

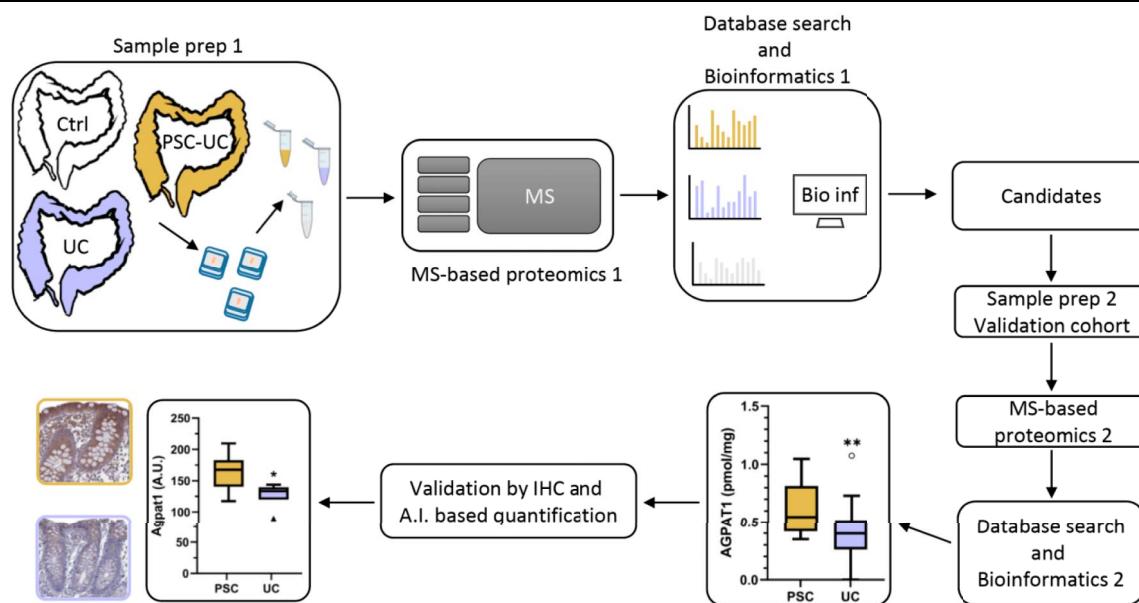
AGPAT1 as a Novel Colonic Biomarker for Discriminating Between Ulcerative Colitis With and Without Primary Sclerosing Cholangitis

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INTRODUCTION: Ulcerative colitis (UC) associated with primary sclerosing cholangitis (PSC-UC) is considered a unique inflammatory bowel disease (IBD) entity. PSC diagnosis in an IBD individual entails a significantly higher risk of gastrointestinal cancer; however, biomarkers for identifying patients with UC at risk for PSC are lacking. We, therefore, performed a thorough PSC-UC biomarker study, starting from archived colonic tissue.

METHODS: Proteins were extracted out of formalin-fixed paraffin-embedded proximal colon samples from PSC-UC (n = 9), UC (n = 7), and healthy controls (n = 7). Patients with IBD were in clinical and histological remission, and all patients with UC had a history of pancolitis. Samples were processed by the multienzyme digestion FASP and subsequently analyzed by liquid chromatography–tandem mass spectrometry. Candidate proteins were replicated in an independent cohort (n: PSC-UC = 16 and UC = 21) and further validated by immunohistochemistry.

AGPAT1 as a novel colonic biomarker in PSC-UC



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RESULTS: In the discovery step, 7,279 unique proteins were detected. The top 5 most differentiating proteins (PSC-UC vs UC) based on linear regression analysis were selected for replication. Of these, 1-acetylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1) was verified as higher in PSC-UC than UC ($P = 0.009$) in the replication cohort. A difference on the group level was also confirmed by immunohistochemistry, showing more intense AGPAT1 staining in patients with PSC-UC compared with UC.

DISCUSSION: We present AGPAT1 as a potential colonic biomarker for differentiating PSC-UC from UC. Our findings have possible implication for future PSC-IBD diagnostics and surveillance.

SUPPLEMENTARY MATERIAL accompanies this paper at <http://links.lww.com/CTG/A801>

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INTRODUCTION

One to 8 percent of patients with inflammatory bowel disease (IBD), in particular ulcerative colitis (UC), will eventually be diagnosed with primary sclerosing cholangitis (PSC) (1,2). Owing to a distinct inflammatory behavior, PSC-associated IBD is considered a unique IBD phenotype, referred to as PSC-IBD or PSC-UC (3,4). In most cases, the bowel disease precedes PSC diagnosis with several years (5), but the subclinical biliary phase is believed to be considerable (6). Furthermore, because elevated liver function tests (LFTs) in most cases are the motive for cholangiography referral, PSC diagnosis is likely to be missed or delayed in cases with normal LFTs.

