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ORIGINAL ARTICLE

# Post-operative Crohn's Disease Recurrence and Infectious Complications: A Transcriptomic Analysis

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## Abstract

**Background** Crohn's disease (CD) is a chronic inflammatory condition affecting the gastrointestinal tract, characterized by complications such as strictures, fistulas, and neoplasia. Despite medical advancements, a significant number of patients with Crohn's disease require surgery, and many experience post-operative complications and recurrence. Previous studies have analyzed gene expression to study recurrence and post-operative complications independently. This study aims to identify overlapping differentially expressed genes and pathways for recurrence and post-operative complications.

**Methods** A dataset including 45 patients with Crohn's disease, including gene expression from ileum and colon tissue, endoscopic recurrence, and intra-abdominal septic complications was analyzed. Gene set enrichment analysis was used to identify gene pathways associated with the outcomes. Finally, a multi-variable logistic regression model was created to assess whether gene pathways were independently associated with both outcomes.

**Results** In ileum tissue, several inflammatory pathways, including interferon alpha and gamma response were upregulated in patients with endoscopic recurrence and intra-abdominal septic complications. In addition, there was upregulation of the epithelial mesenchymal transition pathway. In colon tissue, metabolic processes, such as myogenesis and oxidative phosphorylation were downregulated in both outcomes. In a multivariate model, downregulation of myogenesis in colon tissue was significantly associated with both endoscopic recurrence and intra-abdominal septic complications.

**Conclusion** These findings shed light on the underlying biology of these outcomes and suggest potential biomarkers or therapeutic targets to reduce their occurrence. Further validation and multi-institutional studies are warranted to confirm these results and improve post-operative outcomes for patients with Crohn's disease.

**Keywords** Crohn disease · Inflammatory bowel diseases · Surgical wound infection · Anastomotic leak · Gene expression profiling

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## Introduction

Crohn's disease (CD) is caused by an aberrant immune response to intestinal microbiota, resulting in acute and chronic relapsing inflammation and complications such as strictures, fistulas, and neoplasia [1]. Despite major advances in medical treatment, 30–40% of patients with CD require surgery at some point during their lifetime [2, 3]. Many of these patients experience post-operative CD recurrence, with clinical recurrence affecting up to half of patients at 5 years and endoscopic recurrence occurring in about 70% of patients [4–6]. In addition, up to 20% of patients may require repeat surgery at 10 years [7]. Multiple studies have established risk factors for post-operative recurrence, including fistulizing disease, young age, short disease duration before first surgery, ileocolonic disease, smoking, and the NOD2 gene mutation [6, 8, 9]. However, mounting evidence suggests that post-operative complications, including anastomotic leak, re-laparotomy, and surgical site infection are also significantly correlated with CD recurrence [10–14].

Previous studies have used transcriptomics, or analysis of gene expression through quantifying mRNA transcripts, to study both CD recurrence and anastomotic leak [15–17]. For CD recurrence, differential expression of individual genes, including DUOX2, CXCL5/8, and MMP1, and gene pathways, including JAK/STAT, IL-6, and interferon signaling, have been identified [15]. Similarly, specific genes, including AQP9, HCAR3, and PROC, and gene pathways such as organelle fission and leukocyte activation have been associated with anastomotic leak [17]. Comparisons of serum inflammatory markers suggest that the underlying biological processes driving both CD recurrence and post-operative complications may have significant overlap [18]. However, an analysis of the two outcomes has not been previously performed at the transcriptomic level.

The goal of this study is to identify overlap in differentially expressed genes and pathways for CD recurrence and post-operative complications. In contrast to previous studies of CD recurrence, our approach analyzes both colon and ileum tissue, which may provide more comprehensive results. In addition, we apply normalization techniques for unwanted variation, generating more consistent results [19]. We hypothesize that there will be significant overlap in the transcriptomic profile underlying CD recurrence and post-operative complications. Identification of these genes and gene pathways will improve our understanding of the pathophysiology driving these negative outcomes and will suggest prognostic biomarkers or therapeutic targets to reduce their occurrence.

## Methods

### Datasets and Outcomes

This study was approved by the University of North Carolina Institutional Review Board (Study ID#: 15-0024, 17-0236). An existing tissue bank at the University of North Carolina was used to identify adult patients with CD who underwent surgical resection of the terminal ileum and who had data on endoscopic recurrence available through retrospective chart review. The primary outcome was endoscopic recurrence, based on the first colonoscopy obtained after surgery [20]. To ensure a clear comparison, patients with Rutgeert's endoscopic recurrence score of i0 (no recurrence) vs i3/i4 (recurrence) were selected. Our secondary outcome was intra-abdominal septic complications (IASC), defined as intra-abdominal abscess or anastomotic leak. Additional clinical characterization of these patients, including sex, race, ethnicity, smoking status, Montreal classification (age, disease location, and behavior), pre-operative medication use, duration of disease, previous surgery, and post-operative medication use, was also collected. For medications used pre-operatively, we included medications which were consistently prescribed for 6 months prior to surgery. At our institution, elective resections are timed for at least 2 weeks after last infusion of biologic therapy, while immunomodulators and steroids are generally tapered off after surgery. Post-operatively, medications were restarted at the discretion of the primary gastroenterologist, depending on patient symptoms and laboratory values.

### Specimen, mRNA, and Data Processing

Macroscopically uninflamed biopsies were taken from the terminal ileum and colon at the time of surgery and preserved as formalin-fixed paraffin-embedded (FFPE) samples. FFPE tissue was prepared as previously described, with RNA isolated using the Quick-RNA FFPE MiniPrep, total RNA purified using the MagMAX kit in the KingFisher system, and RNA-seq libraries purified using TruSeq Stranded Total RNA with Ribo-Zero. Paired-end sequencing (50 base-pairs) was performed on the NovaSeq 6000 platform [21].

Sample quality and integrity were assessed using a variety of metrics. First, samples with a low number of transcripts counted were identified ($< 25{,}000$). These sample were confirmed to have a low transcript identity number [22], percentage of sequences aligned, and high duplication percentage. These samples ($n = 4$) were then discarded. Samples with unclear tissue type (colon vs small bowel) based on collected labels and principal component analysis (PCA) were also discarded ($n = 2$).

### Differential Expression Analysis

We used RUVSeq to control for unwanted variation [19]. Control genes were selected by running an uncontrolled differential expression analysis with DESeq2, and selecting genes with a $p$-value > 0.5 [23]. This approach adjusts for all variation that is not due to the outcome of interest, including batch effects, specimen quality, cellular composition, microscopic inflammation, and disease subtype. However, because of clear effects seen in exploratory PCA graphs, we explicitly controlled for sex.

