Transcriptional changes between uninfamed ulcerative colitis and familial adenomatous polyposis pouch mucosa can be attributed to an altered immune response

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A total proctocolectomy with ileal pouch-anal anastomosis (IPAA) is considered the surgery of choice for definitive management of familial adenomatous polyposis (FAP) and some patients with ulcerative colitis (UC). However, this surgical treatment is often associated with pouchitis, a long-term complication that occurs mostly in UC patients. The purpose of this study was to better define the molecular background of pouchitis. A microarray-based survey was performed using pouch mucosal samples collected from 28 and 8 patients undergoing surgery for UC and FAP, respectively. There were 4,770 genes that significantly differentiated uninfamed from inflamed mucosal samples, and their functional features were represented mostly by metabolic and cell proliferation pathways. In contrast, functional analyses of aberrantly expressed genes between UC and FAP samples, irrespective of mucosal inflammation status, revealed multiple pathways and terms that were linked to changes in immune response. Interestingly, the comparison of uninfamed UC and FAP samples identified a set of 29 altered probe sets, including an inflammation-related transcript encoding a Charcot-Leyden crystal (CLC) protein. The most distinct changes in gene expression profiles differentiating uninfamed UC and FAP pouch mucosal samples were attributed to the Gene Ontology category innate immune response. Our study confirmed that alterations in immune responses can be found between patients who underwent surgery for UC and FAP, independent of the pouch inflammation status. This observation may be important when managing IPAA patients. Key words: ulcerative colitis; familial adenomatous polyposis; pouch, gene expression; immune response

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INTRODUCTION

All patients with familial adenomatous polyposis (FAP) and up to 30% of ulcerative colitis (UC) patients with refractory disease or colitis-associated dysplasia require a total colectomy (Mahadevan & Sandborn, 2003; Cosnes et al., 2011; Li & Shen, 2012). Although the restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) decreases the risk of carcinogenesis and improves quality of life by preserving intestinal continuity, side effects occur frequently (Li & Shen, 2012). Pouchitis is the most common long-term complication of IPAA and develops after exposure of the pouch mucosa to fecal stasis (Mahadevan & Sandborn, 2003; Gionchetti et al., 2004; Oghe et al., 2005; Yamamoto et al., 2005; Li & Shen, 2012). The etiology of pouchitis is considered multifactorial.

FAP and UC are two disorders with very different pathogeneses. FAP is a hereditary syndrome, which predisposes towards multiple colonic adenomas that exhibit a 100% risk of malignancy (Stec et al., 2010). UC is an immune-mediated disorder of the large intestine that develops in genetically predisposed individuals (Baumgart & Carding, 2007). Since pouchitis affects up to 50% of patients with UC and only 5% of patients with FAP (Leal et al., 2010a), it is commonly speculated that pouchitis occurring in UC patients may reflect a recurrence of the UC-related inflammatory response to the ileal mucosa.

UC results from abnormal activation of both innate and adaptive immune responses to intestinal flora (Bouguen et al., 2011). When the balance between pro- and anti-inflammatory cytokines and the suppressors of cytokine signaling proteins is impaired, it can mediate interactions between activated immune cells and epithelial and mesenchymal cells that lead to an abnormal inflammatory response (Bouguen et al., 2011). The development of pouchitis is also associated with higher levels of TNF-α, IL-1β, IL-6, and IL-8 and decreased levels of IL-10 (reviewed in Leal et al., 2010b) in UC patients, but not FAP patients. Moreover, even in normal pouch mucosa, significant differences were found in the levels of inflammation activity markers between UC and FAP patients (Leal et al., 2010b; de Paiva et al., 2011). Thus, although the molecular mechanisms underlying both UC and UC-related pouchitis are only partly defined, it is reasonable that pouchitis is a recurrence of UC in the pouch mucosa.