PSC diagnosis entails a distinctly increased incidence of both hepatobiliary cancer (7,8) and IBD-associated colorectal neoplasia (9,10), and risks are already elevated at the time of diagnosis. In line with this, guidelines advocate yearly colonoscopy for patients with IBD with concomitant PSC (11). Recently, published prospective data indicate lower hepatobiliary cancer-related deaths among patients receiving annual imaging evaluation compared with those who do not (12). With increasing support for active colonic and biliary surveillance, the relevance of early PSC detection among patients with IBD will increase.

A limited number of studies have aimed to find colonic PSC-IBD biomarkers. Wohl and et al. (13) reported high IHC expression of the tumor suppressor gene p53 in nondysplastic PSC-UC, and we have previously reported a dampened tissue-factor expression in PSC-UC compared with UC (14). In addition, both Gwela et al. (15) and our group (16,17) have found distinctive immunological features of PSC-UC mucosa. The clinical implications of these observations remain, however, to be determined, and taken together, gastropathologists still have no tool to distinguish PSC-IBD from the much larger groups of "classical" IBD.

Proteomics holds promise for mucosal biomarker discovery in IBD. Previous studies have primarily aimed to help the diagnostic distinction between UC and Crohn's disease or between flare and remission (18). However, the need for fresh or frozen tissue has been a limitation to a broader application of mass spectrometry (MS)-based techniques. For that reason, the ongoing technical refinement of a workflow adapted to formalin-fixed paraffin-embedded (FFPE) biopsies is encouraging. To the best of our knowledge, this technique has never been applied on PSC-IBD samples before.

Here, we compared the mucosal proteome from patients with remissive PSC-UC and UC, using a 2-step LC-MS/MS procedure. 1-acetylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1) was identified and validated as a biomarker differentiating the 2

subtypes, and the following immunohistochemistry (IHC) mirrored the proteomic findings.

METHODS

Study population and sample collection

Study participants were recruited in connection with elective colonoscopy at the Department of Gastroenterology, Uppsala University Hospital, Uppsala, Sweden. All patients with IBD were in clinical remission at the time for colonoscopy. Biopsies were collected from the ascending colon and archived as FFPE samples at the Pathology Department, Uppsala University Hospital. The median archive time was 2 years (range 0–5 years). Only IBD cases without signs of macroscopic inflammation—graded as 0 (normal/inactive) according to the Mayo Endoscopic Score (19)—and no active inflammatory activity at pathologist's assessment were included. Patients with IBD were all biologic naive, and patients on cortisone treatment the last 4 weeks were excluded. UC subjects had normal liver functions test (LFTs), including alkaline phosphatase. All PSC subjects had large duct disease, and no subject had clinical or radiological signs of cirrhosis or had undergone liver transplantation. IBD diagnoses were based on established clinical, endoscopic, and histological criteria (20,21). Healthy controls (HCs) were recruited among patients without previous or present IBD who were examined for anemia, bleeding, or abdominal pain. All controls had normal endoscopy and histology.

Altogether, the discovery cohort consisted of 23 individuals (n : PSC-UC = 9, UC = 7, and HC = 7). For validation, a total of 37 IBD patients (n : PSC-UC = 16 and UC = 21) were recruited. IHC validation was performed on colonic sections from all patients with IBD of the discovery step (total $n = 16$).

Extraction of proteins from FFPE tissue

The method has previously been described (22). In brief, five 10 μ M sections were cut from the same FFPE tissue block and immediately placed in a 1.5 mL collection tube and covered by 0.5 mL heptane. The tubes were vortexed and incubated for 1 hour at room temperature. Twenty-five microliters of methanol was added, and the tubes centrifuged for $\times 20,000g$ for 2 minutes. The supernatant was discarded, and the pellet was air dried for 5 minutes. The proteins were extracted by adding 60 μ L lysis buffer (0.1 M Tris-HCl pH 8.0, 0.1 M DTT, and 4% SDS). After centrifugation (1 minute at $\times 16,000g$), the samples were left in a heating block set to 99 °C with agitation (600 rpm) for 1 hour. The extracts were clarified by centrifugation at $\times 16,000g$ at 18 °C for

10 minutes, and the supernatants were finally transferred to a new tube and stored in -80°C .

Proteomic analysis

The lysates were processed using the multienzyme digestion filter-Aided sample preparation method (23), using consecutive digestion with endoproteinase LysC and trypsin, as described previously (24). Total protein and total peptide were quantified by measuring tryptophan fluorescence using the tryptophan fluorescence (WF) assay (25).

One microgram aliquots of the total peptide were analyzed by LC-MS/MS as described previously (26). In brief, peptides were separated on reverse phase C18 column using a 95-minute acetonitrile gradient. Spectra were acquired using QExactive instrument (Thermo, Palo Alto, CA) and searched using the MaxQuant software. Titers of proteins were calculated by the "total protein approach" using the raw spectral intensities from the MaxQuant output (27).

