.91E-02
Firmicutes	*Ruminococcaceae*	*Subdoligranulum*	4.82E-02	1.11E-01	9.21E-03	1.62E-02
Proteobacteria	*Burkholderiaceae*	*Sutterella*	0.00E+00	0.00E+00	1.40E-02	2.65E-02
Firmicutes	*Peptostreptococcaceae*	*Terrisporobacter*	2.84E-03	7.50E-03	1.70E-03	4.50E-03
Firmicutes	*Erysipelotrichaceae*	*Turicibacter*	5.42E-04	1.42E-03	4.63E-03	8.07E-03
Firmicutes	*Lachnospiraceae*	*Tyzzerella*	0.00E+00	0.00E+00	4.45E-04	8.41E-04
Firmicutes	*Lachnospiraceae*	*Tyzzerella_3*	9.66E-04	1.85E-03	9.01E-04	2.21E-03
Firmicutes	*Lachnospiraceae*	*Tyzzerella_4*	6.58E-03	1.17E-02	1.78E-03	3.17E-03
Firmicutes	*Ruminococcaceae*	*UBA1819*	7.19E-05	1.70E-04	1.71E-04	2.52E-04
Firmicutes	*Lachnospiraceae*	*UC5-1-2E3*	0.00E+00	0.00E+00	2.62E-05	6.92E-05
Firmicutes	*Veillonellaceae*	*Veillonella*	5.39E-03	6.91E-03	3.72E-02	8.55E-02
Firmicutes	*Veillonellaceae*	*Veillonellaceae_f*	1.53E-04	4.06E-04	2.65E-04	4.30E-04
Lentisphaerae	*Victivallaceae*	*Victivallis*	0.00E+00	0.00E+00	1.96E-05	5.19E-05
Firmicutes	*Leuconostocaceae*	*Weissella*	1.20E-03	3.13E-03	5.46E-04	1.44E-03