Abbreviations: IPAA, ileal pouch-anal anastomosis; FAP, familial adenomatous polyposis; UC, ulcerative colitis; PDAI, the pouchitis disease activity index; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, quantitative Reverse-Transcriptase-PCR; FDR, false discovery rate; FC, fold change; K-S, Kolmogorov-Smirnov
Microarray-based studies can identify genes that are differentially expressed between predefined groups of samples; gene subsets with differential expression across predefined classes of genes; and gene expression profiles that determine gene expression signatures among seemingly homogenous phenotypes (Ostrowski & Wywriecz, 2009). Gene expression profiling does not require prior knowledge of the expression profile or a pre-existing hypothesis about the differential gene expression typical for a given biological phenomenon. The results of large-scale measurements can be further analyzed by term enrichment approaches, which may indicate the altered signaling pathways in large-scale cellular networks.

Recently published studies, evaluating molecular profiles in pouch mucosa, have reported that genes significantly associated with UC outcome are involved in regulation of the immune system, modification of the extracellular matrix, and xenobiotic activity (Kabakchev et al., 2014), and gene alterations in pouch inflammation overlap with alterations within proximal small bowel (Yanai et al., 2014) and Crohn’s disease (Ben-Shachar et al., 2013).

In this study, a microarray-based survey was performed to better define the molecular background of UC-related pouchitis and to identify differences in the pouch mucosal defense pathways between UC and FAP.

MATERIALS AND METHODS

**Patients.** Thirty six patients (27 men) with IPAA were enrolled in the study. The patients were recruited at the Outpatient Clinic of the Department of General and Colorectal Surgery during the periodic health evaluation. Among them, 28 patients (median age, 41; range, 27–66 years) underwent surgery for UC and 8 patients (median age, 25; range 19–51 years) for FAP. The median follow-up after the surgery was 24 months; range, 9–142 months, and all the patients had had their ileostomy closed at the time of the study. The Pouchitis Disease Activity Index (PDAI) was used to diagnose or exclude pouchitis (Sandborn et al., 1994; Shen et al., 2002). For each patient, six biopsies were taken from the same areas of the lower part of the pouch mucosa (above the rectal cuff) during endoscopic examination. The reservoir design was the ‘J’ type. Three biopsy specimens taken for molecular testing were immediately snap-frozen and stored at −72°C until use. The other three biopsy specimens were fixed in 10% formalin for histological examination, and paraffin sections (4 µm thick) were routinely stained with hematoxylin and eosin.

The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Medical Sciences Bioethics Committee at Poznan University and all patients signed informed consent before inclusion.

**mRNA extraction.** Total RNA was isolated from mucosal biopsies using the RNeasy Plus Mini Kit (Qiagen, Germany) followed by on-column DNase I digestion. The quality of RNA samples was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA); all samples used for microarray analysis displayed distinct peaks corresponding to intact 28S and 18S ribosomal RNA.

**Quantitative(q) RT-PCR measurements.** QRT-PCR was performed as described before (Mikula et al., 2011) using Sybr Green chemistry. Geometric mean expression of Ubiquitin C — UBC and ribosomal protein P0 — RPLP0 was used as a normalization factor as a result of evaluation of three housekeeping genes (ACTB - actin beta, UBC and RPLP0) with the GeNorm software (Vandesompele et al., 2002). Gene expression results were calculated using ΔΔ Ct method (Livak & Schmittgen, 2001). Primers for the analyzed gene transcripts were taken from PrimerBank (Wäng et al., 2011). The results were analyzed with Mann-Whitney U test using GraphPad Prism 5 software (GraphPad Software, Inc.). P values of less than 0.05 were considered as significant.

**Gene expression microarray analysis.** The average bead signals from the Human HT-12 v4 Expression BeadChip kit (AROS Applied Biotechnology, Aarhus N, Denmark) were quantile normalized with no background correction. All computations were done using R 2.15.0 software with the Bioconductor extension (Genteman et al., 2004). Measurements were filtered according to the relation of interquartile range (IQR) to median. Only probes with IQR/median >1/8 were selected for analysis and 24050 probes out of 47231 passed filtering. Genes with differential expression were selected according to the p-value from the t-test (Welch variant) after correction for multiple hypothesis testing with the Benjamini-Hochberg algorithm. Adjusted p-values <0.05 were considered significant. Hierarchical clustering was performed using a complete method with Euclidian distance as the dissimilarity measure.