IHC and staining assessment

IHC was performed using a primary antibody toward AGPAT1 (HPA073355, Atlas Antibodies AB) (28,29), as described in Supplementary Digital Content (see Supplementary Methods, <http://links.lww.com/CTG/A801>). To allow an objective assessment of AGPAT1 IHC intensity, immunostained and scanned slides were analyzed using the free image software QuPath, version 0.2.3 (30). The software has previously been used successfully for staining evaluation, e.g., in UC studies (31). One representative biopsy was chosen from each patient. The proportion (%) of DAB-stained area within the whole biopsy was measured using the following criteria: resolution: moderate, prefilter: Gaussian, and smoothing sigma: 2. Three different thresholds for positive staining intensity were applied: weak: 0.05–0.14, moderate: 0.15–0.29, and strong: ≥ 0.30 . Finally, a total intensity score was calculated for each biopsy according to the following algorithm: ($\times 1\%$ weakly stained area) + ($\times 2\%$ moderately stained area) + ($\times 3\%$ strongly stained area). For representative images, see Supplementary Figure 1, <http://links.lww.com/CTG/A801>.

Statistical methods

Patient characteristics from the 2 proteomic runs were presented with median and interquartile range for continuous variables and frequencies for discrete variables. Patient characteristics were compared using the Wilcoxon–Mann–Whitney or Kruskal–Wallis test for continuous variables and the Pearson χ^2 test for discrete variables. Differences in protein means were estimated using unadjusted and adjusted linear regression models. We also performed a Wilcoxon–Mann–Whitney test for each protein to confirm that findings in the unadjusted linear models were not heavily influenced by skewed distributions. As a complement to the linear regression analyses, a random forest analysis classifying the study groups was performed on discovery cohort data using R "ranger" package (32). The number of trees was set to 5,000, and mtry (randomly selected variables in each node) was set to square root of the total number of variables. The variable importance was computed as mean decrease in the Gini index, and the 50 highest values were presented with a dot plot. The cohort size for the validation step was calculated based on a *t* test for 5 variables with equal group sizes, 5% significance level (α) after Bonferroni correction, and 80% power, assuming a difference in means of $1.2 \times \text{SD}$.

Overall profiles of protein measurements were presented using scatter plots of the first 2 components from the principal component analysis (PCA). Meta-analysis was performed using the R "metafor" package assuming a random effects model (33). Differences in the IHC intensity were compared using the Wilcoxon–Mann–Whitney test. *P* levels were considered statistically significant when <0.05 (Figures 1 and 2). Bonferroni-corrected *P* value for the validation cohort was <0.01 (0.05/5) and $<8.2 \times 10^{-6}$ (0.05/6,121) for the meta-analysis. All statistical analyses were performed using R (<http://www.R-project.org/>).

Ethical considerations

The project was approved by the Regional Research Ethics Review Board in Uppsala, Sweden (approval number 2011/065, 2014/166, and 2014/166/1), and all patients gave their written informed consent before participation.

RESULTS

Demographic and clinical characteristics

Clinical characteristics of patients from the discovery and validation cohorts are presented in Table 1. As expected, the PSC-UC and UC groups had male and female predominance, respectively, however, reaching statistical difference only in the discovery cohort. In addition, this cohort had a skewness regarding age, with the UC cohort being older. Except for alkaline phosphatase (higher in PSC-UC, not shown), LFTs (alanine aminotransferase (ALT), aspartate aminotransferase (AST), International normalized ratio (INR), and bilirubin) were similar between all groups, for both steps. In line with clinical guidelines, only patients with PSC-UC (44% and 50%, respectively) were on treatment with ursodeoxycholic acid.

Discovery cohort proteomics identifies 5 candidate colonic biomarkers

A total of 7,279 unique proteins were detected in the discovery run. Top 50 proteins in the linear regression analysis (PSC-UC vs UC) from the discovery step are presented in Supplementary Digital Content 1 (see Supplementary Table 1, <http://links.lww.com/CTG/A801>). The top 5 most differentiating proteins based on linear regression were chosen for validation: CD47, TMEM192, LSM7,