First, the filterbyExpression function from EdgeR was used to select genes with at least 10 read counts in 70% of samples [24]. The number of RUVSeq factors was chosen based on optimization of a several factors. First, we sought to balance variation seen in relative log expression plots across samples. Second, we ensured that correlation between included factors and the outcome was low. Third, we selected combinations of factors which included a broad overlap in differentially expressed genes with other factor combinations [19]. This resulted in 19 factors used for ileum tissue and 18 factors for colon tissue.

Differential expression analysis was then performed using DESeq2 with false discovery rate (FDR) adjusted $p$-value ($p$-adj) of < 0.05 considered significant. Adjustment for multiple comparisons was performed using the Benjamini–Hochberg procedure [23]. Pathway analysis was performed using the Molecular Signatures Database hallmark gene set collection and fgsea [25, 26]. Volcano plots were generated using EnhancedVolcano [27]. Heatmaps were created using pheatmap and the Molecular Signatures Database hallmark gene sets [26, 28]. To determine the association between gene pathways and post-operative complications, while controlling for pre-operative characteristics, we used the GSVA library to calculate single-sample gene set enrichment and created multi-variable logistic regression models including gene pathways and possible confounders [29]. Clinical risk factors were selected for inclusion in the multivariate model with a $p$-value threshold of < 0.05. Differentially expressed genes that were found in both colon and ileum tissues were identified and grouped by whether genes were upregulated or downregulated in both tissues. Enrichr was used to perform gene set enrichment on each set of genes (up- vs downregulated genes differentially expressed in both tissues) [30]. Pathways from the Molecular Signatures Database hallmark gene sets with an adjusted $p$-value of < 0.05 were considered significant.

Correlation of gene expression levels between FFPE and fresh frozen (FF) conditions for five patient biopsy samples was performed using the lengthScaledTPM count matrix outputs generated by tximport from Salmon quantification. For each set of samples, only genes present in both conditions were included in the analysis. Pearson's correlation coefficient was calculated for FFPE and FF gene expression levels using log10 transformed gene count matrices and plotted using ggscatter from ggpubr$^{+}$ [31]. All analyses were performed in R (v4.3) [32]. Code to reproduce this work is available at https://github.com/gomezlab/cd_postop.

## Results

### Dataset Characteristics

The CD recurrence dataset included 45 patients, of whom 23 (51%) experienced endoscopic recurrence. Post-operative colonoscopy to assess Rutgeert's score occurred at a mean of 6.5 months after resection. The average age was 29 and 58% of patients were female. In terms of race/ethnicity, 80% of patients were Non-Hispanic White and 13.3% were Non-Hispanic Black (Table 1). Four (17.4%) patients with recurrent disease had experienced IASC, compared with 3 (13.6%) patients without recurrent ($p = 1.0$). Recurrence was less common in patients who received anti-TNF therapy post-operatively (39.1% vs 68.2%, $p = 0.09$). No significant associations were seen between disease location, behavior, pre-operative medication use, duration of disease, or history of surgery and disease recurrence.

### Post-operative Crohn's Disease Recurrence

#### Ileum Tissue

Differential expression analysis across CD recurrence revealed a total of 940 differentially expressed genes (Supplemental Table 1). The genes with the highest upregulation in patients experiencing recurrence included genes previously associated with CD such as glutathione transferase (GSTM1), a tryptophan metabolite receptor (HCAR3), and inflammatory genes such as IL1B and CXCL11 [33, 34]. Highly downregulated genes included the nucleosome gene H3C14 and transcription factor ONECUT2 (Fig. 1A). Inflammatory and immune pathways showed the highest upregulation, including interferon gamma response, TNF-alpha signaling, and IL-6/JAK/STAT3 signaling. In contrast, metabolic processes, such as oxidative phosphorylation, adipogenesis, and myogenesis were downregulated (Fig. 1B). A heatmap based on the top 15 genes showed a strong pattern of differential expression between the two outcomes (Fig. 1C).

#### Colon Tissue

Differential expression analysis across CD recurrence revealed a total of 813 differentially expressed genes

**Table 1** Demographic and clinical characteristics of crohn's disease recurrence dataset

| | | Overall | Non-recurrent | Recurrent | *p*-value |
|---|---|---|---|---|---|
| *n* | | 45 | 22 | 23 | |
| Age, mean (SD) | | 28.8 (9.9) | 31.2 (12.1) | 26.5 (6.9) | 0.120 |
| Sex, *n* (%) | Female | 26 (57.8) | 13 (59.1) | 13 (56.5) | 1.000 |
| | Male | 19 (42.2) | 9 (40.9) | 10 (43.5) | |
| Race, *n* (%) | Asian | 1 (2.2) | 0 (0.0) | 1 (4.3) | 0.561 |
| | Black | 6 (13.3) | 3 (13.6) | 3 (13.0) | |
| | Unknown | 2 (4.4) | 1 (4.5) | 1 (4.3) | |
| | White | 36 (80.0) | 18 (81.8) | 18 (78.3) | |
| Ethnicity, *n* (%) | Non-Hispanic | 44 (97.8) | 21 (95.5) | 23 (100.0) | 0.489 |
| | Unknown | 1 (2.2) | 1 (4.5) | | |
| Smoking Status, *n* (%) | Never | 31 (68.9) | 16 (72.7) | 15 (65.2) | 0.262 |
| | Previous | 6 (13.3) | 4 (18.2) | 2 (8.7) | |
| | Current | 8 (17.8) | 2 (9.1) | 6 (26.1) | |
| Duration of disease (months), mean (SD) | | 7.2 (7.2) | 8.5 (8.7) | 5.9 (5.2) | 0.229 |
| Age Classification, *n* (%) | A1 | 13 (28.9) | 6 (27.3) | 7 (30.4) | 0.335 |
| | A2 | 30 (66.7) | 14 (63.6) | 16 (69.6) | |
| | A3 | 2 (4.4) | 2 (9.1) | | |
| Disease Location, *n* (%) | L1 | 12 (26.7) | 6 (27.3) | 6 (26.1) | 0.232 |
| | L2 | 3 (6.7) | | 3 (13.0) | |
| | L3 | 29 (64.4) | 16 (72.7) | 13 (56.5) | |
| | L4 | 1 (2.2) | | 1 (4.3) | |
| Disease Behavior, *n* (%) | B1 | 5 (11.1) | 4 (18.2) | 1 (4.3) | 0.249 |
| | B2 | 24 (53.3) | 12 (54.5) | 12 (52.2) | |
| | B3 | 16 (35.6) | 6 (27.3) | 10 (43.5) | |
| Perianal Disease, *n* (%) | Absent | 36 (80.0) | 18 (81.8) | 18 (78.3) | 1.000 |
| | Present | 9 (20.0) | 4 (18.2) | 5 (21.7) | |
| History of surgery, *n* (%) | Yes | 3 (6.7) | 0 (0.0) | 3 (13.0) | 0.233 |
| | No | 42 (93.3) | 22 (100.0) | 20 (87.0) | |
| Anti-TNF alpha use, *n* (%) | Yes | 21 (46.7) | 8 (36.4) | 13 (56.5) | 0.291 |
| | No | 24 (53.3) | 14 (63.6) | 10 (43.5) | |
| Immunomodulator use, *n* (%) | Yes | 28 (62.2) | 13 (59.1) | 15 (65.2) | 0.908 |
| | No | 17 (37.8) | 9 (40.9) | 8 (34.8) | |
| Steroid use, *n* (%) | Yes | 32 (71.1) | 16 (72.7) | 16 (69.6) | 1.000 |
| | No | 13 (28.9) | 6 (27.3) | 7 (30.4) | |
| Post-operative steroid use, *n* (%) | Yes | 15 (33.3) | 7 (31.8) | 8 (34.8) | 1.000 |
| | No | 30 (66.7) | 15 (68.2) | 15 (65.2) | |
| Post-operative anti-TNF alpha use, *n* (%) | Yes | 24 (53.3) | 15 (68.2) | 9 (39.1) | 0.098 |
| | No | 21 (46.7) | 7 (31.8) | 14 (60.9) | |
| Intra-abdominal septic complication, *n* (%) | Yes | 7 (15.6) | 3 (13.6) | 4 (17.4) | 1.000 |
| | No | 38 (84.4) | 19 (86.4) | 19 (82.6) | |

