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Abnormal Small Intestinal Epithelial Microvilli in Patients With Crohn Disease

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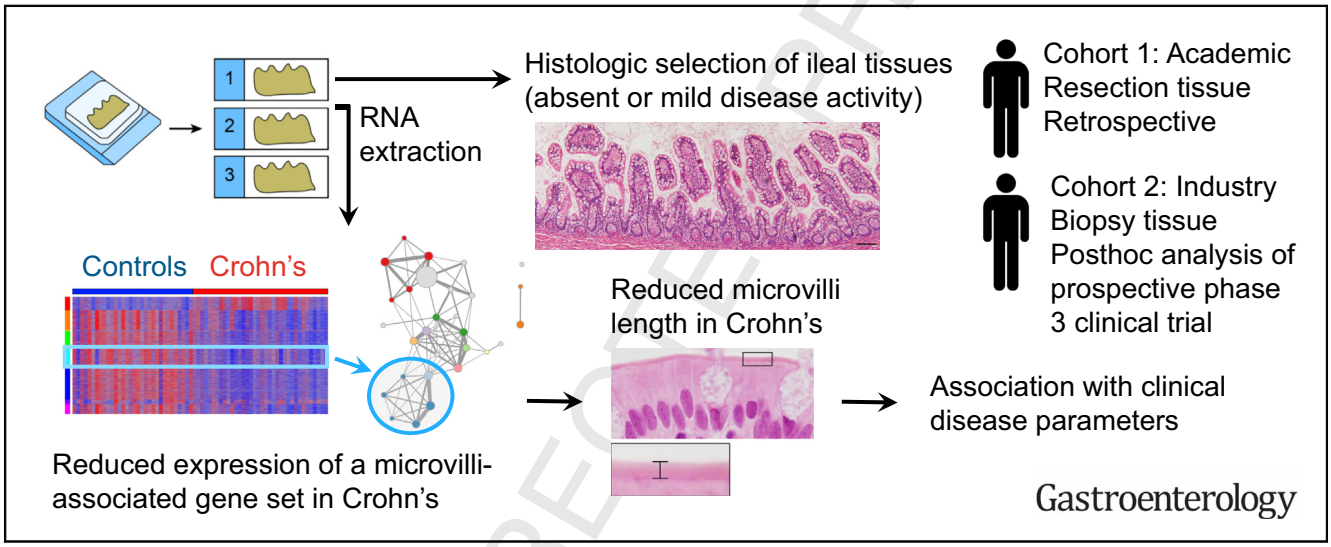

BACKGROUND & AIMS: Crohn disease (CD) presents as chronic and often progressive intestinal inflammation, but the contributing pathogenic mechanisms are unclear. We aimed to identify alterations in intestinal cells that could contribute to the chronic and progressive course of CD. **METHODS:** We took an unbiased system-wide approach by performing sequence analysis of RNA extracted from formalin-fixed paraffin-embedded ileal tissue sections from patients with CD (n = 36) and without CD (controls; n = 32). We selected relatively uninflamed samples, based on histology, before gene expression profiling; validation studies were performed using adjacent serial tissue sections. A separate set of samples (3 control and 4 CD samples) was analyzed by transmission electron microscopy. We developed methods to visualize an overlapping modular network of genes dysregulated in the CD samples. We validated our findings using biopsy samples (110 CD samples for gene expression analysis and 54 for histologic analysis) from the UNITI-2 phase 3 trial of ustekinumab for patients with CD and healthy individuals (26 samples used in gene expression analysis). **RESULTS:** We identified gene clusters that were altered in nearly all CD samples. One cluster encoded genes associated with the enterocyte brush border, leading us to investigate microvilli. In ileal tissues from patients with CD, the microvilli were of decreased length and had ultrastructural defects compared with tissues from controls. Microvilli length correlated with expression of genes that regulate microvilli structure and function. Network analysis linked the microvilli cluster to several other down-regulated clusters associated with altered intracellular trafficking and cellular metabolism. Enrichment of a core microvilli gene set also was lower in the UNITI-2 trial CD samples compared with controls; expression of microvilli genes was correlated with microvilli length and endoscopy score and was associated with response to treatment. **CONCLUSIONS:** In a transcriptome analysis of formalin-fixed and paraffin-embedded ileal tissues from patients with CD and controls, we associated transcriptional alterations with histologic alterations, such as differences in microvilli length. Decreased microvilli length and decreased expression of the microvilli gene set might contribute to epithelial malfunction and the chronic and progressive disease course in patients with CD.