Illumina identifiers were mapped to genes and Kyoto Encyclopedia of Genes and Genomes (KEGG) identifiers using the lumiHumanAll.db (1.16.0), KEGG.db (2.6.1), and lumi (2.6.0) (Du et al., 2008) packages. The significance of expression changes related to each KEGG pathway was assessed with the Kolmogorov-Smirnov (K-S) test using probe lists sorted by the appropriate p-value as input. Mapping to Gene Ontology (GO) (Ashburner et al., 2000) terms was performed with the GO.db (2.6.1) package (Lin et al., 2008). The significance of expression changes related to the GO terms (biological function branch) was assessed with the hypergeometric test. Results from the K-S and hypergeometric tests were corrected for multiple hypothesis testing with the Benjamini-Hochberg algorithm.

Microarray data were deposited in Gene Expression Omnibus under GSE50788.

RESULTS

**Signaling pathways that differed between uninflamed and inflamed pouch mucosa.**

At the time of the study, uninflamed pouch mucosa was found in 15 and 5 patients with IPAA who underwent surgery for UC and FAP, respectively, by both endoscopic and histopathologic evaluation (the PDAI histology score = 0), and pouchitis was diagnosed in 13 UC and 3 FAP patients. In the latter patients mild to moderately active pouchitis was diagnosed clinically (increased stool frequency, feal urgency or abdominal cramp, fever, rectal bleeding), endoscopically (24 findings of granularity, edema, friability, loss of vascular pattern, mucosal hemorrhage, or ulceration), and histologically (varying degrees of changes in mucosal architecture, infiltration by acute and chronic inflammatory cells, ulceration, and crypt abscesses; mean PDAI histology score was 2.62 ± 1.31) (Shen et al., 2002). On endoscopy, the neoterminal ileum above the pouch was normal in all patients.

To compare gene expression profiles corresponding to tissue morphology, microarray data sets were analyzed using pair-wise comparisons between 20 uninflamed and
16 inflamed pouch mucosal samples from both UC and FAP patients. A total of 24050 probe sets passed our filtration criterion (IQR/median >1/8). Of these, 7152 probes (4770 genes) significantly differentiated \( (p<0.05, \text{FDR adjusted}) \) uninflamed from inflamed samples and 584 (437 genes) had expression fold changes (FC) greater than 2 (Table S1 at www.actabp.pl). The unsupervised hierarchical clustering grouped mucosa samples according to their inflammation status; although, the distinction between two branches was not absolutely concordant with histological evaluation. As shown in Fig. 1, the inflamed mucosa branch represented 10 out of 13 UC samples and one out of three FAP samples.

Differentially regulated transcripts in comparison of uninflamed and inflamed samples were further evaluated using qRT-PCR. Eight most discriminative genes were chosen for evaluation: four upregulated in inflamed tissue (carcinoembryonic antigen-related cell adhesion molecule 7 — CEACAM7; V-set and immunoglobulin domain containing 2 — VSIG2; carbonic anhydrase 2 — CA2; membrane-spanning 4-domains, subfamily A, member 12 — MS4A12) and four downregulated (cubilin — CUBN; sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 — SULT2A1; flavin containing monoxygenase 1 — FMO1; pancreatic lipase-related protein 2 — PNLIPRP2) (Table S1 at www.actabp.pl). QPCR measurements confirmed differences in mRNA abundances indicated on microarrays for all selected genes (Fig. 2).

