Urine Proteomics Identifies Biomarkers for Diagnosis and Fibrosis Severity in Pediatric Chronic Pancreatitis

Madalyn G. Moore, MS[1,2], Vineet Garlapally, MS[3,4], Katelyn B. Brusach, PhD[5], Venkata S. Akshintala, MD[6], Lynn Babcock, MD[7], Juan Gurria, MD[8], Lindsey Hornung, MS[9], Michelle Saad, MD, MS[3,4], Rachel Sheridan, MD[10,11], Andrew Trout, MD[12,13,14], Liwen Zhang, PhD[15], Zobeida Cruz-Monserrate, PhD[16], Brian C. Searle, PhD[1,2,17,18] and Maisam Abu-El-Haija, MD, MS[3,4]

INTRODUCTION: **Reliable biomarkers for the diagnosis of chronic pancreatitis (CP) and pancreatic fibrosis severity are lacking, hindering effective treatment and management. Histologic fibrosis is a hallmark of late-stage CP, but noninvasive methods to evaluate fibrosis progression are limited. We used urine proteomics to discover biomarkers that identify patients with CP and predict fibrosis severity.**

METHODS: **We performed a cross-sectional study of 130 total subjects (CP n = 50) selected based on clinical criteria in a tertiary care setting. Urine proteomics samples were quantified using data-independent acquisition mass spectrometry. Differential biomarker candidates were identified with false discovery rate-corrected pairwise comparisons. These proteins were validated with an independent paired urine and plasma sample cohort (n = 36). Machine learning was used to develop a protein panel that predicted Ammann scores for patients with histologic fibrosis.**

RESULTS: **We found 34 proteins consistently differentially expressed between CP and controls in pairwise false discovery rate-controlled tests. Of these, 25 urine proteins outperformed 19 previously suggested CP blood-based biomarkers in an independent validation cohort. Isocitrate dehydrogenase (IDH1), calcyphosin (CAPS), synuclein gamma (SNCG), and protein S100-P (S100P) all produced receiver operator curve area under the curve values >0.95, while the best plasma marker was interleukin 2 receptor subunit alpha (receiver operator curve area under the curve = 0.80). A 12-protein panel of identified markers predicted fibrosis severity with a linear correlation R^2 value of 0.61.**

DISCUSSION: **We identified a panel of proteins that may diagnose CP in children and developed a model to predict pancreatic fibrosis severity, offering promising tools for improving diagnostics and patient care.**

KEYWORDS: protein biomarkers; pancreas; chronic pancreatitis; histologic fibrosis; proteomics

ABBREVIATIONS: AP, acute pancreatitis; AUC, area under the curve; CP, chronic pancreatitis; EPI, exocrine pancreatic insufficiency; FC, fold change; FDR, false discovery rate; FRC, fracture pain controls; HC, healthy controls; INSPPIRE, International Study Group of Pediatric Pancreatitis, In Search for a Cure; IRB, Institutional Review Board; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MS, mass spectrometry; PROBE, prospective specimen collection, retrospective blinded evaluation;

[1]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio, USA; [2]Pelotonia Institute for Immuno-Oncology, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, USA; [3]Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati, Ohio, USA; [4]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [5]Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, Ohio, USA; [6]Division of Gastroenterology, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA; [7]Department of Pediatrics, Division of Emergency Medicine, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA; [8]Division of General and Thoracic Pediatric Surgery, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA; [9]Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [10]Division of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [11]Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [12]Department of Radiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA; [13]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [14]Department of Radiology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [15]Campus Chemical Instrument Center Mass Spectrometry and Proteomics Facility, Ohio State University, Columbus, Ohio, USA; [16]Division of Gastroenterology, Hepatology and Nutrition, The James Comprehensive Cancer Center, The Ohio State University Wexner Medical Center, Columbus, Ohio, USA; [17]Department of Biomedical Informatics, The Ohio State University, Columbus, Ohio, USA; and [18]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, Minnesota, USA. **Correspondence:** Brian C. Searle, PhD. E-mail: Searle.brian@mayo.edu. Maisam Abu-El-Haija, MD, MS. E-mail: Maisam.Haija@cchmc.org.

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REDcap, research electronic data capture; ROC, receiver operator curve; STROBE, strengthening the reporting of observational studies in epidemiology; t-SNE, t-distributed stochastic neighbor embedding

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/B461

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INTRODUCTION

Chronic pancreatitis (CP) is a progressive inflammatory disorder of the pancreas that is characterized by painful end-stage sequelae, with tissue fibrosis leading to loss of endocrine and exocrine function and morphological changes on imaging. Long-term chronic inflammation from CP is a risk factor for pancreatic cancer. It has been estimated that up to a third of patients with acute pancreatitis (AP) are at risk of developing acute recurrent pancreatitis, which is a risk for CP in their lifetime (1). Most adult CP cases arise from excessive alcohol use or smoking, while CP in children is tightly linked to hereditary causes (e.g., PRSS1 and SPINK1 mutations), with genetics playing a major role in the pancreatitis course (2). Given the different risk profiles for the 2 groups, pediatric and adult CP should be studied separately (3). In all cases, however, the presence of fibrotic tissue that leads to a decrease in pancreas function is a diagnostic hallmark of CP, but tissue histology is rarely obtained from patients with suspected CP due to the need for invasive biopsy. Diagnosis of CP is challenging because current techniques include imaging that can overrepresent or underrepresent the extent of pancreatic fibrosis and disease progression, potentially leading to incorrect or delayed medical intervention (4). There remains a need to develop a diagnostic method for CP to effectively treat patients.

The Ammann scoring system (5) attempts to quantify interlobular and perilobular fibrosis by grading both the extent and distribution of fibrosis within the pancreas on histology. Despite its utility, the application of histopathologic fibrosis scoring in clinical practice is limited because of the invasive nature of obtaining pancreatic tissue samples, which is rarely performed in patients with suspected CP, especially in the pediatric population. Moreover, most existing studies focus on adult CP cohorts, leaving a significant gap in our understanding of fibrosis development and severity in pediatric cases, where etiologies often differ, and genetic factors play a more prominent role. To date, no reliable circulating biomarkers have been established for assessing pancreatic fibrosis in patients with CP.

Proteomics offers an advantageous methodology for identifying candidate marker proteins that could be disease biomarkers. Proteomics is a method by which proteins and peptides can be quantified using mass spectrometry (MS). Using recent developments in data-independent acquisition MS, biomarker protein identification has been shown to have improved reproducibility across large cohorts. Our prior research has identified CEL2A as a novel urine protein biomarker for the diagnosis of AP (6). Previous studies have attempted to identify novel diagnostic biomarker candidates for CP using biofluids but have shown confounding variables between sample groups or have lacked validation (7). In addition, there have been no attempts to identify markers that exclude age and lifestyle-related variables, such as alcohol use and smoking. We hypothesized that, in children, urine proteomics would differentiate patients with CP from patients with AP and controls and would reveal correlated results with tissue fibrosis.

METHODS

Study approval

The study had subjects enrolled in 2 study cohorts approved by the Institutional Review Board (IRB) at Cincinnati Children's Hospital Medical Center for the acute pancreatitis registry (IRB 2012–4050), and the Total Pancreatectomy Cohort Study (IRB 2016–9510). All patients provided written informed consent for participation in the study. The protocol and informed consent forms were approved by the institutional ethics committees before enrollment. The study was designed using principles in the STROBE guidelines for Strengthening the Reporting of Observational Studies in Epidemiology.