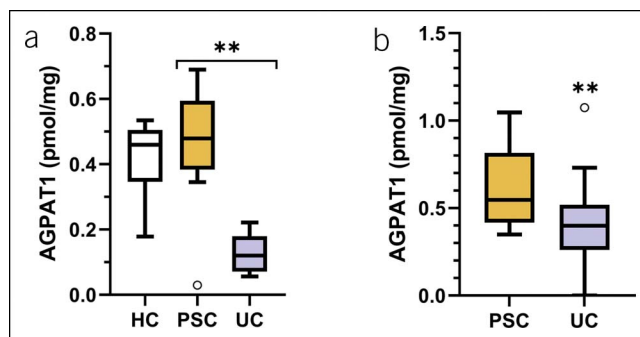


Figure 1. AGPAT1 concentrations according to LC-MS/MS proteomics. The Tukey test for AGPAT1 concentrations in colonic biopsies according to LC/MS-MS analysis for (a) the discovery step (n: PSC-UC = 9, UC = 7, and HCs = 7) and (b) the validation step (n: PSC-UC = 16 and UC = 21). Results are presented as boxplots; horizontal line representing median value, box from first quartile to third quartile, and whiskers represents max/min with outliers excluded. **Indicates $P < 0.01$. AGPAT1, 1-acetylgllycerol-3-phosphate O-acyltransferase 1; HCs, healthy controls; PSC, ulcerative colitis with concomitant primary sclerosing cholangitis; UC, ulcerative colitis.

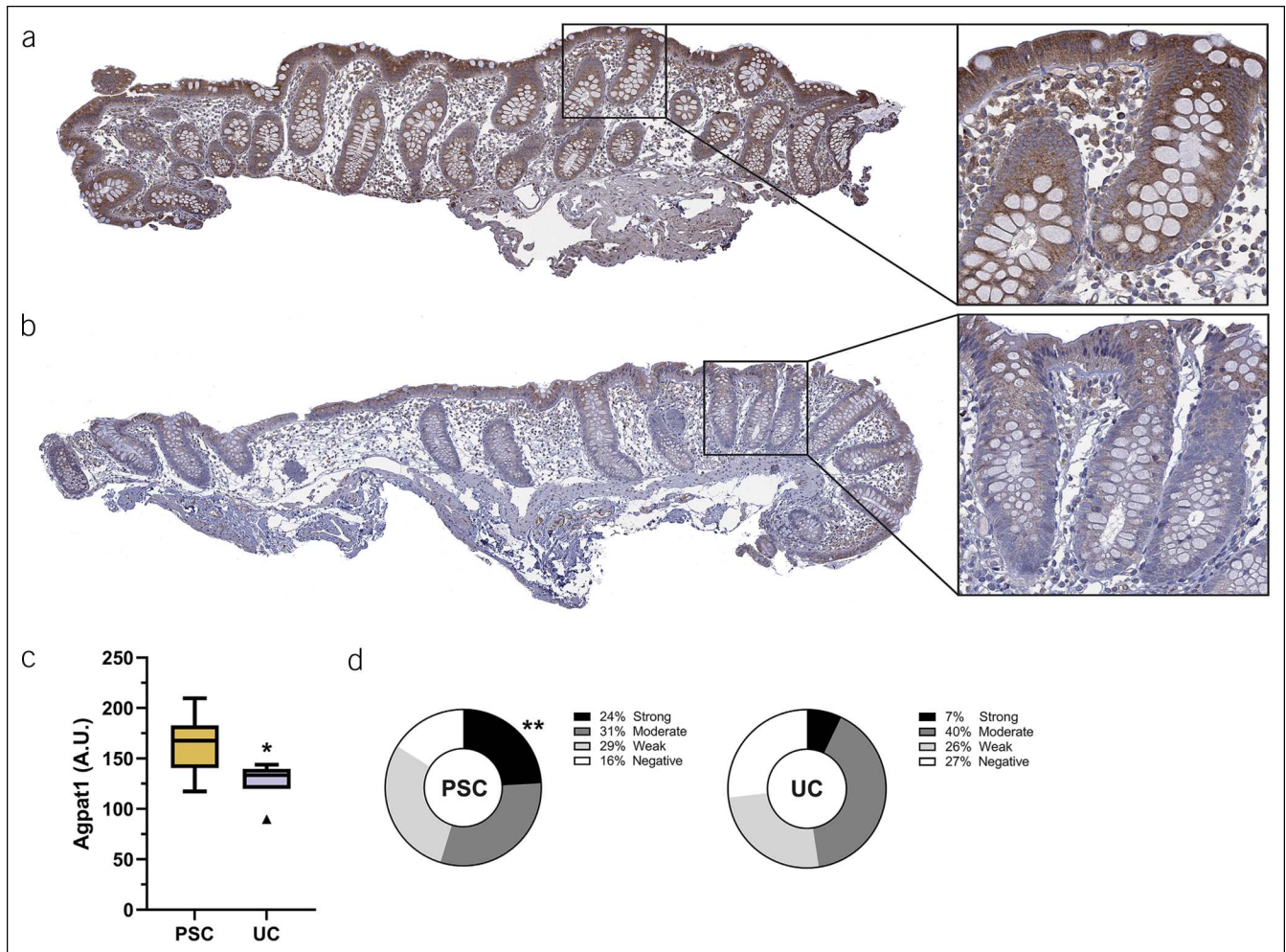


Figure 2. Immunohistochemistry staining for AGPAT1 confirms proteomic findings. **(a, b)** Images of representative colon biopsies from a) PSC-UC and **(b)** UC, respectively, where differences in staining intensity and distribution are visualized. Magnification $\times 40$ and $\times 400$, respectively. **(c)** Global AGPAT1 staining quantification for PSC-UC ($n = 9$) and UC ($n = 7$). Results are presented as boxplots; horizontal line representing median value, box from first quartile to third quartile, and whiskers represents max/min with outliers excluded. **(d)** Donut charts showing proportions of AGPAT1 staining categories for PSC-UC (left) and UC (right). AGPAT1, 1-acetyl-glycerol-3-phosphate O-acyltransferase 1; PSC, ulcerative colitis with concomitant primary sclerosing cholangitis; UC, ulcerative colitis.