While most of the pathways were associated with metabolic processes, the notable exceptions included cell cycle, DNA replication, and RNA transport pathways and the B cell receptor signaling pathway, the single KEGG pathway related to immunological processes. Genes involved in metabolism pathways were usually down-regulated in inflamed specimens, while genes from DNA replication and RNA transport were up-regulated. The probe sets that were differentially expressed between uninflamed and inflamed mucosa samples (with corrected \( p \)-values <0.05) were then used for the calculation of their attribution to GO terms. GO analyses uncovered 38 significant terms (Table S3 at www.actabp.pl) that confirmed the findings based on KEGG.

Signaling pathways that distinguish between UC and FAP-related pouch mucosa

Next, similar comparative analyses were performed on microarray data of the mucosal samples, representing the two groups of disorders, UC and FAP, independent of inflammation status. This pair-wise comparison re-

![Figure 1. Hierarchical clustering of 36 samples based on their gene expression.](image1)

Hierarchical cluster analysis of the whole dataset shows that inflamed samples cluster together. FAP, familial adenomatous polyposis; UC, ulcerative colitis.

![Figure 2. QRT-PCR validation of genes with highest magnitude of changes between uninflamed and inflamed samples in microarray survey.](image2)

Relative levels for carcinoembryonic antigen-related cell adhesion molecule 7 — CEACAM7; V-set and immunoglobulin domain containing 2 — VSIG2; carbonic anhydrase 2 — CA2; membrane-spanning 4-domains, subfamily A, member 12 — MS4A12; cubilin — CUBN; sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 — SULT2A1; flavin containing monoxygenase 1 — FMO1 and pancreatic lipase-related protein 2 — PNLIPRP2 were measured in RNA extracted from uninflamed (n=20) and inflamed (n=16) biopsies. Geometric mean expression of UBC and RPLP0 was used for normalization between samples. The box border represents the interquartile range, and the horizontal line in the box is the median. The whiskers denote the largest/smallest observation. The logarithmic scale was used to accommodate the magnitude of expression changes. Statistical significances of differences were assessed by the Mann–Whitney test and \( P \) values <0.05 were considered as significant (*)

*bilin — CUBN; sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1 — SULT2A1; flavin containing monoxygenase 1 — FMO1; pancreatic lipase-related protein 2 — PNLIPRP2 (Table S2 at www.actabp.pl).
A. Paziewska and others revealed only 48 probe sets (40 genes) as significantly different \( (p < 0.05, \text{FDR adjusted}) \) between the two groups of samples (Table S4 at www.actabp.pl). To validate the expression of the most altered genes in UC and FAP comparison the qRT-PCR was performed to ascertain their discriminative status. We selected two upregulated genes differentiating UC and FAP samples in microarray survey.