*TNF* tissue necrosis factor

(Supplemental Table 2). Similar to ileum tissue, HCAR3 showed strong upregulation, as well as immune/inflammatory genes (IGLV3-27, CXCL5). Hyaluronic linking protein HAPLN4 and apolipoproteins APOA1 and APOC3 were downregulated. (Fig. 2A). Pathway enrichment analysis showed that immune/inflammatory regulatory pathways, such as allograft rejection, interferon gamma response, and IL-6/JAK/STAT3 signaling had the highest upregulation, while metabolic and wound healing processes including oxidative phosphorylation, myogenesis, adipogenesis, and apical junction pathways were downregulated (Fig. 2B). A heatmap based on the top 15 genes also showed a pattern of differential expression between the two outcomes (Fig. 2C). In a multivariable

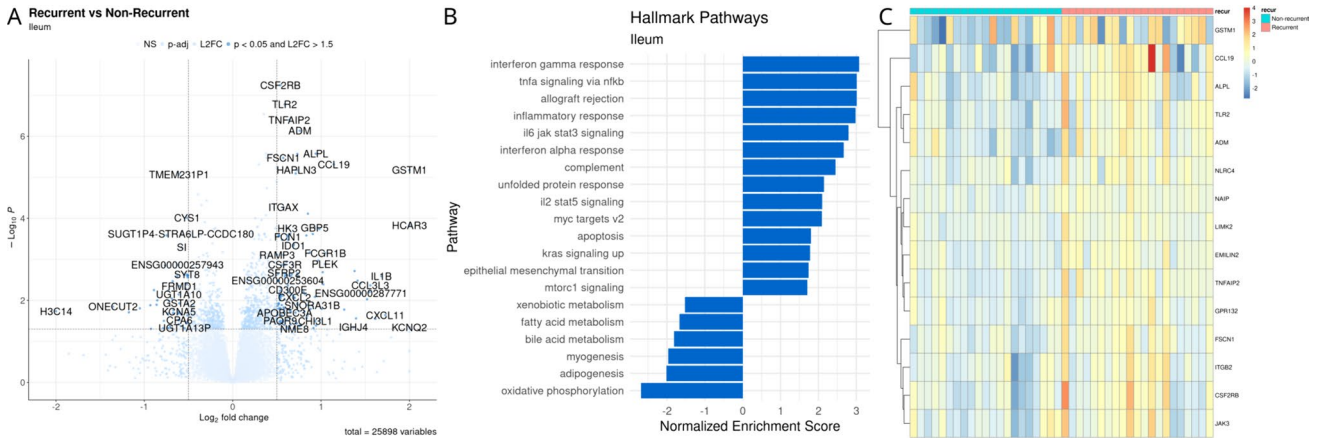


**Fig. 1** Differential expression analysis of Crohn's disease recurrence in ileum tissue. **A** Volcano plot of differentially expressed genes, filtered by adjusted p-value and fold change. **B** Pathway enrichment analysis based on the hallmark gene sets. **C** Heatmap of gene expression based on top 15 differentially expressed genes (by adjusted *p*-value)

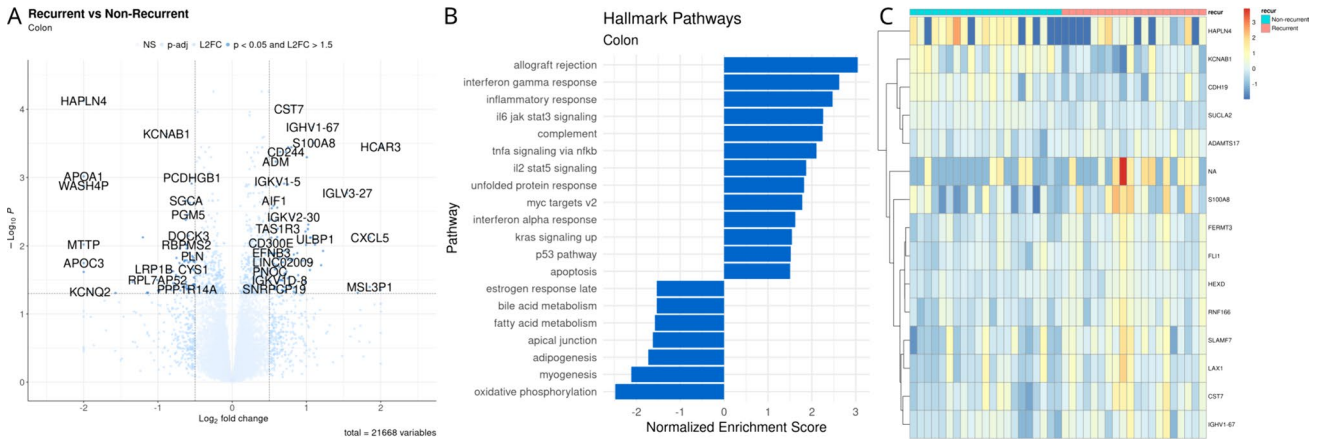


**Fig. 2** Differential expression analysis of Crohn's disease recurrence in colon tissue. **A** Volcano plot of differentially expressed genes, filtered by adjusted p-value and fold change. **B** Pathway enrichment analysis based on the hallmark gene sets. **C** Heatmap of gene expression based on top 15 differentially expressed genes (by adjusted *p*-value)