NDUFA4, and AGPAT1. As further statistical support for the candidate protein selection, each of the 5 proteins were among the top 50 proteins being of highest discriminative importance according to the random forest analysis (see Supplementary Figure 2, <http://links.lww.com/CTG/A801>). For all 5 proteins, differences remained significant after adjusting for age and the use of thiopurines (see Supplementary Table 2, <http://links.lww.com/CTG/A801>). The use of ursodeoxycholic acid in the PSC-UC group did not influence the outcome (data not shown). AGPAT1 levels for HC were significantly different compared with UC but not PSC-UC.

AGPAT is confirmed in the validation cohort

When comparing colonic candidate biomarker concentrations for patients in the validation cohort, AGPAT1 was again statistically confirmed as being higher in PSC-UC than in UC. The remaining 4 candidate failed to be replicated. A statistical summary for the 5 biomarkers selected for validation is presented in Table 2, and AGPAT1 concentrations from both proteomic steps are presented in Figure 1a,b.

Meta-analysis supports biomarker selection

As a complement to the step-by-step comparison, we also conducted a meta-analysis for the protein concentrations from both proteomic steps. Only proteins that had more than 5 nonzero values in each step were included. In total, 6,121 proteins fulfilled the criteria, of which AGPAT1 had the lowest P value ($3.6e-06$) (see Supplementary Table 3, <http://links.lww.com/CTG/A801>). Significance was retained after adjusting for multiple testing ($3.6e-06 \times 6,121$ tests, $P = 0.02$). Furthermore, AGPAT1 was still highly significant ($P = 8.2e-05$) in a meta-analysis of variables adjusted for age and the use of thiopurines.

IHC AGPAT1 staining mirrors proteomic results

To further validate mass spectrometry data, sections of archival FFPE colonic specimens from each patient with IBD in the discovery cohort were subjected to IHC. The use of a validated antibody toward AGPAT1 resulted in a general cytoplasmic staining of all epithelial and inflammatory cells. The surface epithelial cells displayed a generally more intense staining, as compared with the

Table 1. Patient characteristics

	Discovery cohort			P values	Validation cohort		P values
	PSC-UC n = 9	UC n = 7	Controls n = 7		PSC-UC n = 16	UC n = 21	
Male sex, n (%)	7 (78)	1(14)	5 (71)	0.012 ^a , 0.040 ^b	11 (69)	17 (81)	0.391 ^a
Age, yr (median, range)	38 (36–49)	61 (51–66)	50 (24–74)	0.030 ^c , 0.043 ^d	41 (33–54)	36 (32–44)	0.529 ^c
IBD duration, yr (median, range)	12 (9–19)	30 (5–37)	NA	0.711 ^c	18 (10–23)	14 (12–20)	0.866 ^c
PSC duration, yr (median, range)	9 (5–12)	NA	NA	—	8 (4–14)	—	—
Endoscopic remission, n (%)	9 (100)	7 (100)	NA	—	16 (100)	21 (100)	—
Histologic remission, n (%)	9 (100)	7 (100)	NA	—	16 (100)	21 (100)	—
Medication, n (%)							
5-ASA or SSZ	8 (89)	6 (86)		0.375 ^a	16 (100)	18 (86)	0.115 ^a
Thiopurines	4 (44)	1(14)		0.197 ^a	4 (25)	7 (33)	0.583 ^a
Biologics	0 (0)	0 (0)		—	0 (0)	0 (0)	—
Steroids	0 (0)	0 (0)		—	0 (0)	0 (0)	—
Ursodeoxycholic acid	4 (44)	0 (0)		<0.005 ^a	8 (50)	0 (0)	<0.001 ^a

CI, confidence interval; NA, not applicable; PSC-UC, ulcerative colitis with associated primary sclerosing cholangitis; SSZ, sulfasalazine; UC, ulcerative colitis; 5-ASA, 5-aminosalicylate.
^aTests used: Pearson χ^2 test (PSC-UC vs UC).
^bPearson χ^2 test (PSC-UC vs UC vs Ctrl).
^cWilcoxon–Mann–Whitney (PSC-UC vs UC).
^dKruskal–Wallis test (PSC-UC vs UC vs Ctrl).

epithelial cells within the crypts, varying from weak–moderate to moderate–strong between individual patients (Figure 2a,b). An automated global staining quantification was made using bioimage software (as described in Methods). AGPAT1 staining intensity mirrored the proteomics findings, with higher composite scores in PSC-UC as compared with UC (Figure 2c). As for the different staining intensity categories, PSC-UC had significantly higher proportion of "strong" AGPAT1 staining than UC (Figure 2d). Taken together, these data verify the findings from MS and highlight the potential of combining routine imaging technologies, such as IHC, with an artificial intelligence-based scoring.