Study design and patient population

This was a cross-sectional PRoBE phase 1 biomarker study (8) based on a cohort of pediatric and young adults less than 21 years of age presenting to a large tertiary freestanding Children's Hospital. Some measurements from a subsection of our cohort were previously published (6) in a study focused on identifying AP biomarkers. This work builds on that preliminary data. Our study included both male and female pediatric subjects, and findings are reported for both sexes. The cohorts identified included patients with CP, patients with AP, healthy controls (HC), or an acute extremity fracture (fracture pain controls [FRC]) injury. CP was defined as per the published International Study Group of Pediatric Pancreatitis: In Search for a CuRE (INSPPIRE) criteria and required imaging features of CP including the presence of imaging findings indicative of CP including but not limited to ductal calcifications, pancreatic ductal dilation plus one of the following: (i) pain consistent with pancreatic origin, (ii) endocrine dysfunction, and (iii) exocrine dysfunction (9,10). Based on this, AP was defined by meeting 2 of 3 criteria (pain, laboratory evidence by lipase or amylase of more than 3× normal levels, and imaging findings). Physicians completed surveys regarding clinical data for patients with the characteristics of AP presentations. The nonpancreatitis groups included individuals without chronic pain syndromes or significant chronic diseases. The fracture group comprised patients presenting to the emergency department within 48 hours of a painful extremity fracture confirmed by imaging. Samples were obtained from AP patients within 48 hours of presentation, and samples from patients with CP were obtained on the day of surgery for total pancreatectomy with islet cell transplantation (TPIAT). Data were stored in the Research Electronic Data Capture database (REDcap, Nashville, TN).

Statistical analysis

Patient demographic data were collected at the time of admission and detailed in the Results section. Cohort biostatistical data were analyzed using SAS, version 9.4 (SAS Institute, Cary, NC). Owing to skewed distributions and/or small sample sizes, continuous variables were summarized as medians and interquartile ranges. Categorical variables were presented as counts and percentages. χ^2 or Fisher exact tests were used for group comparisons of

categorical variables. For continuous data, nonparametric Kruskal-Wallis tests were used for group comparisons.

Proteomics methods

Urine samples were processed by acetone precipitation and digested on suspension traps using trypsin. Plasma samples were processed using the Mag-net protocol (11) followed by on-bead digestion. Peptides were analyzed with a block randomized design using data-independent acquisition MS. The discovery cohort used a Thermo Fusion, while the validation cohort used an Exploris 480 mass spectrometer configured with a chromatogram library workflow (12) and interpreted using Prosit predicted spectra (13,14). Quantification used median normalization, and statistical significance was assessed using Bonferroni-corrected *t*-tests to control the familywise error rate, with the goal of preventing any false positives (familywise error rate < 0.05). Full protocols, including reagent compositions, instrument settings, and software parameters, are detailed in the Supplementary Methods (http://links.lww.com/CTG/B461).

Fibrosis and pathology scoring

At the time of TPIAT, pancreas tissue was collected for clinical examination per standard protocol and was sent to the pathology laboratory for review by a single pathologist for internal data consistency. Hematoxylin-eosin-stained slides were created for each case and labeled from the head, body, or tail of the pancreas. In 80% of cases, samples from 2 of these regions were available. The Ammann scoring system was followed to systematically grade fibrosis (5,15). Briefly, perilobular and intralobular fibrosis were graded separately. Fibrosis involving less than 50% (focal) of the pancreatic parenchyma was staged as 1 (mild), 2 (moderate), and 3 (marked). Fibrosis in more than 50% (diffuse) of the pancreatic parenchyma was staged as 4 (mild), 5 (moderate), and 6 (marked). The final fibrosis score was the sum of 2 components, perilobular (scored from 1 to 6) and intralobular (scored from 1 to 6), and was further categorized into 3 groups: 0–4 (low), 5–8 (medium), and 9–12 (high). Examples of these scores are illustrated in Figure 5a.

Machine learning modeling

Quantitative values from 46 CP samples were used to develop a linear model to predict Ammann scores. Twelve CP-specific proteins were selected based on minimum redundancy, and 10,000 multiple linear regression models were built using bootstrapping. Coefficients from these models were averaged to generate a single predictive model. This process was then repeated 10,000 times using 12 randomly selected proteins to determine a background correlation distribution.

Data availability

Data, analytic methods, and study materials will be made available to other researchers on request. Raw proteomics data are publicly available on MassIVE using data set identifier MSV000096607 (reviewer password: "pancreas") and at ProteomeXchange using the data set identifier PXD058660. Raw proteomics data file annotations are listed in Supplementary Data 1 (http://links.lww.com/CTG/B461).

Table 1. Baseline participant characteristics of the discovery and validation cohorts

Discovery cohort[a] n = 130				
	CP n = 50	**AP n = 28**	**Controls n = 52**	***P* value**
Age (yr)	14.5 (10.9–17.0)	14.0 (11.0–17.0)	11.0 (7.3–13.8)	0.0006
Sex				
Male	26 (52%)	18 (64%)	27 (52%)	0.51
Female	24 (48%)	10 (36%)	25 (48%)	
BMI percentile	81.9 (43.2–97.1) n = 49	75.6 (34.1–96.0) n = 27	91.4 (71.6–99.0) n = 17	0.08
BMI percentile ≥85 (overweight)	23/49 (47%)	11/27 (41%)	11/17 (65%)	0.29
Validation cohort[a] n = 36				
	CP n = 15	**AP n = 10**	**Controls n = 11**	***P* value**
Age (yr)	11.4 (7.3–16.4)	9.0 (5.0–13.0)	9.0 (8.0–13.0)	0.29
Sex				
Male	11 (73%)	5 (50%)	4 (36%)	0.18
Female	4 (27%)	5 (50%)	7 (64%)	
BMI percentile	59.6 (24.8–97.4) n = all	61.1 (21.9–97.6) n = all	60.6 (35.6–91.7) n = 8	0.93
BMI percentile ≥85 (overweight)	6/15 (40%)	3/10 (30%)	2/8 (25%)	0.80

Data presented as median (25th–75th percentile) or n (%).
AP, acute pancreatitis; BMI, body mass index; CP, chronic pancreatitis; HC, healthy control.
[a]Controls (31 extremity fracture patients, no pancreatitis history, and 21 HCs in the discovery cohort and 11 HCs in the validation cohort). Complete data unless otherwise specified by n = or stated denominator. In addition, 21 of 36 patients with CP had plasma and urine samples available from the same subject (14 CP, 3 AP, 4 HC).

Table 2. Clinical features of patients with CP with available fibrosis histology scores

CP fibrosis cohort n = 52	Low (0–4) n = 4	Medium (5–8) n = 22	High (9–12) n = 26	*P* value
Age (yr)	14.9 (13.8–16.2)	13.9 (10.9–17.0)	13.4 (8.6–15.8)	0.59
Sex				
Male	2 (50%)	12 (55%)	16 (62%)	0.51
Female	2 (50%)	10 (45%)	10 (38%)	
Body mass index percentile	93.6 (61.1–96.0) n = all	88.6 (57.9–97.6) n = all	66.3 (38.6–95.2) n = all	0.37

Data presented as median (25th–75th percentile) or n (%). Complete data unless otherwise specified by stated denominator.

RESULTS

Clinical characteristics of discovery and validation cohorts

The discovery cohort included 130 individuals, 28 with AP, 50 with CP, and 52 controls (21 HC and 31 FRC), and included some previously published proteomics results (6), where previous measurements were expanded on with fibrosis data. The validation cohort included an additional 36 individuals, 10 with AP, 15 with CP, and 11 HCs, measured 2 years later with an alternative proteomics platform. The sample sizes for the discovery cohort were chosen based on power analysis from a 6-sample test data set, while the sizes for the validation cohort were based on a power analysis of the discovery cohort data set. Baseline characteristics for both cohorts of the groups are presented in Table 1. Of the validation cohort, we also had time-matched plasma samples from 5 AP, 14 CP, and 4 HC. Of the 65 total individuals with CP, 52 had pancreas tissues available for fibrosis scoring measurements. Clinical characteristics were included for the 3 fibrosis groupings: low, medium, and high (Table 2). We processed samples from these subjects according to the experimental outline in Figure 1.