**Table 2** Multivariable analysis of association between clinical risk factors, myogenesis pathway, and Crohn's disease recurrence

| | Intercept | Odds ratio | *p*-value | 95% confidence interval | |
|---|---|---|---|---|---|
| | | 0.25 | 0.89 | − 3.16 | 3.65 |
| Sex (ref = female) | Male | 0.26 | 0.80 | − 3.95 | − 0.77 |
| Age | | 0.9 | − 0.12 | 0.11 | 0.9 |
| Smoking (ref = non-smoker) | Smoker | − 3.05 | 0.03 | − 5.76 | − 0.33 |
| Post-op anti-TNF alpha inhibitor (ref = none) | | 1.04 | 0.27 | − 0.81 | 2.89 |
| Myogenesis pathway | | 10.32 | 0.001 | 3.97 | 16.68 |

model including age, sex, smoking status, and post-operative TNF-alpha inhibitor use, the myogenesis pathway showed a strong association with CD recurrence with an OR of 10.3 and $p$ value = 0.001 (Table 2).

### Genes Differentially Expressed Across Tissues

To determine pathways acting similarly in both colon and ileum tissues to affect CD recurrence, we identified 117 genes that were differentially upregulated and 53 genes

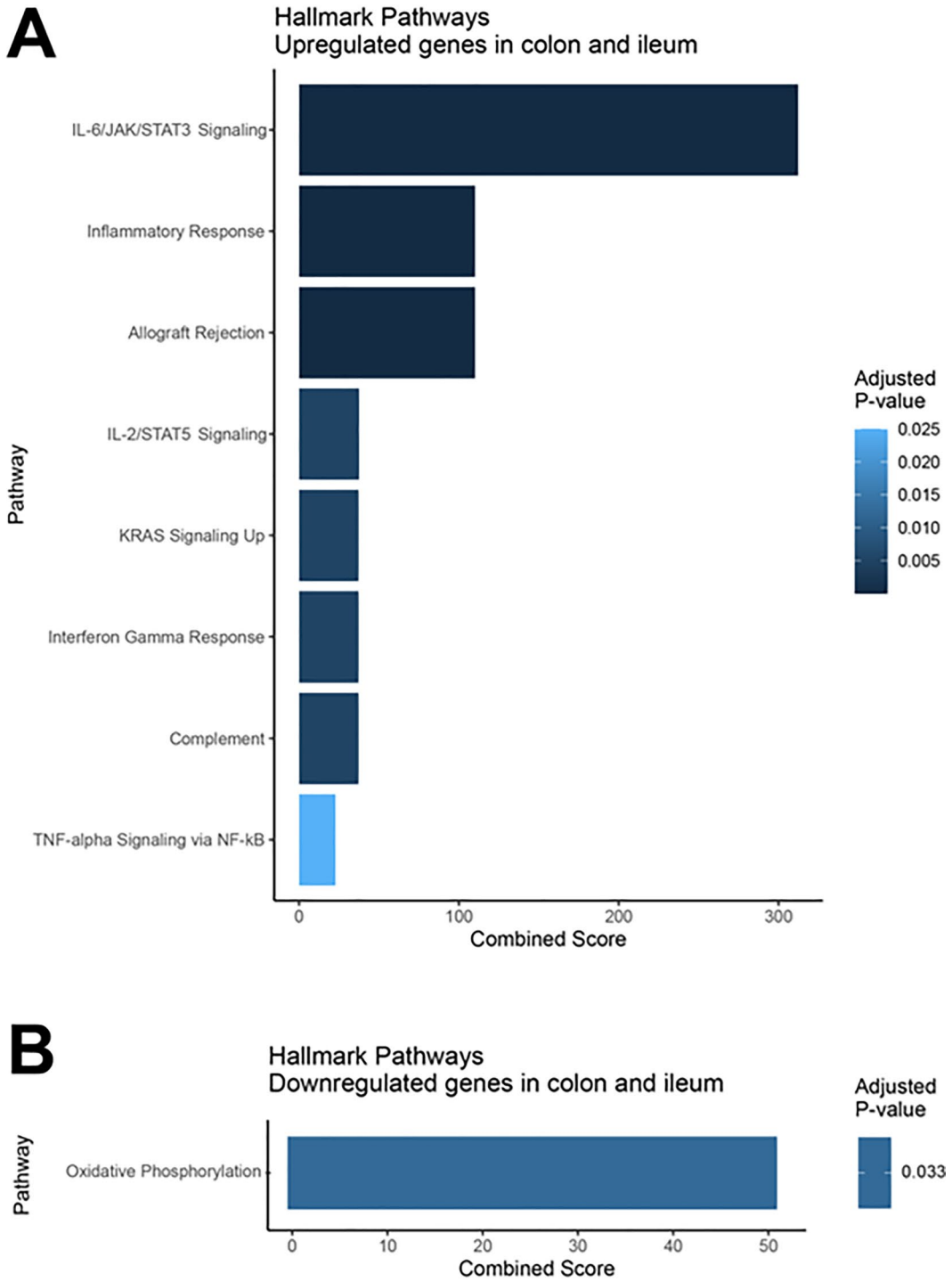


**Fig. 3** Differentially expressed genes across tissues. **A** Pathway enrichment analysis of genes differentially upregulated in both colon and ileum tissue based on the hallmark gene sets. **B** Pathway enrichment analysis of genes differentially downregulated in both colon and ileum tissue based on hallmark gene sets. In both, the combined score indicates the combination of the p-value and odds ratio

that were downregulated in both tissues (Supplemental Tables 3 and 4). Gene set enrichment analysis identified immune and inflammatory pathways, including IL-6/JAK/STAT3 signaling, inflammatory response, and allograft rejection, for upregulated genes (Fig. 3A and Supplemental Table 5). For genes downregulated in both tissues, only oxidative phosphorylation reached the significance threshold (Fig. 3B and Supplemental Table 6).