Limited overall PSC-IBD vs UC proteome separation

We also compared the overall protein profiles between PSC-UC and UC for both proteomic runs, separately, using PCA. For the

discovery cohort, no apparent separation of clusters was seen for any of the 3 groups (PSC-IBD, UC, and controls) (Figure 3a). PCA for the validation data set revealed a small proteome separation for PSC-IBD and UC clusters, reaching statistical significance for the second component (Figure 3b). However, in summary, our results indicate a high degree of biological similarity between the 2 diseases, when in remission.

DISCUSSION

There is a growing support for active and early initiated cancer surveillance in PSC and PSC-IBD; however, clinically useful biomarkers to discriminate PSC-UC from UC before the biliary disease turns clinically overt are still lacking. Lately, development of MS workflow has enabled high-resolution proteomics on FFPE tissue, facilitating biomarker studies on hard-recruited IBD

Table 2. Statistical summary of proteomics for colonic candidate biomarkers chosen for validation

Protein	UniProt ID	Discovery cohort			Validation cohort			Validated
		Mean diff	95% CI	P value, t-test	Mean diff	95% CI	P value, t-test	
CD47	Q08722	0.908	0.549 to 1.268	0.0002	−0.486	−0.765 to −0.207	0.0016 ^a	No
TMEM192	Q8IY95	0.206	0.121 to 0.290	0.0003	ND	—	—	No
LSM7	Q9UK45	0.954	0.531 to 1.377	0.0006	−0.188	−0.918 to 0.542	0.6169	No
NDUF4F4	Q9P032	0.708	0.393 to 1.022	0.0006	0.259	−0.071 to 0.588	0.1326	No
AGPAT1	Q99943	−0.332	−0.482 to −0.182	0.0007	−0.214	−0.367 to −0.061	0.0094	Yes

AGPAT1, 1-acetylgllycerol-3-phosphate O-acyltransferase 1; CD47, leukocyte surface antigen CD47; CI, confidence interval; ND, not detected; NDUFAF4 NADH dehydrogenase ubiquinone 1 alpha subcomplex assembly factor 4; TMEM192, transmembrane protein 192, LSM7, U6 SnRNA-associated Sm-like protein LSM7; Uniprot, Universal Protein Resource.
^aOpposite value of effect.