Urine proteomics of discovery and validation cohorts

We detected a total of 2,137 library proteins with global urine proteomics of the discovery cohort, representing >95% of the observable human urine proteome (16). Of these proteins, we identified

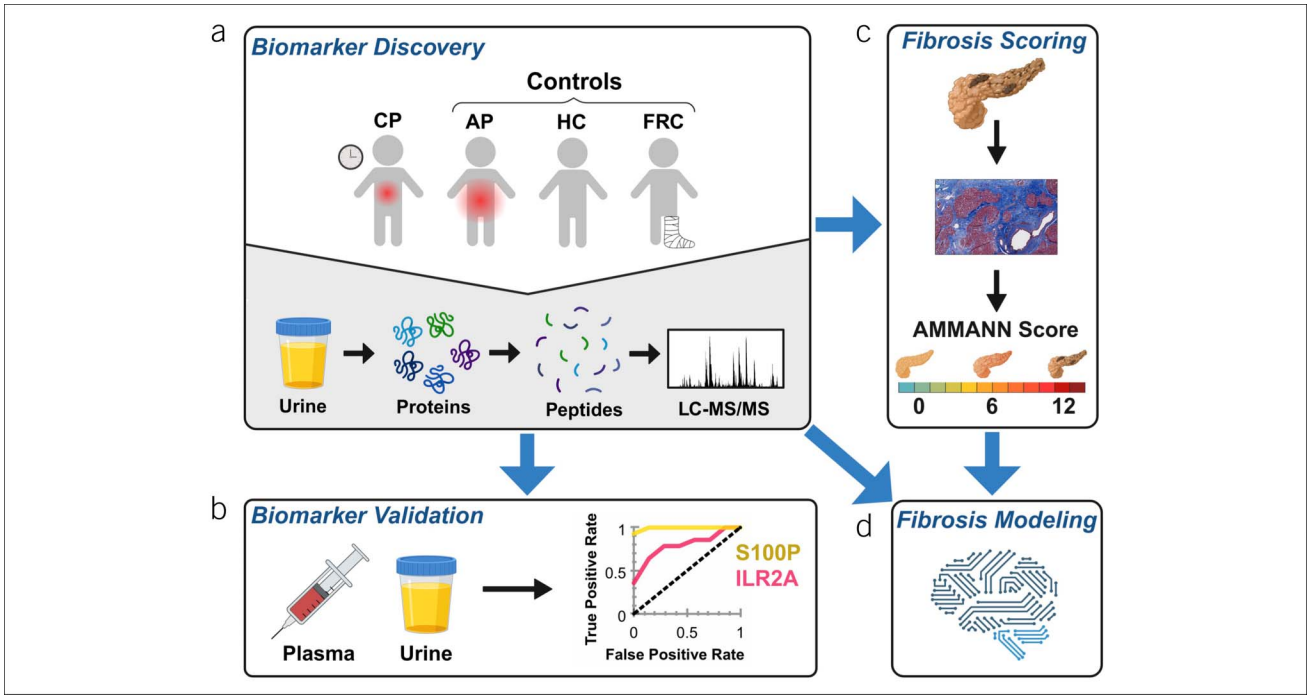

Figure 1. Experimental workflow. (**a**) The discovery cohort urine samples underwent a bottom-up proteomics sample preparation before mass spectrometry analysis for global protein quantification. (**b**) The validation cohort plasma and urine samples were processed, and significance was determined based on previous CP biomarker findings for blood studies. An example receiver operator curve plot for the best urine (S100P) and plasma (IL2RA) markers is shown. (**c**) CP patient samples who underwent TPIAT provided tissue samples, and these samples were given an Ammann score based on histology results. (**d**) Finally, the proteomics data were combined with the Ammann scores, and machine learning was used to generate a linear regression model between the 2 variables. Created in BioRender. AP, acute pancreatitis; CP, chronic pancreatitis; HC, healthy controls; FRC, fracture pain controls; IL2RA, interleukin 2 receptor subunit alpha; LC-MS/MS, liquid chromatography-tandem mass spectrometry; S100P, Protein S100-P; TPIAT, total pancreatectomy with islet cell transplantation.

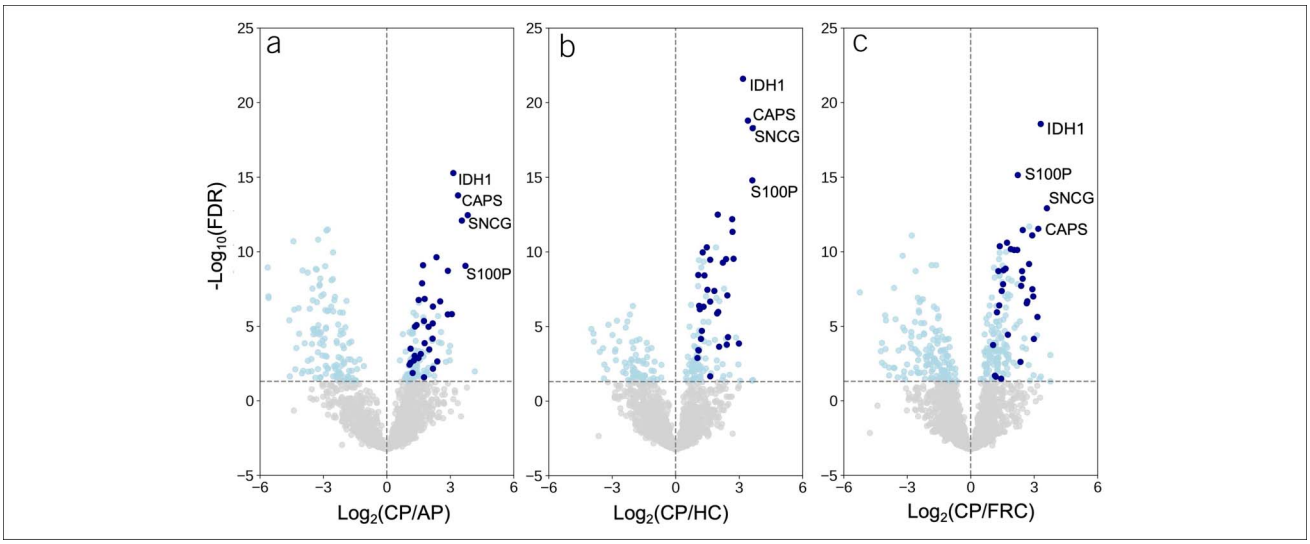

Figure 2. Volcano plots showing significant (familywise error rate <0.05) proteins that were either downregulated or upregulated in the urine discovery cohort (light blue) relative to nonsignificant proteins (gray). A total of 34 proteins were significantly upregulated within pairwise tests of CP to all individual groups (dark blue) relative to AP (**a**), HC (**b**), and FRC (**c**). The 4 consistently most significant proteins are labeled (IDH1, S100P, SNCG, and CAPS). AP, acute pancreatitis; CAPS, calcyphosin; CP, chronic pancreatitis; HC, healthy controls; FRC, fracture pain controls; FDR, false discovery rate; IDH1, isocitrate dehydrogenase; S100P, protein S100-P; SNCG, synuclein gamma.

1,760 across all 130 samples, where 34 had <0.05 Bonferroni-corrected false discovery rate (FDR) and >2× fold change in pairwise comparisons with every control group (AP, HC, and FRC). This approach was intentionally stringent to eliminate non-replicating expression changes. When samples from individuals with CP were compared with samples from controls, we found 4 proteins that were significantly upregulated above others consistently: isocitrate dehydrogenase (IDH1), calcyphosin (CAPS), synuclein gamma (SNCG), and protein S100-P (S100P) (Figure 2 and Supplemental Figure 1, http://links.lww.com/CTG/B461). In the validation cohort with analysis performed in a different laboratory, we similarly detected 2,010 proteins across 36 samples but could only quantify 29 of the 34 previously identified. We found that all of these proteins remained significantly different (Figure 3a) and that the cohorts tracked together in relative expression levels (Figure 3b).