**Table 1.** List of genes differentially expressed (adjusted \( p \)-value ≤0.05) between uninflamed UC and FAP samples. In bold are the genes expressed with a fold change >1.5.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Symbol</th>
<th>Adj. ( p )-value</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>lysosomal-associated membrane protein 2</td>
<td>LAMP2</td>
<td>0.032</td>
<td>0.83</td>
</tr>
<tr>
<td>BRCA2 and CDKN1A interacting protein</td>
<td>BCCIP</td>
<td>0.032</td>
<td>0.80</td>
</tr>
<tr>
<td>Charcot-Leyden crystal protein</td>
<td>CLC</td>
<td>0.032</td>
<td>1.54</td>
</tr>
<tr>
<td>VAMP (vesicle-associated membrane protein A, 33kDa)</td>
<td>VAPA</td>
<td>0.032</td>
<td>0.80</td>
</tr>
<tr>
<td>dual specificity phosphatase 2</td>
<td>DUSP2</td>
<td>0.032</td>
<td>1.30</td>
</tr>
<tr>
<td>metallo-beta-lactamase domain containing 2</td>
<td>MBLAC2</td>
<td>0.032</td>
<td>0.86</td>
</tr>
<tr>
<td>transcriptional adaptor 3</td>
<td>TADA3</td>
<td>0.032</td>
<td>1.33</td>
</tr>
<tr>
<td>ubiquitin-conjugating enzyme E2 variant 1</td>
<td>UBE2V1</td>
<td>0.032</td>
<td>1.36</td>
</tr>
<tr>
<td>heat shock protein 90kDa alpha (cytosolic), class A member 1</td>
<td>HSP90AA1</td>
<td>0.032</td>
<td>0.78</td>
</tr>
<tr>
<td>family with sequence similarity 110, member A</td>
<td>FAM110A</td>
<td>0.039</td>
<td>1.41</td>
</tr>
<tr>
<td>inositol 1,4,5-trisphosphate receptor interacting protein-like 2</td>
<td>ITPRIPL2</td>
<td>0.039</td>
<td>1.97</td>
</tr>
<tr>
<td>p21 protein (Cdc42/Rac)-activated kinase 7</td>
<td>PAK7</td>
<td>0.039</td>
<td>0.85</td>
</tr>
<tr>
<td>acidic (leucine-rich) nuclear phosphoprotein 32 family, member C</td>
<td>ANP32C</td>
<td>0.039</td>
<td>0.77</td>
</tr>
<tr>
<td>inhibitor of growth family, member 3</td>
<td>ING3</td>
<td>0.039</td>
<td>1.35</td>
</tr>
<tr>
<td>proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional peptidase 7)</td>
<td>PSMB8</td>
<td>0.039</td>
<td>1.35</td>
</tr>
<tr>
<td>nuclear transcription factor, X-box binding 1</td>
<td>NFX1</td>
<td>0.039</td>
<td>1.29</td>
</tr>
<tr>
<td>microtubule associated serine/threonine kinase 3</td>
<td>MAST3</td>
<td>0.043</td>
<td>1.36</td>
</tr>
<tr>
<td>ring finger protein 38</td>
<td>RNF38</td>
<td>0.045</td>
<td>0.82</td>
</tr>
<tr>
<td>component of oligomeric golgi complex 5</td>
<td>COGS</td>
<td>0.045</td>
<td>1.72</td>
</tr>
<tr>
<td>ubiquitin protein ligase E3C</td>
<td>UBE3C</td>
<td>0.045</td>
<td>0.85</td>
</tr>
<tr>
<td>sperm antigen with calponin homology and coiled-coil domains 1</td>
<td>SPECC1</td>
<td>0.045</td>
<td>1.40</td>
</tr>
<tr>
<td>leucine rich repeat containing 58</td>
<td>LRRCS8</td>
<td>0.045</td>
<td>0.66</td>
</tr>
<tr>
<td>NIEAP endocytosis associated 2</td>
<td>NECAP2</td>
<td>0.047</td>
<td>1.27</td>
</tr>
<tr>
<td>dimethylarginine dimethylaminohydrolase 1</td>
<td>DDAH1</td>
<td>0.047</td>
<td>0.72</td>
</tr>
<tr>
<td>Der1-like domain family, member 3</td>
<td>DERL3</td>
<td>0.047</td>
<td>1.22</td>
</tr>
</tbody>
</table>

**Figure 3.** QRT-PCR validation of genes differentiating UC and FAP samples in microarray survey. Relative levels for interferon induced transmembrane protein 2 — IFITM2, butyrophilin, subfamily 3, member A2 — BTN3A2, dimethylarginine dimethylaminohydrolase 1 — DDAH1 and sorting nexin 4 — SNX4 were measured in RNA extracted from UC \( (n=28) \) and FAP \( (n=8) \) biopsies. Gene expression calculation was performed as in Figure 2. The box border represents the interquartile range, and the horizontal line in the box is the median. The whiskers denote the largest/smallest observation. Statistical significances of differences were assessed by the Mann–Whitney test and \( P \) values <0.05 were considered as significant (*).
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...and two downregulated (dimethylarginine dimethylaminohydrolase 1 — DDAH1 and sorting nexin 4 — SNX4) genes (Table S4 at www.actabp.pl). QPCR survey confirmed significantly changed mRNA abundance of IFITM2, DDAH1 and SNX4 gene (Fig. 3).