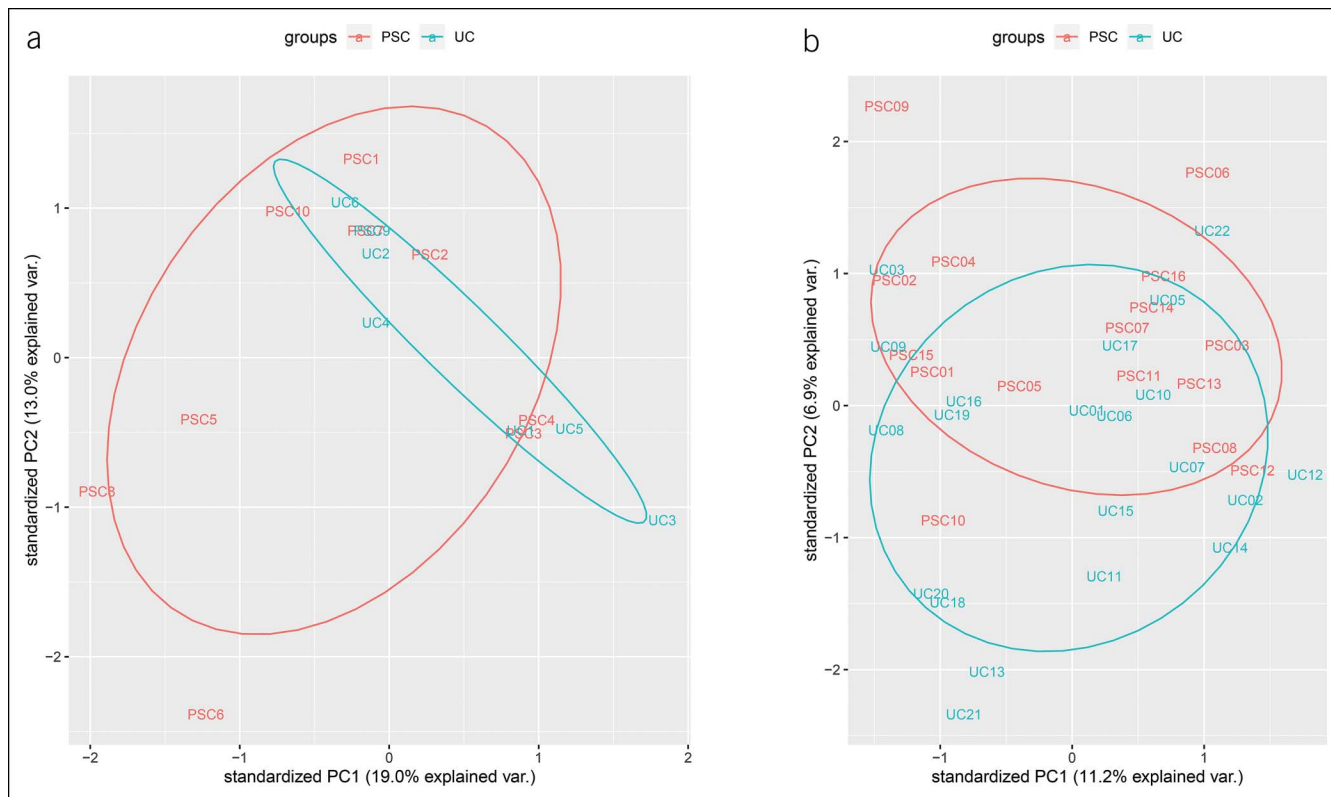


Figure 3. Limited overall mucosal proteome differences between PSC-UC and UC. Principal component plots of proteomic findings in (a) the discovery step (7,279 proteins; n: PSC-UC = 9 and UC = 7) and (b) the verification step (7,706 proteins; n: PSC-UC = 16 and UC = 21). Codes represents individual IBD subjects. Red ellipse represents PSC-UC, and blue represents UC, where each ellipse represents 95% confidence interval. Results are plotted according to the first 2 components, with the proportion of variation explained by the respective axis. Corresponding statistical tables is found under each plot, where the first 5 components are presented with proportion of explained variance (%), odds ratio (OR), and confidence interval (CI). PC, principal component; PSC, ulcerative colitis associated with primary sclerosing cholangitis; UC, ulcerative colitis.

subgroups such as PSC-UC. In this LC-MS/MS based biomarker study, using archival biopsies from the proximal colon of patients with remissive IBD, we were able to identify and validate AGPAT1 as a potential colonic biomarker to differentiate PSC-UC from UC.

The concept of biomarkers in IBD is attractive, for example, in facilitating the diagnostic distinction between IBD subgroups, or as a complement to endoscopy in staging inflammatory behaviour (34). Despite the obvious benefits offered by plasma or serum, no proposed circulating biomarkers have yet been implemented in clinical practice (35). As an example, repeated observations support a high prevalence of antineutrophil cytoplasmic antibodies positivity in patients with PSC (36); however, testing for antineutrophil cytoplasmic antibodies has not proved to add strength in the prediction of the IBD subtype (37). The use of blood fluids as the starting point for unbiased proteomic biomarker studies have also certain limitations (18,38). In intestinal tissue, candidate biomarkers may be more concentrated but also less subjected to impact from non-IBD-related factors. Several MS-based studies have investigated the colonic proteome in UC and/or Crohn's disease during the last decade (18), but in most cases, findings are yet to be validated before possible clinical usefulness can be assessed.

To the best of our knowledge, this is the first PSC-IBD biomarker report using colonic FFPE. The method, described in 2010 by Ostasiewicz et al. (19), enables high-resolution proteome

analysis of archived biological samples. The FFPE concept was recently proven technically and practically feasible in a retrospective IBD report, however, with a different MS methodology than used in our study (39). Our objective was to perform an unbiased biomarker search on colonic tissue. For this purpose, we chose a 2-step LC-MS/MS approach where a full proteome comparison was followed by validation in a new cohort, using the same method. By repeating LC-MS/MS, we believe we reduced the risk of false positive findings considerably. Only 1 of 5 candidate markers was confirmed—a result which we believe is consistent with biological variance, reasonably most critical for detection of low-abundance proteins.

We present 1-acylglycerol-3-phosphate O-acyltransferase 1 (AGPAT1) as a candidate biomarker for discriminating between "classical" UC and PSC-associated UC. The main finding is further strengthened by the fact that AGPAT1 also was top ranked in the meta-analysis, after combining the 2 proteomic data sets. AGPAT1 is an enzyme, responsible for the conversion of phospholipid lysophosphatic acid into phosphatic acid (40). There is a growing interest in the role for lipid metabolism in IBD, as an actor in immune regulation, but also as a potential target for biomarker discovery (41). Moreover, several lipid metabolic pathways with potential importance for colorectal cancer development have been detected (42). Interestingly, AGPAT1 has previously been acknowledged as a negative prognostic marker for the outcome in patients with colorectal cancer (43), also confirmed by prognostic analysis of data

from The Cancer Genome Atlas data (<https://www.proteinatlas.org/ENSG00000204310-AGPAT1/pathology/colorectal+cancer>).