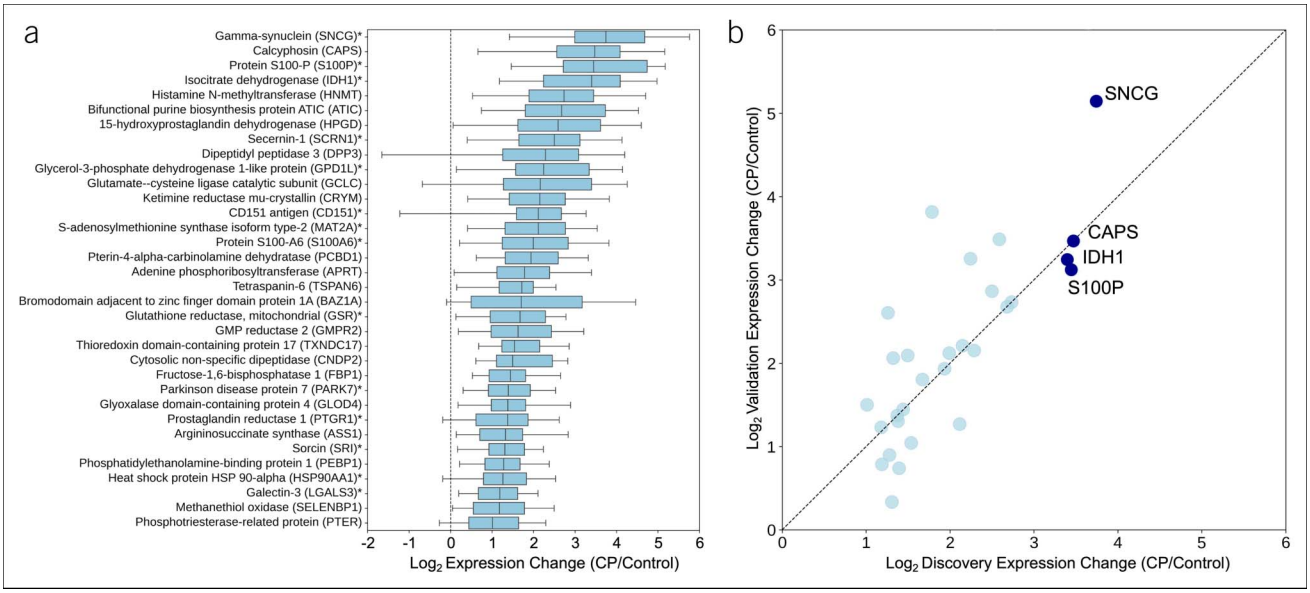

Figure 3. (**a**) Urine proteome analysis box plots showing the delta of $\log_2$ normalized intensities of CP samples and control sample median for each of the 34 significantly upregulated proteins (FC > 1, familywise error rate <0.05) in the discovery cohort. *Existing literature indicates significantly high expression in pancreatic ductal adenocarcinoma compared with controls. (**b**) Regression showing the median delta of the $\log_2$ normalized intensities of CP and control sample median for the validated proteins found in both the urine discovery and validation cohorts. Control groups include AP, HC, and FRC in the discovery cohort and AP and HC in the validation cohort. AP, acute pancreatitis; CP, chronic pancreatitis; FC, fold change; HC, healthy controls; FRC, fracture pain controls.

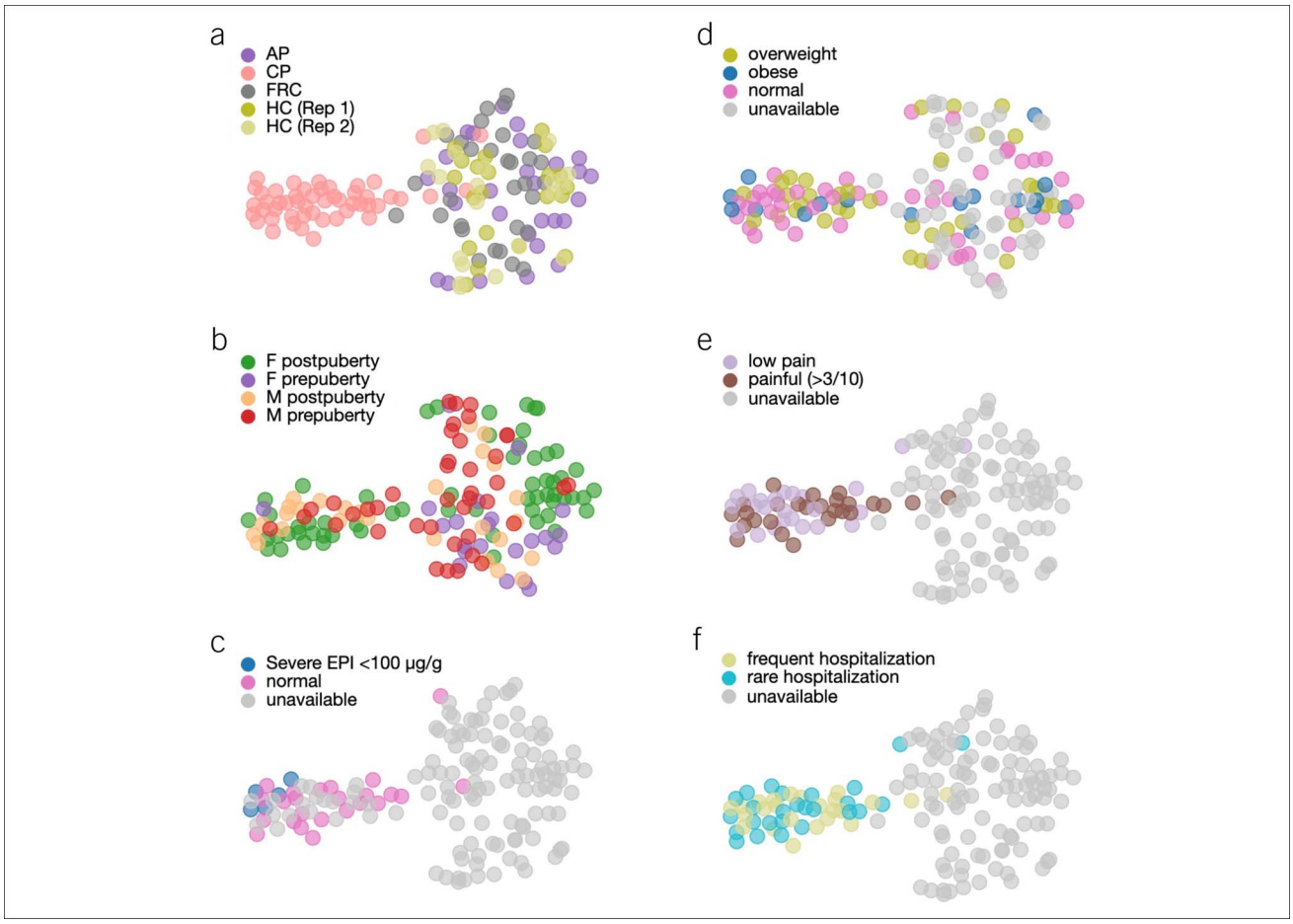

Figure 4. Protein expression clustering using t-SNE for the 34 significantly upregulated proteins based on condition (**a**), age and sex (**b**), elastase levels (**c**), obesity (**d**), pain (**e**), and hospitalization rate (**f**). Puberty was assumed to be ≥ 10 yo for female patients and ≥11 yo for male patients. Individuals with fecal elastase levels below 100 µg/g were considered to have exocrine pancreatic insufficiency. Individuals with body mass index *Z*-scores >1 were considered overweight (84th percentile), while individuals with *Z*-scores >2 were considered obese (98th percentile). Pain at the time of sample collection was self-reported on a scale of 0–10. Individuals with frequent hospitalization rates were admitted more than 3 times in an 18-month period. Pain and hospitalization rate data are only available for patients with CP. AP, acute pancreatitis; CP, chronic pancreatitis; HC, healthy controls; FRC, fracture pain controls; t-SNE, t-distributed stochastic neighbor embedding.