## Intra-abdominal Septic Complications

### Ileum Tissue

We next identified differentially expressed genes in ileum tissue between patients who experienced post-operative IASC and those who did not. Inflammatory genes were upregulated including IL1B (Interleukin-1 beta), CXCL11 (CXC chemokine), and IGHJ4 (immunoglobulin component). These included the pathways interferon alpha response, interferon gamma response, TNF-alpha signaling, and IL6/JAK/STAT3 signaling. In addition, there was alteration of pathways related to wound healing, including upregulation of epithelial mesenchymal transition and downregulation of myogenesis, adipogenesis, and oxidative phosphorylation (Fig. 4B).

### Colon Tissue

We also analyzed differentially expressed genes for IASC in colon tissue. There was downregulation of inflammatory pathways including inflammatory response, TNF-alpha signaling, and IL2/STAT5 signaling. There was also downregulation of tissue remodeling pathways, including the epithelial mesenchymal transition, myogenesis, and apical junction pathways (Fig. 5B). In these pathways, the genes MMP23 (a matrix metallopeptidase), TPM1 (a component of actin filaments), and EPYC (a regulator of collagen fibrillogenesis) showed strong downregulation (Fig. 5A). Univariate comparisons of clinical variables with IASC showed that only smoking status was associated with the outcome, while pre-operative steroid and TNF-alpha inhibitor use was not (Table 3). In a multivariate model, the myogenesis pathway also showed association with IASC (OR − 17.6, $p$-value = 0.04) (see Table 3).

## Correlation of Gene Expression in Fresh Frozen Tissue Versus FFPE

RNA isolated from FFPE tissue is known to be highly degraded compared to Fresh Frozen (FF) tissue [35]. To provide confidence in the accuracy of data from FFPE samples, we compared expression data from five patient biopsies in which tissue was preserved both as FFPE and FF specimens and used for RNA-sequencing. Pearson's correlation coefficient ranged from $R = 0.67$ to $R = 0.89$, indicating a positive linear relationship in gene expression levels across specimen preparation methods (Fig. 6A–E).

## Discussion

This study identified differentially expressed genes and gene pathways in intestinal tissue for patients with CD experiencing endoscopic recurrence and intra-abdominal septic complications. Patients experiencing both outcomes showed downregulation of metabolic and wound healing pathways, such as oxidative phosphorylation, myogenesis, and apical junction formation. In a multivariable model

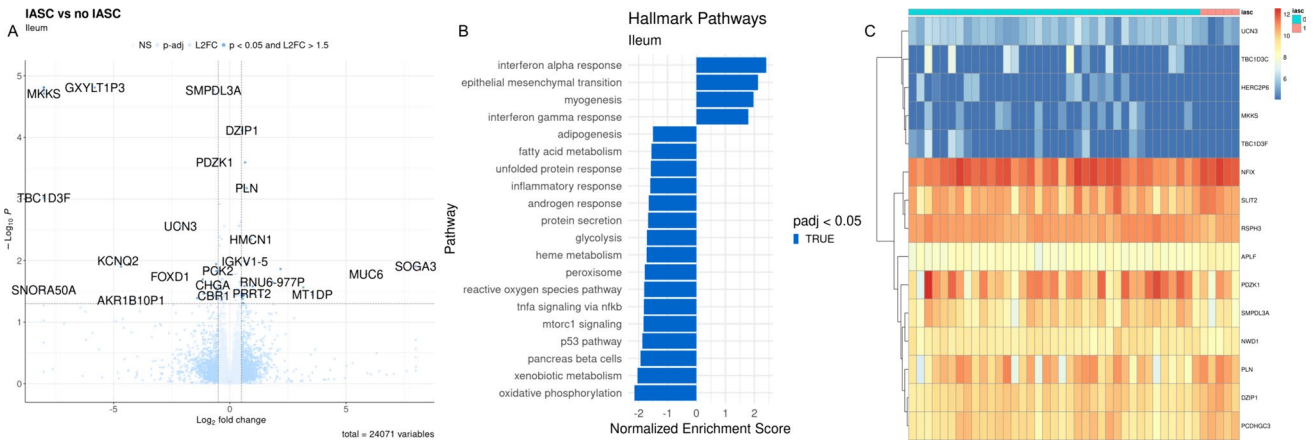


**Fig. 4** Differential expression analysis of intra-abdominal septic complications in ileum tissue. **A** Volcano plot of differentially expressed genes, filtered by adjusted p-value and fold change. **B** Pathway enrichment analysis based on the hallmark gene sets. **C** Heatmap of gene expression based on top 15 differentially expressed genes (by adjusted $p$-value). *IASC* Intra-abdominal septic complication

**Table 3** Univariate and multivariate analyses of association between clinical risk factors, myogenesis pathway, and intra-abdominal septic complication

| | | Overall | No IASC | IASC | *p*-value | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|
| *n* | | 44 | 38 | 6 | | |
| Sex, *n* (%) | Female | 24 (54.5) | 22 (57.9) | 2 (33.3) | 0.387 | |
| | Male | 20 (45.5) | 16 (42.1) | 4 (66.7) | | |
| Race, *n* (%) | Asian | 1 (2.3) | 1 (2.6) | | 0.970 | |
| | Black | 6 (13.6) | 5 (13.2) | 1 (16.7) | | |
| | Unknown | 2 (4.6) | 2 (5.2) | | | |
| | White | 35 (79.5) | 30 (78.9) | 5 (83.3) | | |
| Ethnicity, *n* (%) | Non-Hispanic | 43 (97.7) | 37 (97.4) | 6 (100.0) | 1.000 | |
| | Unknown | 1 (2.3) | 1 (2.6) | | | |
| Smoking, *n* (%) | Non-smoker | 35 (79.5) | 32 (84.2) | 3 (50.0) | 0.089 | 2.9 (− 1.9–7.8) |
| | Smoker | 9 (20.5) | 6 (15.8) | 3 (50.0) | | |
| Pre-operative steroids, *n* (%) | 0 | 31 (70.5) | 26 (68.4) | 5 (83.3) | 0.652 | |
| | 1 | 13 (29.5) | 12 (31.6) | 1 (16.7) | | |
| Pre-operative anti-TNF, *n* (%) | 0 | 21 (47.7) | 18 (47.4) | 3 (50.0) | 1.000 | |
| | 1 | 23 (52.3) | 20 (52.6) | 3 (50.0) | | |
| Age, mean (SD) | | 29.0 (10.1) | 28.4 (9.8) | 32.3 (12.2) | 0.484 | |
| Myogenesis Pathway, mean (SD) | | 0.5 (0.3) | 0.6 (0.2) | 0.1 (0.0) | < 0.001 | − 17.6 (− 34.1–1.0) |