While the K-S test used for the annotation of gene expression measurements to pre-defined KEGG signaling pathways showed their attribution to 22 pathways (Table S5 at www.actabp.pl), a small number of differentiating genes below the applied significance threshold made it impossible to select GO terms using the hypergeometric test. The disparity between KEGG and GO functional analyses stems from different statistical approaches; the latter one requires a set of differentiating genes fulfilling certain statistical conditions.

To further explore if GO terms could portray UC vs. FAP functional changes in pouch mucosa, similarly to KEGG, we used the over-representation method for testing the top five percent of probe sets, sorted according to their p-value, as the input for GO analyses. This approach identified 31 GO terms that were in close agreement with the KEGG findings. The annotation results showed mostly immunologically related KEGG pathways, including those associated with B-cell, T-cell, and toll-like (TLR) receptors (Table S5 at www.actabp.pl) and GO categories associated with immune response (Table S6 at www.actabp.pl).

**Signaling pathways that distinguish normal pouch mucosa between UC and FAP patients**

Pair wise comparison of uninflamed UC and FAP samples identified that there was altered expression of 29 probe sets (p<0.05, FDR adjusted) belonging to 25 genes (Table 1). The expression status of three selected genes (inositol 1,4,5-trisphosphate receptor interacting protein 2 — ITPRIPL2, component of oligomeric golgi complex 5 (COG5) and Charcot-Leyden crystal protein (CLC) were measured in RNA extracted from uninflamed UC (n=15) and FAP (n=5) biopsies. Gene expression calculation was performed as in Fig. 2. The box border represents the interquartile range, and the horizontal line in the box is the median. The whiskers denote the largest/smallest observation. Statistical significances of differences were assessed by the Mann–Whitney test and P values <0.05 were considered as significant (*).
protein-like 2 — ITPRIPL2; component of oligomeric golgi complex 5 — COG5 and Charcot-Leyden crystal protein — CLC), that demonstrated expression greater than 1.5-fold, was independently validated by qRT-PCR. While the direction of expression changes of two genes, COG5 and CLC, was consistent with microarray measurement, only the expression of CLC mRNA was significantly changed by 3.1-fold between uninflamed UC and FAP samples (Fig. 4).

Under standard testing conditions, no functional processes or terms could be selected using KEGG or GO analyses, respectively. However, when using the top five percent of probe sets (according to their p-value) differentiating uninflamed mucosa from UC and uninflamed mucosa from FAP patients, the GO analysis indicated an inflammation related term: innate immune response (GO:0045087) (Table 2).

**DISCUSSION**

The frequency of pouchitis is much lower in patients surgically treated for FAP than for UC, suggesting that inherent molecular properties of ileal mucosa have a critical role in its pathogenesis. If so, determination of the molecular makeup of FAP and UC pouches may provide clues to understanding the mechanisms underlying the development of pouchitis. The frequencies of pouchitis in FAP and UC suggested there are pathophysiological similarities related to immune response in UC and pouchitis after surgery. This hypothesis was previously tested in a few published studies (Leal et al., 2010; de Paiva et al., 2011) that aimed to connect molecular changes within the UC pouch with inflammation. It was determined that the signal transducer IFN-γ and an activator of cytokine expression STAT1 were increased in the normal pouch mucosa of UC patients (Leal et al., 2010). Furthermore, UC exhibited significantly higher levels of TLR4 protein than FAP patients; TLR4 is a receptor involved in the recognition of extracellular cues from bacteria and activation of the innate immune system (de Paiva et al., 2011). Although these studies were performed on singular elements involved in immune response, the results led to the same conclusion that inflammatory-like properties of the UC pouch could explain the more frequent onset of pouchitis.