Attempts were made to limit common CP comorbidities when possible. The incidence rate of diabetes in the CP cohort was low enough to not be considered as a confounding factor (discovery: 1/50, validation: 3/15). Similarly, efforts were made to limit the effect of recurrent AP (recurrent pancreatitis) on the analysis of AP as a control group (discovery: 0/28, validation: 3/10). Considering t-distributed stochastic neighbor embedding (t-SNE) clustering of urinary protein expression in the 34 significant proteins from the discovery cohort, CP clearly differentiated from the other groups (Figure 4a). Age was a significant differentiator ($P = 0.0006$) of CP, specifically in the female fraction of the cohort, where nearly all female patients in the discovery cohort were postpuberty ($P = 0.0032$), whereas this was not true for male patients (Figure 4b). For this analysis, puberty was assumed to be ≥10 yo for females (17) and ≥11 yo for males (18). Further work is needed to explore how menstrual cycles are associated with pancreatitis. Of the 50 CP individuals in the discovery cohort, fecal elastase was reported for 29. Of these, 5 were determined to have exocrine pancreatic insufficiency (EPI) based on elastase levels below 100 µg/g stool. Although these individuals seem to have more extreme molecular phenotypes as shown by increased within-cluster distances (Figure 4c), the low number of EPI cases did not allow subgroup analysis in a meaningful fashion. We observed no significant bias based on obesity (Figure 4d, overweight: *Z*-score>1, obese: *Z*-score>2), reported pain (Figure 4e, 0–10), or hospitalization rate (Figure 4f, frequent rate defined as hospitalized >3 times in 18 months). Like others (19), we found that the fecal elastase level did not correlate with either scored pain or number of hospitalizations.

Proteomics of paired plasma validation cohort samples

We measured 21 paired urine and plasma samples from the validation cohort with global proteomics (14 CP, 3 AP, and 4 HC) to compare newly discovered urine markers with existing blood-based markers. For this, we measured 19 proteins previously studied in blood for CP diagnostic ability: interleukin 2 receptor subunit alpha (IL2RA) (20), MMP7 (21), ICAM1 (22), GDF15 (23), LGALS3BP (24), HP (25), PON1 (26), APOA2 (22), MMP9 (27), CX3CL1 (28), APOC1 (29), LTF (22), RETN (30), THBS1 (22), RARRES2 (31), AZGP1 (22), CRP (27), ADIPOQ (32), and PPBP (22). Although

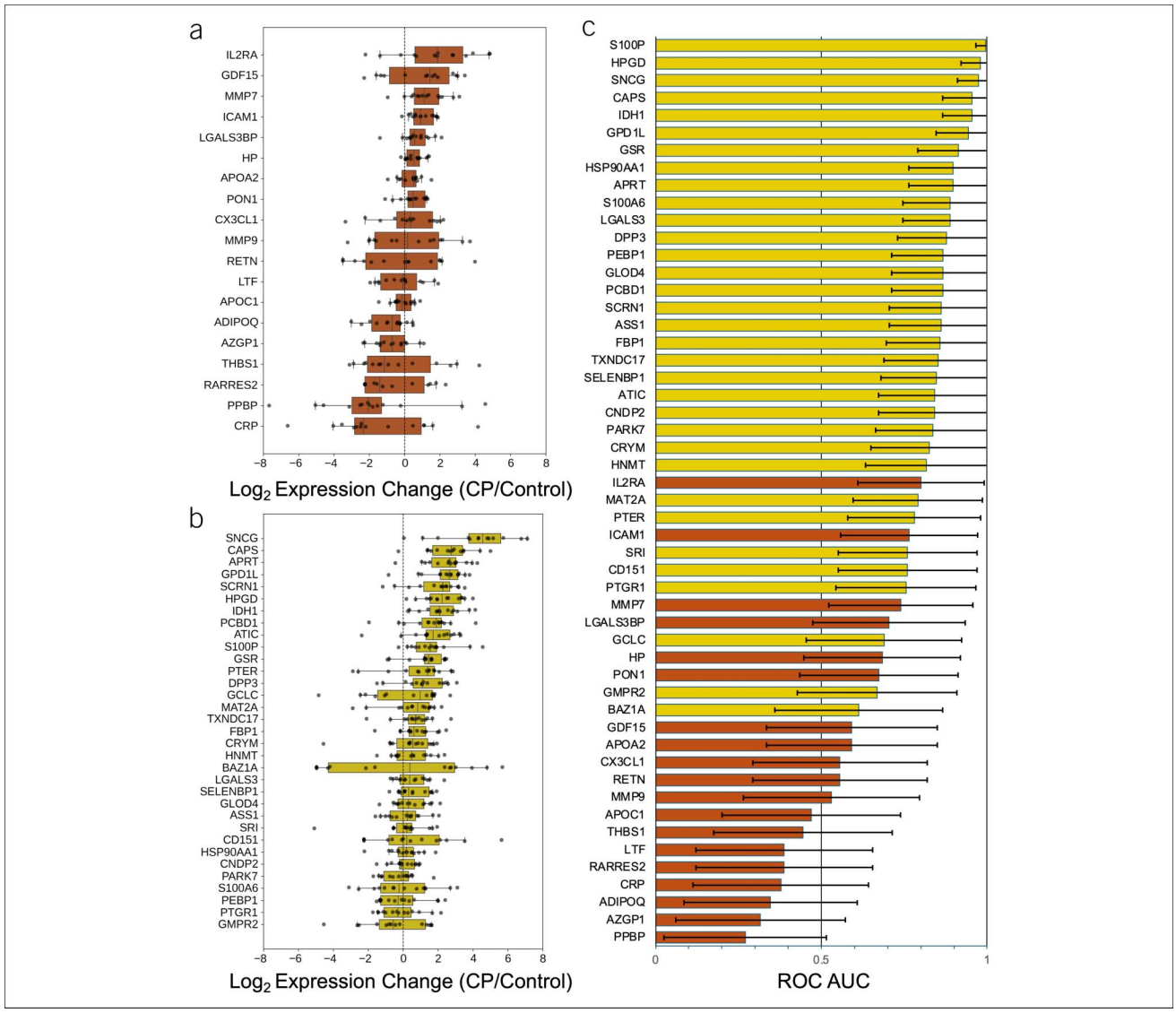

Figure 5. Box plots showing the delta of $\log_2$ normalized intensities of CP samples and control sample median for (**a**) each of the 19 proteins previously studied as CP biomarkers in serum, plasma, or whole blood found in the paired validation cohort plasma samples and (**b**) urine protein biomarker candidates found in the paired validation cohort urine samples. (**c**) The ROC AUC values for each of these proteins, where yellow denotes the novel urine proteins and red denotes the previously studied blood proteins. Error bars indicate a 95% confidence interval. The top performing proteins in urine were S100P (AUC: 0.994), HPGD (AUC: 0.979), SNCG (AUC: 0.974), CAPS (0.954), and IDH1 (0.954). AUC, area under the curve; CAPS, calcyphosin; CP, chronic pancreatitis; IDH1, isocitrate dehydrogenase; ROC, receiver operator curve; S100P, protein S100-P; SNCG, synuclein gamma.

some of these proteins were previously studied only in serum, many were first measured in plasma. In our work, we found that 10 of 19 proteins produced higher expression levels in CP than the median control expression, where no proteins had higher than a tenfold median increase (Figure 5a). By contrast, 27 urine proteins had CP median expression values above the median control expression, where SNCG had >25× median fold change (Figure 5b). We generated receiver operator curves (ROCs) for each protein in their respective biofluid and found that 25 urine proteins had a larger area under the curve (AUC) than all the previously studied blood proteins (Figure 5c). Error bars use 95% confidence intervals consistent with the Hanley and McNeal method (33). Consistent with previous data, we found that S100P, IDH1, CAPS, and SNCG were top-performing diagnostic markers, and all had ROC-AUC values >0.95. The best performing plasma marker was IL2RA (ROC-AUC = 0.80). Surprisingly, although we were able to measure these proteins in plasma, we found that they performed poorly when measured in urine (Supplemental Figure 2, http://links.lww.com/CTG/B461).