*IASC* intra-abdominal septic complication

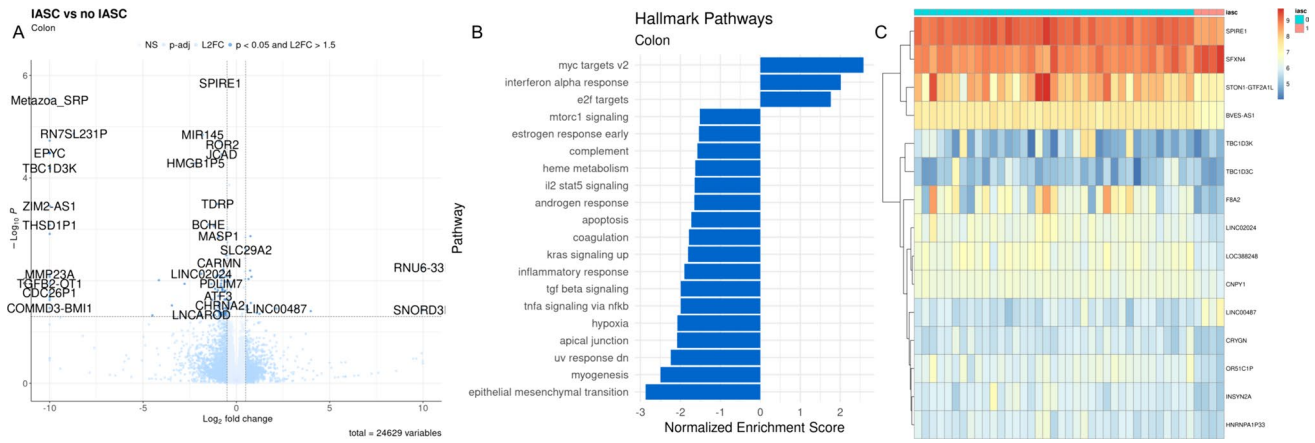


**Fig. 5** Differential expression analysis of intra-abdominal septic complications in colon tissue. **A** Volcano plot of differentially expressed genes, filtered by adjusted *p*-value and fold change. **B** Pathway enrichment analysis based on the hallmark gene sets. **C** Heatmap of gene expression based on top 15 differentially expressed genes (by adjusted *p*-value). *IASC* intra-abdominal septic complication

including clinical risk factors, downregulation of the myogenesis pathway in colon tissue was independently associated with CD recurrence and IASC.

Our results largely agree with previous studies of differential expression in post-operative CD recurrence. A 2019 study including 65 patients showed enrichment of the pathways toll-like receptor, NOD-like receptor, and TNF signaling [16]. A 2022 study including 149 patients identified upregulation of TNF-alpha, IFN-gamma, IL23A and IL17A, as well as activation of the JAK/STAT3 pathway. Our results confirm the importance of these genes and gene pathways in the underlying biological processes that drive CD recurrence. In particular, they externally validate upregulation of the JAK/STAT3 pathway and dysfunction in cellular metabolism. Importantly, our results for CD recurrence analyses are based on FFPE tissue and we show that data generated from these samples is highly correlated with data generated from fresh frozen tissue biopsied at the same time. The ability to use

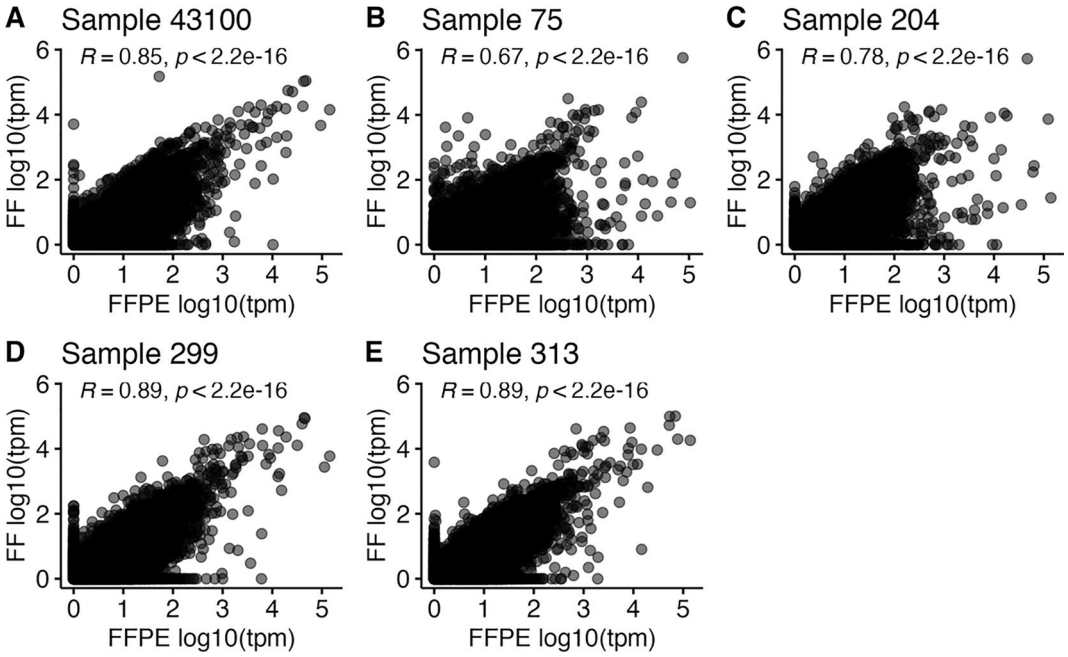


**Fig. 6** Correlation of gene expression for fresh frozen tissue and FFPE tissue. **A**–**E** Five patient colon samples were sequenced as both Fresh Frozen tissue (FF) and as Formalin-Fixed Paraffin-Embedded tissue (FFPE). The Pearson coefficient of correlation was calculated on log10 normalized TPM (transcript per million) gene expression profiles for each of the five patient samples

FFPE tissue will allow for creation of broader retrospective cohorts.