Keywords: Inflammatory Bowel Diseases; Formalin-Fixed; Paraffin-Embedded; Next-Generation Sequencing; RNA-Seq.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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IMPACT

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Crohn disease (CD) is a form of inflammatory bowel disease (IBD) primarily characterized by clinical symptoms and pathologic signs, such as transmural influx of inflammatory cells.[1] Even with treatment, patients with CD experience periods of remission and periods of active inflammation. Factors driving the chronic and relapsing nature of CD can include dysbiosis of the intestinal microbiome, which is a persistent pathologic feature of the CD intestine,[2] but whether analogous persistent defects occur in host cells is less clear. We propose that uninflamed intestinal tissue regions will be a rich source for uncovering such defects that could contribute to the chronic nature of CD.

We adopted a molecular profiling approach to predict alterations in small intestinal host cells because this platform provides an unbiased system-wide view of disease-associated processes and has been successfully applied to personalized medicine approaches for other diseases.[3] However, the interpretation of transcriptional data from patients with complex inflammatory diseases is challenging, because the gene expression levels obtained from a heterogeneous sample are influenced by disease state and by proportional abundances of constituent cells and relative transcript abundances within these cells.[4] Immune cell infiltration and tissue damage or remodeling that can occur downstream of active inflammation influence bulk gene expression data. This confounding effect has been clearly demonstrated in molecular profiling studies of several complex inflammatory diseases, including scleroderma,[5] rheumatoid arthritis,[6] and CD.[7–9] To overcome this challenge, we adopted a molecular profiling approach that allows researchers to match intact tissue morphology to gene expression data.

Formalin-fixed paraffin-embedded (FFPE) tissue is the standard preservation method used for routine surgical pathology. Recent technical developments have enabled extraction of DNA, RNA, and protein from FFPE tissues,[10] thereby providing an abundant tissue source for genomic analyses of clinical samples. Several studies have compared transcriptional profiles generated from freshly frozen and FFPE samples from the same individual and found that the 2 preservation methods produce concordant data, despite the higher degree of RNA degradation in the FFPE samples.[11–14] A key advantage of FFPE molecular profiling for complex inflammatory disease samples is that it allows for definitive selection using histologic criteria of the exact samples to be profiled. Traditionally, selection of relatively uninflamed intestinal tissues for CD molecular profiling studies relies on gross endoscopic evaluation with or without subsequent histologic assessment of a tissue site near to the one used for RNA isolation. Furthermore, although the entire tissue sample is lysed during traditional RNA extraction methods, only a small portion of the FFPE tissue sample is used for RNA extraction. Thus, serial FFPE tissue sections are available to correlate gene expression findings with histologic features predicted to be altered.

In this study, we generated RNA-seq transcriptional profiles using standard archival FFPE pathology tissue specimens from patients with CD and those without IBD (non-IBD; control) who underwent ileocolic resection surgery. We chose resection samples because these contain whole-thickness tissue, including the muscularis propria, and thus all intestinal host cells could be examined (in contrast to biopsy samples). Histologic assessment before molecular profiling was performed to select samples only from tissue regions uninvolved in active inflammatory disease based on current clinical guidelines used by pathologists. We surmised that this prescreening process would minimize molecular signatures driven by altered proportional abundances of cell subsets and potentially unmask those driven by underlying defects in host cells, especially if these consisted of small-magnitude gene expression changes. Such defects might contribute to the chronic and progressive nature of CD. We identified several common gene signatures altered in CD intestine compared with controls, one of which led us to identify previously unrecognized morphometric features of CD, namely shorter length and altered ultrastructure of epithelial microvilli. We validated the decreased enrichment of this gene signature and its correlation with microvilli length in an independent cohort of patients with CD and biopsy samples. Together, these data supported the hypothesis that there are persistent alterations of host cells in relatively uninflamed CD intestinal tissue.

Abbreviations used in this paper: **CD, Crohn disease; CDAI, Clinical Disease Activity Index; FFPE, formalin-fixed, paraffin-embedded; H&E, hematoxylin and eosin; I-Wk0, induction baseline; I-Wk8, induction week 8; IBD, inflammatory bowel disease; M-Wk44, maintenance week 44; non-IBD, control subjects without inflammatory bowel disease; SES-CD, Simple Endoscopic Score for Crohn's Disease; TEM, transmission electron microscopy; UST, ustekinumab; VIL1, villin 1.**

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