Correlation of urine proteins with fibrosis score

We obtained 46 CP pancreas tissue samples from patients in the discovery cohort and 6 from patients in the validation cohort. Examples of the histology for varying degrees of fibrosis can be seen in Figure 6a. Since the validation cohort contained only a small range of scored samples, we considered the combined cohorts as a single fibrosis data set of 52 individuals. We built a linear regression model for predicting fibrosis using bootstrapping from 12 proteins with minimal redundancy (S100A6, GSR, GPD1L, IDH1, CAPS, SNCG, HPGD, HNMT, CRYM, GMPR2, PCBD1, and ATIC). More than 10,000 samples, these 12

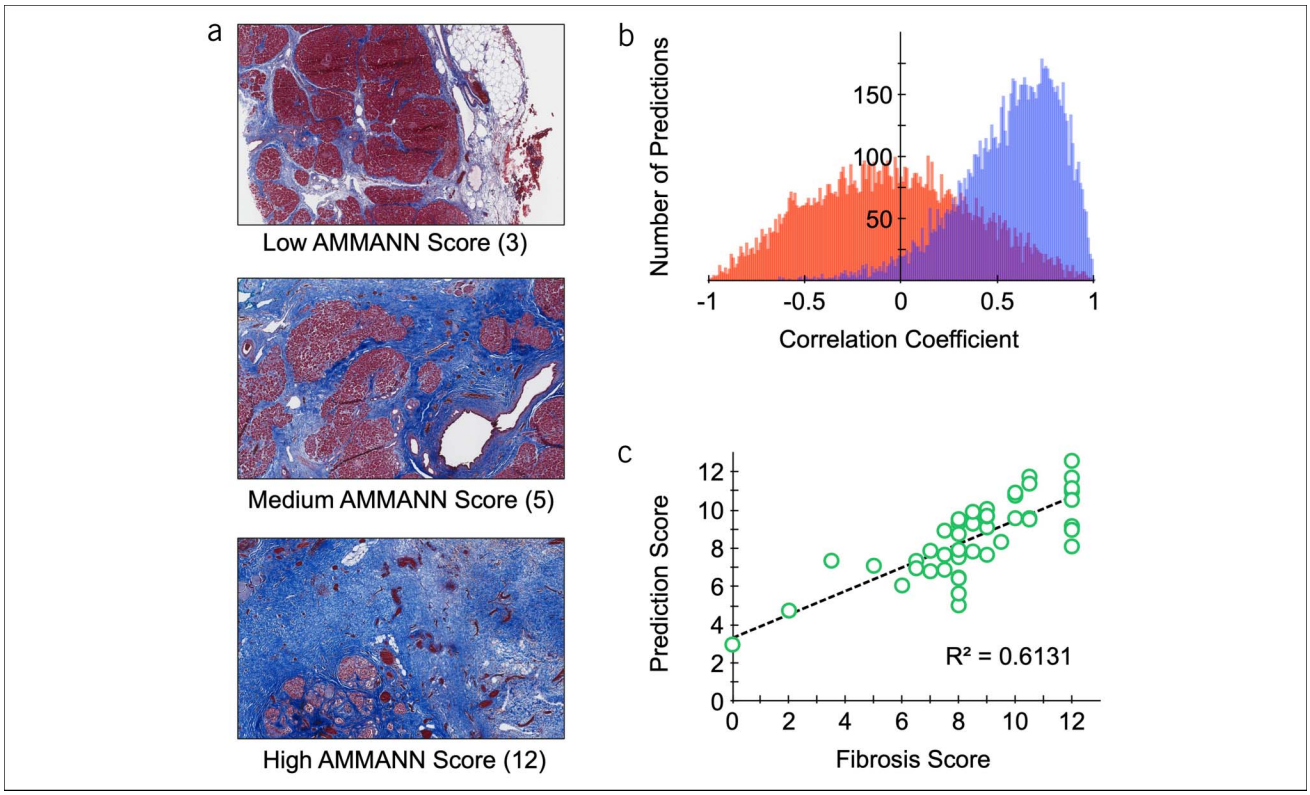

Figure 6. (**a**) Representative photomicrographs of trichrome-stained pancreatic tissue samples. Blue-colored areas indicate fibrosis in CP samples of low, medium, and high fibrosis score groups (4× magnification). (**b**) Correlation distributions of predicted fibrosis scores based on the 12 significant CP proteins determined to correlate with fibrosis (blue) and 12 randomly selected proteins (red). (**c**) Regression model showing the prediction scores of proteins as they predict the fibrosis score in CP. CP, chronic pancreatitis.

proteins performed significantly better than linear regression models generated from 12 randomly selected proteins (Figure 6b). We found that most of the 10,000 bootstrap models were positively correlated with fibrosis, with an average Pearson correlation coefficient >0.6. The aggregate linear model predicted Ammann fibrosis scores with $R^2 = 0.6131$ (Figure 6c).

DISCUSSION

Our study took a novel approach to studying CP in children, where genetic risk factors are more prevalent, by performing proteomics analysis of minimally invasive biofluids (urine and blood) in our cohort. These experiments derived potential biomarkers that may help differentiate patients with CP from patients with AP and patients without pancreatitis (healthy subjects and extremity fracture conditions). Widely accepted biomarkers for CP do not currently exist because it has been historically difficult to separate CP-specific proteins from proteins altered by other confounding factors, such as alcohol use and smoking, as well as from other disease states. The fibrosis scoring data as a measure of tissue destruction in the CP cohort add to the novelty of our findings and show that a panel of proteins performs well as a disease diagnostic biomarker and a measure of fibrosis in pancreatic tissues. We confirmed previously known proteins in CP and discovered a new data set in childhood CP, with top-performing proteins being S100P, IDH1, CAPS, and SNCG.

Urine is an attractive, noninvasive biomarker source that reflects protein changes caused by disease state (34). Although urine can be challenging for protein-level measurements because of protein concentration variability, our study used precipitation followed by protein concentration normalization to ensure equal protein in each sample regardless of the original biological levels. Moreover, to eliminate effect of variations in kidney excretion, none of our CP subjects had a diagnosis for an underlying kidney disease, and the urine samples were provided after an overnight fasting state. Previous studies attempted to characterize the urine proteome of adults with CP but were limited by a lack of validation (35) or sample size (35,36). Previously, we reported robust urine marker proteins for AP in a pediatric sample population (6). In this work, we reinvestigated this data set for CP markers and identified 34 potential candidates. Of these, 7 proteins have previously been shown as upregulated in CP through adult cohort studies using fluids and tissue (PCBD1 (37), HNMT (37), MAT2A (37), SCRN1 (37), CD151 (38), HSP90AA1 (39), and PARK7 (40)). Gene Ontology (GO) protein set enrichment revealed that 16 of 34 significantly changing proteins were involved in cation binding (FDR = 5.3E-03) and 12 were involved in oxidoreductase activity (FDR: 4.3E-06). Interestingly, 14 of these proteins are highly expressed in pancreatic ductal adenocarcinoma (PDAC) subjects compared with controls in fluids and tissue (GPD1L (41), CD151 (38), GSR (22), HSP90AA1 (39), IDH1 (42), LGALS3 (43), MAT2A (37), PARK7 (40), PTGR1 (44), S100P (45), S100A6 (45), SCRN1 (46), SRI (47), and SNCG (48)), underlining a possible link to PDAC in individuals with hereditary pancreatitis (49,50) or CP to PDAC (51).