Our results also reveal novel pathways, including upregulation of the epithelial mesenchymal transition pathway, which has previously been associated with severe disease phenotypes in pediatric CD [36]. Compared with previous studies, our results may show a sharper difference in gene expression because we focused on i0 vs i3/i4 Rutgeert's scores for endoscopic recurrence, while previous studies included i1 and i2 in their analyses, which are less strongly correlated with clinical and surgical recurrence [4]. In addition, we use RUVSeq normalization to control for unwanted sources of variation, which is necessary for the analysis of heterogenous gene expression data [19]. Finally, our study performed analyses using both colon and ileum sample data.

Our results for IASC largely agreed with a previous transcriptomic analysis of colorectal anastomotic leak. The previous study showed that the genes AQP9, HCAR3, and MMP10, along with the gene pathways leukocyte activation, inflammatory response, angiogenesis, and DNA repair were downregulated in anastomotic leak [17]. While we also found that DNA repair and inflammatory/immune processes were downregulated in colon tissue, pathways regarding myogenesis were not specifically identified in the previous study. However, a key difference with our dataset is that it is more heavily weighted toward IBD (60% of patients), rather than colorectal cancer. It is possible that IASC in IBD is more driven by inflammation and dysregulation in wound healing rather than angiogenesis and cell division. In addition, the previous study also included a large number of patients with an implanted investigational device, with an unusually high rate of anastomotic leak (24%). Further studies, including a larger cohort of patients to capture more events, are needed to further explore the biological processes underlying anastomotic leak and intra-abdominal abscess.

Our results also raise interesting questions regarding biologic therapies. The importance of the JAK/STAT pathway in Crohn's disease recurrence suggests that kinase inhibitors, including tofacitinib and upadacitinib, may be particularly helpful. In addition, the association between pre-operative targeted therapy use and post-operative complications, especially SSI and anastomotic leak, is controversial [37–42]. However, the downregulation of pathways in IASC that are targeted by biologics and kinase inhibitors relevant to IBD, such as infliximab and adalimumab for TNF-alpha, and tofacitinib and upadacitinib for JAK/STAT, suggests that they may play a role in this outcome. Future studies focusing on differential expression in the setting of pre-operative targeted therapies and association with post-operative outcomes are needed to elucidate these effects.

Our study does have limitations. Most importantly, it uses a relatively small, single-institution dataset with a very limited number of patients with IASC. While our findings largely confirm the results of other studies, future studies will benefit from larger, multi-institutional datasets to ensure generalizability. Second, analysis of both inflamed and uninflamed tissue may provide additional insights.

Third, additional molecular data types, such as small RNA, chromatin biology, or serum markers would add to differential expression analyses. Of particular interest for future studies is the microbiome, which has previously been demonstrated to be associated with both CD recurrence and anastomotic leak [43, 44]. Multi-omic analyses including transcriptomic and microbial data are an exciting direction for future research. Fourth, though our correlation analysis of gene expression for five patients from whom we had both FFPE and FF tissue indicate a positive linear correlation of expression values across tissue preservation methods, two of the samples had Pearson correlation coefficients below $R = 0.8$. This indicates RNA degradation in a few individual FFPE samples may lead to imperfect correlation with FF tissue.

## Conclusion

This study identified differentially expressed genes and gene pathways, including upregulation of interferon alpha and gamma signaling, and downregulation of oxidative phosphorylation and myogenesis, associated with both post-operative infectious complications and endoscopic recurrence. Strong downregulation of myogenesis was seen in IASC and recurrence and remained an independent risk factor when controlling for clinical variables. These results suggest biological mechanisms underpinning the clinical association between post-operative complications and CD recurrence. Future studies, including external and experimental validation, focusing on the differentially expressed genes identified in this study may identify new biomarkers or targeted therapies which could reduce the risk of both complications and CD recurrence.

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**Data availability** Data associated with this study will be published in an online repository upon publication of this and other related manuscripts.

## Declarations

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the University of North Carolina Institutional Review Board (Study ID#: 15-0024, 17-0236).



## References

1. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet (London, England).* 2017;389:1741–1755.
2. Hancock L, Mortensen NJ. How often do IBD patients require resection of their intestine? *Inflamm Bowel Dis.* 2008;14:S68–S69.
3. Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? *Gut.* 2011;60:1178–1181.
4. Rivière P, Vermeire S, Irles-Depe M, Van Assche G, Rutgeerts P, Denost Q et al. Rates of postoperative recurrence of Crohn's disease and effects of immunosuppressive and biologic therapies. *Clin Gastroenterol Hepatol.* 2021;19:713-720.e1.
5. Regueiro M, Feagan BG, Zou B, Johanns J, Blank MA, Chevrier M et al. Infliximab reduces endoscopic, but not clinical, recurrence of Crohn's disease after ileocolonic resection. *Gastroenterology.* 2016;150:1568–1578.
6. Regueiro M. Management and prevention of postoperative Crohn's disease. *Inflamm Bowel Dis.* 2009;15:1583–1590.