Most disorders result from the dysfunction of signaling pathways in large-scale cellular networks. Therefore, analyses of disrupted cellular components and the related aberrant signaling may be the key factors in studies on the molecular basis of diseases. A comparison of the gene expression profiles corresponding to the inflamed mucosal morphology showed numerous genes with altered expression, which met the FDR threshold criteria for being considered differentially expressed (Table S1 at www.actabp.pl). Probe sets sorted according to significant differences between uninflamed and inflamed pouch mucosal samples uncovered several GO categories related to very similar metabolic dysfunctions based on KEGG annotations (Table S2 and S3 at www.actabp.pl). Surprisingly, the vast majority of the altered pathways were not related to immune response. Further KEGG and GO analyses of probe sets with expression differences between UC and FAP samples, irrespective of inflammation status, revealed multiple pathways (Table S5 at www.actabp.pl) and terms (Table S6 at www.actabp.pl), respectively, linked to immune response. When comparing uninflamed UC and FAP samples, we identified 25 genes with differential expression (Table 1). However, only 3 of these genes demonstrated expression greater than 1.5-fold, indicating that the established set of differentially expressed transcripts reflect modest changes. Further validation with qRT-PCR confirmed altered expression of mRNA encoding CLC gene (Fig. 4). The CLC protein has lysophospholipase activity and is expressed in eosinophils and basophils. Its level has been shown to correlate with eosinophil density and the degree of inflammation in celiac disease (De Re et al., 2009). Furthermore, its mRNA abundance was 10-times higher in early versus late-onset colorectal tumors (Ågesen et al., 2011), where early tumors displayed higher overall expression of immune-relevant genes. Of note, the most distinct changes in gene expression profiles differentiating uninflamed UC and FAP mucosa samples could be attributed to the GO category innate immune response (Table 2), but not the KEGG pathway, when using the top five percent of differentiating probe sets. Therefore, by using the whole transcriptome survey, for the first time, we provide indirect evidence that molecular mechanisms underlying inflammation of ileal pouch mucosa in UC patients can mimic those associated with the development of immune-mediated inflammation in the large intestine. Interestingly, previous work comparing the whole transcriptomes of biopsies obtained from UC patients with and without active large bowel inflammation also provided evidence for the molecular signature of the “pre-inflammatory state” in histologically uninflamed UC colonic specimens (Olsen et al., 2009).

While the microarray technology allows surveying changes in gene expression in genome scale, the results are often required to be verified using qPCR, specifically when potential biomarkers of disease need to be validated. QPCR technique is considered as a gold standard in gene expression measurements, however the concordance of qPCR and microarray results may be significantly influenced by the variability of technical procedures inherent to each method. This problem has already been explored and discussed in the literature (Morey et al., 2006). Among the other factors, a small degree of gene expression change, typically of less than 2-fold, has been determined as the feature lowering correlations between microarrays and qPCR (Etienne et al., 2004; Morey et al., 2006). This could be reflected by the reproducibility of microarrays and qRT-PCR results for a set of alternative genes in two comparisons performed on pouch mucosas: uninflamed vs. inflamed (Fig. 2) and CU vs. FAP (Fig. 3), where the magnitude of expression changes and the number of differentiating genes for each set was substantially different. We were able to confirm the expression concordance of all genes selected for uninflamed vs. inflamed samples comparison (Fig. 2). At the same time for UC and FAP comparison, where the most discriminative gene (IFITM2) had the expression FC of 2, we confirmed altered expression of three out of four genes tested (Fig. 3). Consequently, poor reproduction of microarrays by qRT-PCR observed for comparison of normal pouch mucosa between UC and FAP patients could be attributed to both the modest changes in expression of the assessed genes (Table 1 and Fig. 4) and the different methodology behind these two techniques.

In summary, the full discovery and understanding of biological processes underlying UC-related pouchitis on a genomic scale would require an extensive knowledge of signaling mechanisms underlying the development of inflammation in UC patients. This knowledge is currently not available. However, our analyses indicate there are significant molecular differences between patients undergoing surgery for UC and FAP, independent of the
pouch inflammation status. This observation may be important when managing IPAA patients.

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