Since most proposed diagnostic protein markers for CP have been reported in blood (7), we measured a set of paired urine and

plasma samples for 21 individuals collected in the same visit. In addition to the 34 candidate urine markers, we measured 19 previously reported plasma or serum CP protein markers: adipokines/chemokines/cytokines, glycoproteins/lipoproteins, and other potential protein biomarkers. Unsurprisingly, markers discovered in blood had poor performance in urine; the reverse was also true. Of the plasma proteins we targeted, soluble IL-2 receptor (20) (IL2RA) was the top-performing protein with an AUC of 0.801 to predict CP. By contrast, the best urine candidate, S100P, produced a nearly perfect AUC of 0.995 to predict CP.

In this work, we attempted to associate quantitative protein abundances with histology scoring as a proxy for disease progression. Although no individual protein successfully predicted the severity of fibrosis in CP, a panel of multiple proteins may be useful for classifying patients. In addition, proteomics could be used as a preassessment to screen candidates for fibrosis, which currently requires invasive biopsies to fully assess the progression of CP. By using histology scoring on pancreas tissues, our quantitative proteomics results can be directly compared with disease progression. Compared with other studies, our population had a higher fibrosis score than another published study (52), which could be attributed to the nature of the referral center as a TPIAT center and the frequency of genetic mutations in children leading to higher fibrosis scores. Methods for measuring pancreas fibrosis previously included endoscopic ultrasound with elastography by measuring tissue stiffness (53). However, this technique has challenges in differentiating fibrosis from inflammation and neoplasia, operator dependency, and accessibility considerations. Our studies show promise for circulating protein measures and pancreas fibrosis grading.

Our study has the limitations of being a single-center study with a small cohort, albeit relatively large for a pediatric study. One weakness is that at the time of cohort design, sex and age were not considered to have significant associations. Only after finalizing the full cohort, we observed that female patients exhibiting pancreatitis in our study were significantly older than male patients or other control populations (Table 1). In addition, we do not have longitudinal data to assess progression from early to late CP, and the range of fibrosis histology is biased toward severe fibrosis. In particular, since fibrosis histology was only performed immediately before a scheduled TPIAT surgery, our cohort contained a very few individuals graded for low fibrosis. These weaknesses are offset by having distinct derivation and validation cohorts and same-day-matched urine, plasma, and histology scoring data sets. However, owing to limited independent validation of fibrosis data availability, future validation of fibrosis prediction using urine biomarkers is necessary to assess reproducibility. Some proteins are either difficult or impossible to measure with global proteomics, either due to having low abundance relative to the sample dynamic range or due to incompatibilities with sample preparation methods. Human trypsinogen (PRSS1), for example, is challenging to measure because porcine trypsin is used to digest proteins before MS analysis.

In conclusion, we have found several protein markers that perform well for distinguishing patients with CP from patients with AP and HCs in a pediatric cohort. Furthermore, we have found a set of proteomic markers in the urine that correlate with fibrosis scoring on pancreas tissues, suggesting potential for building future diagnostic criteria. These results may help unravel the underlying mechanisms of CP, present potential therapeutic targets, and identify insights into the linkage of pancreatitis to PDAC. These results underline a need for future multicenter and larger cohort studies to deeply characterize CP-specific proteins in pediatric and adult cohorts.

CONFLICTS OF INTEREST

Guarantor of the article: Brian C. Searle, PhD, and Maisam Abu-El-Haija, MD, MS.

Specific author contributions: M.G.M., B.C.S., M.A.-E.-H.: writing, first draft. All authors: writing, editing, and approval of the final submitted version. L.B., J.G., M.A.-E.-H.: sample collection and supervision of the cohorts. M.G.M., V.G.: sample preparation. V.G., M.S., R.S., A.T.: clinical data collection. M.G.M., L.Z., B.C.S.: molecular data collection. M.G.M., K.B.B., B.C.S., L.H.: data analysis. B.C.S., M.A.-E.-H.: supervision and funding.

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Study Highlights

WHAT IS KNOWN

- ✓ Chronic pancreatitis is an inflammatory condition often diagnosed with imaging, which is unreliable for early detection.
- ✓ No validated noninvasive biomarkers exist for diagnosing chronic pancreatitis or monitoring fibrosis, especially in children.

WHAT IS NEW HERE

- ✓ This study identifies specific urine proteins that outperform existing blood-based markers in diagnosing.
- ✓ A subset of these urine biomarkers tracks with fibrosis severity, indicating their predictive power.
- ✓ By developing a multiprotein urine panel, we provide a measurable, noninvasive tool for tracking disease progression.
- ✓ These findings could guide new diagnostic protocols that improve early intervention, reducing reliance on invasive procedures.

REFERENCES

1. Sankaran SJ, Xiao AY, Wu LM, et al. Frequency of progression from acute to chronic pancreatitis and risk factors: A meta-analysis. Gastroenterology 2015;149(6):1490–500.e1.
2. Kumar S, Ooi CY, Werlin S, et al. Risk factors associated with pediatric acute recurrent and chronic pancreatitis: Lessons from INSPPIRE. JAMA Pediatr 2016;170(6):562–9.
3. Abu-El-Haija M, Kumar S, Quiros JA, et al. The management of acute pancreatitis in the pediatric population: A clinical report from the NASPGHAN Pancreas Committee. J Pediatr Gastroenterol Nutr 2018;66(1):159–76.
4. Forsmark CE. The early diagnosis of chronic pancreatitis. Clin Gastroenterol Hepatol 2008;6(12):1291–3.
5. Ammann RW, Heitz PU, Klöppel G. Course of alcoholic chronic pancreatitis: A prospective clinicomorphological long-term study. Gastroenterology 1996;111(1):224–31.
6. Akshintala VS, Moore MG, Cruz-Monserrate Z, et al. Urine proteomics profiling identifies novel acute pancreatitis diagnostic biomarkers in a pediatric population. Gastroenterology 2024;167(5):1019–21.e2.