In addition, for healthy subjects, AGPAT1 was higher than for non-PSC-UC. However interesting from a pathophysiological perspective, the finding was considered of minor relevance for the main objective, why no controls were included in the replication step.

In line with previous AGPAT1 IHC characterizations, a general cytoplasmatic staining was observed (29), however, clearly most accentuated in surface epithelial cells. Intensity scores for AGPAT1 staining, as assessed by an open access image software, mirrored proteomic findings with the highest scores found in the PSC-UC cohort. Compared with MS-based proteomics on FFPE tissue, IHC is a cheap method, already incorporated in clinical practice. Therefore, despite the limited statistical basis, we consider the AGPAT1 IHC data encouraging.

The overall proteome comparison revealed a high biological overlap between the 2 UC subtypes. Albeit a tendency of differential clustering was found for the validation cohort, our results not only stand in contrast to the phenotypical differences (3,4) but also to the GWAS studies showing surprisingly limited overlap between UC and PSC-UC susceptibility loci (44). In addition, our observation diverges from new data from the United Kingdom where significant differences in the mucosal transcriptomic landscape were found between PSC-IBD and IBD (45). However, since our analysis included more than 7,000 unique findings, it cannot be ruled out that a more targeted approach, for example, applying PCA to the top distinguishing proteins found in the meta-analysis, would have rendered more apparent clustering.

We acknowledge several limitations of this study. First, the relatively small patient groups, although at the same magnitude, as in many of the previous MS-based biomarker studies in this field. Second, by not including patients on biologics, the results per-se cannot be extrapolated to this subgroup, which is likely to have had a more difficult-to-treat course. Third, as in all MS-based proteomic methods, there is a definite risk of considerable variations, including an important impact of preanalytical factors (46,47). These are likely to be further enhanced when combining separate runs, why for example meta-analysis data must be interpreted with caution. Finally—as well as an inherent limitation of all PSC-IBD studies using non-PSC-IBD subjects as controls—as this study did not include cholangiography at baseline, we cannot rule out isolated cases of subclinical PSC among the UC cohort.

At the same time, we believe that this biomarker study has strengths. These include the phenotypically balanced IBD groups. The interpretations of previous UC proteomic studies have been hampered by clinical heterogeneity. In our study, we exclusively included patients with IBD in clinical and histological remission. In addition, with the intent to minimize impact of different disease extension, the UC cohort was confined to patients with pancolitis and biopsies were consistently taken from the ascending colon (in accordance with the PSC-UC right-side inflammatory predominance). Furthermore, by not including patients on biologics and/or steroids, we believe we reduced the influence by potent medications as far as feasible. Last but not least, as many biomarker studies lack a validation cohort, we consider the use of a second cohort for MS validation, together with the subsequent IHC confirmation, as a definite strength.

In summary, we were able to identify a novel colonic biomarker with a possible capacity to differentiate PSC-UC from UC.

The study may be considered proof-of-concept for coming PSC-IBD studies, and the findings warrant further validation in larger independent IBD cohorts before possible clinical application can be addressed.

CONFLICTS OF INTEREST

Guarantor of the article: Mikael Åberg, PhD.

Specific author contributions: Fredrik Rorsman, MD, PhD, and Mikael Åberg, PhD, contributed equally to this work. J.V., M.C., F.R., and M.Å. conceived the study. Patients were recruited by J.V., F.R., and M.C. M.Å. extracted proteins, and J.W. and K.Z. performed the L.C. MS/MS analysis. C.L. performed the IHC. J.V., F.R., M.Å., M.C., N.E., K.G., and A.W. analyzed and interpreted the data. J.V. and M.Å. wrote the article with help from all authors. All authors have approved the final draft of the article before submission.

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Potential competing interests: None to report.

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Study Highlights

WHAT IS KNOWN

- ✓ Primary sclerosing cholangitis (PSC) is a severe disease and strongly associated to inflammatory bowel disease (IBD) and foremost ulcerative colitis.
- ✓ IBD precedes the biliary disease in most cases; however, colonic biomarkers for identifying patients with IBD at risk are lacking.

WHAT IS NEW HERE

- ✓ 1-acetylglycerol-3-phosphate O-acyltransferase 1 is overexpressed in PSC-ulcerative colitis (UC) colon compared with classical UC.
- ✓ Colonic 1-acetylglycerol-3-phosphate O-acyltransferase 1 is quantifiable using artificial intelligence-aided immunohistochemistry reading.
- ✓ Proteomics using archived PSC-UC tissue is technically feasible.

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