7. de Buck van Overstraeten A, Eshuis EJ, Vermeire S, Van Assche G, Ferrante M, D'Haens GR et al. Short- and medium-term outcomes following primary ileocaecal resection for Crohn's disease in two specialist centres. *Br J Surg.* 2017;104:1713–1722.
8. Cleynen I, González JR, Figueroa C, Franke A, McGovern D, Bortlík M et al. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut.* 2013;62:1556–1565.
9. Michelassi F, Balestracci T, Chappell R, Block GE. Primary and recurrent Crohn's disease experience with 1379 patients. *Ann Surg.* 1991;214:230–240.
10. Scarpa M, Ruffolo C, Bertin E, Polese L, Filosa T, Prando D et al. Surgical predictors of recurrence of Crohn's disease after ileocolonic resection. *Int J Colorectal Dis.* 2007;22:1061–1069.
11. Welsch T, Hinz U, Löffler T, Muth G, Herfarth C, Schmidt J et al. Early re-laparotomy for post-operative complications is a significant risk factor for recurrence after ileocaecal resection for Crohn's disease. *Int J Colorectal Dis.* 2007;22:1043–1049.
12 Crowell KT. Risk factors and implications of anastomotic complications after surgery for Crohn's disease. *World J Gastrointest Surg.* 2015;7:237.
13. Bachour SP, Shah RS, Rieder F, Qazi T, Achkar JP, Philpott J et al. Intra-abdominal septic complications after ileocolic resection increases risk for endoscopic and surgical postoperative Crohn's disease recurrence. *J Crohns Colitis.* 2022;16:1696–1705.
14 Carvello M, D'Hoore A, Maroli A, Cuenca C, Vermeire S, Danese S et al. Postoperative complications are associated with early and increased rate of disease recurrence after surgery for Crohn's disease. *Dis Colon Rectum.* 2022. https://doi.org/10.1097/DCR.0000000000002446.
15. Ngollo M, Perez K, Hammoudi N, Gorelik Y, Delord M, Auzolle C et al. Identification of gene expression profiles associated with an increased risk of post-operative recurrence in Crohn's disease. *J Crohn's Colitis.* 2022;16:1269–1280.
16. Cushing KC, McLean R, McDonald KG, Gustafsson JK, Knoop KA, Kulkarni DH et al. Predicting risk of postoperative disease recurrence in Crohn's disease: Patients with indolent Crohn's disease have distinct whole transcriptome profiles at the time of first surgery. *Inflamm Bowel Dis.* 2019;25:180–193.
17. Van Praagh JB, De Wit JG, Olinga P, De Haan JJ, Nagengast WB, Fehrmann RSN et al. Colorectal anastomotic leak: transcriptomic profile analysis. *Br J Surg.* 2021;108:326–333.
18. Mullin G, Zager Y, Anteby R, Jacoby H, Kent I, Ram E et al. Inflammatory markers may predict post-operative complications and recurrence in Crohn's disease patients undergoing gastrointestinal surgery. *ANZ J Surg.* 2022;92:2538–2543.
19 Risso D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat Biotechnol.* 2014. https://doi.org/10.1038/nbt.2931.
20. Rutgeerts P, Geboes K, Vantrappen G, Beyls J, Kerremans R, Hiele M. Predictability of the postoperative course of Crohn's disease. *Gastroenterology.* 1990;99:956–963.
21. Chen KA, Nishiyama NC, Kennedy Ng MM, Shumway A, Joisa CU, Schaner MR et al. Linking gene expression to clinical outcomes in pediatric Crohn's disease using machine learning. *Sci Rep.* 2024;14:1–10.
22. Wang L, Nie J, Sicotte H, Li Y, Eckel-Passow JE, Dasari S et al. Measure transcript integrity using RNA-seq data. *BMC Bioinformatics.* 2016;17:1–16.
23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15:1–21.
24. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010;26:139–140.
25. Sergushichev AA. An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. *bioRxiv.* 2016. https://doi.org/10.1101/060012.
26. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. *Cell Syst.* 2015;1:417.
27. Blighe K, Rana S, Lewis M. EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. R package version 1.14.0. 2022. https://github.com/kevinblighe/EnhancedVolcano
28. Kolde R. Pheatmap: pretty heatmaps. 2019. 2012; 726.
29. Hänzelmann S, Castelo R, Guinney J. GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinform.* 2013;14:1–15.
30 Xie Z, Bailey A, Kuleshov MV, Clarke DJB, Evangelista JE, Jenkins SL et al. Gene set knowledge discovery with enrichr. *Curr Protoc.* 2021. https://doi.org/10.1002/CPZ1.90.
31. Kassambara A. ggpubr: "ggplot2" Based Publication Ready Plots. 2023. https://rpkgs.datanovia.com/ggpubr/.
32. 3.6.3 RDCT. A Language and Environment for Statistical Computing. R Found Stat Comput. 2020. https://www.R-project.org.
33. Kaddoura R, Ghelani H, Alqutami F, Altaher H, Hachim M, Jan RK. Identification of specific biomarkers and pathways in the treatment response of infliximab for inflammatory bowel disease: in-silico analysis. *Life.* 2023;13:680.
34 Zhou YJ, Zhao BL, Qian Z, Xu Y, Ding YQ. Association of glutathione S-transferase M1 null genotype with inflammatory bowel diseases: a systematic review and meta-analysis. *Medicine (United States).* 2019. https://doi.org/10.1097/MD.0000000000017722.
35. Jacobsen SB, Tfelt-Hansen J, Smerup MH, Andersen JD, Morling N. Comparison of whole transcriptome sequencing of fresh, frozen, and formalin-fixed, paraffin-embedded cardiac tissue. *PLoS ONE.* 2023. https://doi.org/10.1371/JOURNAL.PONE.0283159.
36. Chen KA, Nishiyama N, Ng MMK, Shumway A, Joisa CU, Schaner MR et al. Linking gene expression to clinical outcomes in pediatric Crohn's disease using machine learning. *bioRxiv.* 2022;39:S48.
37. Waterland P, Athanasiou T, Patel H. Post-operative abdominal complications in Crohn's disease in the biological era: Systematic review and meta-analysis. *World J Gastrointest Surg.* 2016;8:274.
38. Rosenfeld G, Qian H, Bressler B. The risks of post-operative complications following pre-operative infliximab therapy for Crohn's disease in patients undergoing abdominal surgery: a systematic review and meta-analysis. *J Crohn's Colitis.* 2013;7:868–877.
39. Nørgård BM, Nielsen J, Qvist N, Gradel KO, De Muckadell OBS, Kjeldsen J. Pre-operative use of anti-TNF-α agents and the risk of post-operative complications in patients with Crohn's disease—a nationwide cohort study. *Aliment Pharmacol Ther.* 2013;37:214–224.
40. Cohen BL, Fleshner P, Kane SV, Herfarth HH, Palekar N, Farraye FA et al. Prospective cohort study to investigate the safety of preoperative tumor necrosis factor inhibitor exposure in patients with inflammatory bowel disease undergoing intra-abdominal surgery. *Gastroenterology.* 2022;163:204–221.
41 Law CCY, Bell C, Koh D, Bao Y, Jairath V, Narula N. Risk of postoperative infectious complications from medical therapies in inflammatory bowel disease. *Cochrane Database Syst Rev.* 2020. https://doi.org/10.1002/14651858.CD013256.pub2.
42. Cira K, Weber M-C, Wilhelm D, Friess H, Reischl S, Neumann P-A. Clinical medicine. The effect of anti-tumor necrosis factor-alpha therapy within 12 weeks prior to surgery on postoperative

complications in inflammatory bowel disease: a systematic review and meta-analysis. *J Clin Med.* 2022;2022:6884.
43. Van Praagh JB, De Goffau MC, Bakker IS, Van Goor H, Harmsen HJM, Olinga P et al. Mucus microbiome of anastomotic tissue during surgery has predictive value for colorectal anastomotic leakage. *Ann Surg.* 2019;269:911–916.
44. Zhuang X, Tian Z, Li N, Mao R, Li X, Zhao M et al. Gut microbiota profiles and microbial-based therapies in post-operative Crohn's disease: a systematic review. *Front Med.* 2021;7:1081.