7. Cruz-Monserrate Z, Gumpper K, Pita V, et al. Biomarkers of chronic pancreatitis: A systematic literature review. Pancreatology 2021;21(2):323–33.
8. Yadav D, Conwell DL, Pandol SJ, et al. Diagnostic and prognostic biomarkers of chronic pancreatitis: A conceptual framework based on the PRoBE design. Gastroenterology 2024;166(6):957–62.e3.
9. Morinville VD, Husain SZ, Bai H, et al. Definitions of pediatric pancreatitis and survey of present clinical practices. J Pediatr Gastroenterol Nutr 2012;55(3):261–5.
10. Abu-El-Haija M, Kumar S, Szabo F, et al. Classification of acute pancreatitis in the pediatric population: Clinical report from the NASPGHAN pancreas committee. J Pediatr Gastroenterol Nutr 2017; 64(6):984–90.
11. Wu CC, Tsantilas KA, Park J, et al. Enrichment of extracellular vesicles using Mag-Net for the analysis of the plasma proteome. Nat Commun 2025;16(1):5447.
12. Searle BC, Pino LK, Egertson JD, et al. Chromatogram libraries improve peptide detection and quantification by data independent acquisition mass spectrometry. Nat Commun 2018;9(1):5128.
13. Gessulat S, Schmidt T, Zolg DP, et al. Prosit: Proteome-wide prediction of peptide tandem mass spectra by deep learning. Nat Methods 2019;16(6): 509–18.
14. Searle BC, Swearingen KE, Barnes CA, et al. Generating high quality libraries for DIA MS with empirically corrected peptide predictions. Nat Commun 2020;11(1):1548.
15. Klöppel G, Maillet B. Pseudocysts in chronic pancreatitis: A morphological analysis of 57 resection specimens and 9 autopsy pancreata. Pancreas 1991;6(3):266–74.
16. Swensen AC, He J, Fang AC, et al. A comprehensive urine proteome database generated from patients with various renal conditions and prostate cancer. Front Med (Lausanne) 2021;8(548212):548212.
17. Cabrera SM, Bright GM, Frane JW, et al. Age of thelarche and menarche in contemporary US females: A cross-sectional analysis. J Pediatr Endocrinol Metab 2014;27(1-2):47–51.
18. Herman-Giddens ME, Steffes J, Harris D, et al. Secondary sexual characteristics in boys: Data from the Pediatric Research in Office Settings Network. Pediatrics 2012;130(5):e1058–68.
19. Talley NJ, Holtmann G, Nguyen QN, et al. Undiagnosed pancreatic exocrine insufficiency and chronic pancreatitis in functional GI disorder patients with diarrhea or abdominal pain. J Gastroenterol Hepatol 2017; 32(11):1813–7.
20. Manes G, Spada OA, Rabitti PG, et al. Neopterin serum levels in pancreatic adenocarcinoma. Int J Pancreatol 1999;25(1):31–7.
21. Manjari KS, Jyothy A, Kumar PS, et al. Association of matrix metalloproteinase-7 (-181A/G) promoter polymorphism in chronic pancreatitis. Indian J Med Res 2014;140(5):609–15.
22. Pan S, Chen R, Crispin DA, et al. Protein alterations associated with pancreatic cancer and chronic pancreatitis found in human plasma using global quantitative proteomics profiling. J Proteome Res 2011;10(5): 2359–76.
23. Kaur S, Chakraborty S, Baine MJ, et al. Potentials of plasma NGAL and MIC-1 as biomarker(s) in the diagnosis of lethal pancreatic cancer. PLoS One 2013;8(2):e55171.
24. Maekawa T, Kamada Y, Ebisutani Y, et al. Serum Mac-2 binding protein is a novel biomarker for chronic pancreatitis. World J Gastroenterol 2016; 22(17):4403–10.
25. Ueda M, Kamada Y, Takamatsu S, et al. Specific increase in serum core-fucosylated haptoglobin in patients with chronic pancreatitis. Pancreatology 2016;16(2):238–43.
26. Zhang L, Lin B. Decreased serum paraoxonase activity in patients with chronic pancreatitis. Am J Med Sci 2013;346(5):363–5.
27. Manjari KS, Jyothy A, Vidyasagar A, et al. Matrix metalloproteinase-9, transforming growth factor-β1, and tumor necrosis factor-α plasma levels in chronic pancreatitis. Indian J Gastroenterol 2013;32(2):103–7.
28. Kozak A, Talar-Wojnarowska R, Kaczka A, et al. Utility of different serum fibrosis markers in diagnosing patients with chronic pancreatitis and pancreatic adenocarcinoma. World J Gastrointest Oncol 2016;8(8):635–41.
29. Ni Q, Yun L, Xu R, et al. Correlation between blood lipid levels and chronic pancreatitis: A retrospective case-control study of 48 cases. Medicine (Baltimore) 2014;93(28):e331.
30. Kamath MG, Pai CG, Kamath A, et al. Monocyte chemoattractant protein-1, transforming growth factor-beta1, nerve growth factor, resistin and hyaluronic acid as serum markers: Comparison between recurrent acute and chronic pancreatitis. Hepatobiliary Pancreat Dis Int 2016;15(2): 209–15.
31. Adrych K, Stojek M, Smoczynski M, et al. Increased serum chemerin concentration in patients with chronic pancreatitis. Dig Liver Dis 2012; 44(5):393–7.
32. Adrych K, Smoczynski M, Stelmanska E, et al. Serum adiponectin and leptin concentrations in patients with chronic pancreatitis of alcoholic and nonalcoholic origin. Pancreas 2008;36(2):120–4.
33. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143(1):29–36.
34. Fliser D, Novak J, Thongboonkerd V, et al. Advances in urinary proteome analysis and biomarker discovery. J Am Soc Nephrol 2007;18(4):1057–71.
35. Paulo JA, Kadiyala V, Brizard S, et al. Short gel, long gradient liquid chromatography tandem mass spectrometry to investigate the urine proteome of chronic pancreatitis. Open Proteomics J 2013;6(1):1–13.
36. Weeks ME, Hariharan D, Petronijevic L, et al. Analysis of the urine proteome in patients with pancreatic ductal adenocarcinoma. Proteomics Clin Appl 2008;2(7-8):1047–57.
37. Chen R, Brentnall TA, Pan S, et al. Quantitative proteomics analysis reveals that proteins differentially expressed in chronic pancreatitis are also frequently involved in pancreatic cancer. Mol Cell Proteomics 2007; 6(8):1331–42.
38. Gesierich S, Paret C, Hildebrand D, et al. Colocalization of the tetraspanins, CO-029 and CD151, with integrins in human pancreatic adenocarcinoma: Impact on cell motility. Clin Cancer Res 2005;11(8): 2840–52.
39. Chen CC, Chen LL, Li CP, et al. Myeloid-derived macrophages and secreted HSP90α induce pancreatic ductal adenocarcinoma development. Oncoimmunology 2018;7(5):e1424612.
40. He XY, Liu BY, Yao WY, et al. Serum DJ-1 as a diagnostic marker and prognostic factor for pancreatic cancer. J Dig Dis 2011;12(2):131–7.
41. Hu D, Ansari D, Pawłowski K, et al. Proteomic analyses identify prognostic biomarkers for pancreatic ductal adenocarcinoma. Oncotarget 2018;9(11):9789–807.
42. Brody JR, Yabar CS, Zarei M, et al. Identification of a novel metabolic-related mutation (IDH1) in metastatic pancreatic cancer. Cancer Biol Ther 2018;19(4):249–53.
43. Schaffert C, Pour PM, Chaney WG. Localization of galectin-3 in normal and diseased pancreatic tissue. Int J Pancreatol 1998;23(1):1–9.
44. Restifo D, McDermott JR, Cvetkovic D, et al. Conditional dependency of LP-184 on prostaglandin reductase 1 is synthetic lethal in pancreatic cancers with DNA damage repair deficiencies. Mol Cancer Ther 2023; 22(10):1182–90.
45. Crnogorac-Jurcevic T, Missiaglia E, Blaveri E, et al. Molecular alterations in pancreatic carcinoma: Expression profiling shows that dysregulated expression of S100 genes is highly prevalent. J Pathol 2003;201(1):63–74.
46. Lu SY, Hua J, Liu J, et al. Construction of a paclitaxel-related competitive endogenous RNA network and identification of a potential regulatory axis in pancreatic cancer. Transl Oncol 2022;20(101419):101419.
47. Gong J, Li X, Feng Z, et al. Sorcin can trigger pancreatic cancer-associated new-onset diabetes through the secretion of inflammatory cytokines such as serpin E1 and CCL5. Exp Mol Med 2024;56(11):2535–47.
48. Li Z, Sclabas GM, Peng B, et al. Overexpression of synuclein-gamma in pancreatic adenocarcinoma. Cancer 2004;101(1):58–65.
49. Raimondi S, Lowenfels AB, Morselli-Labate AM, et al. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. Best Pract Res Clin Gastroenterol 2010;24(3):349–58.
50. Duell EJ, Lucenteforte E, Olson SH, et al. Pancreatitis and pancreatic cancer risk: A pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol 2012;23(11):2964–70.
51. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013;144(6):1252–61.
52. Tirkes T, Shah ZK, Takahashi N, et al. Reporting standards for Chronic Pancreatitis by using CT, MRI, and MR cholangiopancreatography: The Consortium for the Study of Chronic Pancreatitis, diabetes, and Pancreatic Cancer. Radiology 2019;290(1):207–15.
53. Iglesias-Garcia J, de la Iglesia-Garcia D, Lariño-Noia J, et al. Endoscopic ultrasound (EUS) guided elastography. Diagnostics (Basel) 2023;13(10):1686.