Title: Comparative analysis of inflamed and non-inflamed colon biopsies reveals strong proteomic inflammation profile in patients with ulcerative colitis

Version: 1 Date: 28 March 2012

Reviewer: Ryan Stidham

Reviewer's report:

Purpose and significance: This study retrospectively evaluates variation in mucosal & submucosal proteomic profiles of patients with ulcerative proctitis, with the intentions of identifying novel biomarkers of disease activity and elucidating new mechanisms and pathways driving inflammation in inflammatory bowel disease. There is a need for non-invasive surrogates of mucosal inflammation and disease activity in ulcerative colitis. I agree that fecal calprotectin, lactoferrin, C-reactive protein, and imaging do not necessarily reflect inflammation specific to ulcerative colitis.

Methods: The authors perform paired analysis of endoscopically inflamed and normal mucosa in those with UC, controlling for individual variation. Sample size is appropriate given bioinformatics necessary for proteomics. I do have reservations about the variation in the UC cohort, specifically that 10/20 were using 5-ASA, 4 were untreated, and two were using renin-angiotensin inhibitors. Further, the authors state (68-70) that inflammatory biomarkers and pathways of interest need to be specific to ulcerative colitis and not other causes of colitis (infectious, neoplastic, medication-induced). An additional comparison group comprised of non-IBD related inflammation (C. diff colitis mucosal biopsies) should be included to clarify pathways specific to ulcerative colitis. Ulcerative proctitis variation in severity impacts histology, cellularity, protein expression, and likely post-translational protein modification. Histology results, namely grade of inflammation is not reported and should be to characterize patient characteristics. Ideally patients would have a consistent grade of inflammation, both endoscopically and histologically, for more uniform results, especially since fold change of protein expression is reported. To demonstrate whether normal control and endoscopically normal UC mucosa are comparable, I would include a direct comparison of proteomic profiles of control left colon and non-inflamed UC left colon mucosal biopsies. Further, the variation in control group protein expression demonstrates the lack of a standard protein profile of colonic mucosal, at least in this study population. Sound statistical methods used do identify 33 unique proteins with at least a two fold expression change between inflamed and non-inflamed mucosa in ulcerative proctitis subjects. These results do highlight metabolic pathways involved in ulcerative colitis, however nothing specific can be concluded. Characterizing the consistent elements of mucosal protein profiles serves an important role for future proteomic investigations in UC, however this study is very preliminary and few conclusions can be drawn.
This study reports unique mucosal proteins differentially expressed in inflamed mucosa compared to normal mucosal in patients with ulcerative proctitis. There is value within the field of suggesting novel metabolic pathways potentially driving inflammation in ulcerative colitis, especially as proteomics becomes more accessible. The results from this study, remain far from having clinical impact. The variance reported, even in glycerol-3-phosphate-dehydrogenase, is small. In addition, it is unclear if any of these markers can be used as a biomarker, as there were no comparisons to stool or serum detection of high-variance compounds by ELISA or other high-throughput assays. A biomarker that non-invasively better correlates to histology or endoscopic appearance of mucosa or predicts response to specific therapies, would be clinically useful.

To strengthen this manuscript I would recommend:

Major Revision
1. Clearly state in the abstract and background that the purpose of this work is to describe the proteomic profile in ulcerative colitis, not the express discovery of new biomarkers.
2. Narrow the UC cohort to identical medical therapy (all 5-ASA, all treatment naïve, etc) including non-IBD medications and reanalyze the data. This should be possible given the small number of patients necessary to enroll.
3. Add a non-IBD inflammation control group (i.e. C diff colitis distal colon biopsies) for comparison and clarification of proteins unique to IBD related inflammation.

Minor Revision
4. Please provide histology inflammation grading for UC cohort.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.