

1 *Supplement*

2 **Identification and validation of a multivariable prediction model based on blood**
3 **plasma and serum metabolomics for the distinction of chronic pancreatitis subjects**
4 **from non-pancreas disease control subjects**

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36 **Suppl. Material and Methods**

37 *Study details*

38 We conducted a type 3 study for multivariable prediction for individual prognosis
39 according to the TRIPOD guidelines [1]. Patients with chronic pancreatitis, liver cirrhosis,
40 healthy blood donors and preoperative patients with non-pancreatic or liver disease were
41 consecutively recruited from university referral centers in Greifswald, Dresden, Berlin, and
42 Bochum, all in Germany.

43 For the identification study, EDTA plasma samples were collected within a case-control
44 study from 80 patients with CP and 80 non-pancreatic control patients, who underwent
45 small, non-pancreas-related surgical procedures under general anesthesia (see below).

46 For the first validation study, 144 chronic pancreatitis patients and 204 non-pancreatic
47 control patients were recruited at three different centers. Because it was acknowledged that
48 CP patients are relatively young and mostly male, during patient recruitment, special care
49 was taken to also recruit younger and mostly male patients for the control group with the
50 aim to achieve a similar average patient age and a similar ratio of male to female subjects
51 in both groups.

52 In the second validation study, a different sample type was utilized: serum samples
53 taken from 49 chronic pancreatitis patients, 56 controls, and 57 liver cirrhosis patients were
54 analyzed. These samples were collected in a fourth independent center. Furthermore, the
55 control group consisted of healthy blood donors instead of patients waiting to undergo a
56 small surgery.

57 The key study dates for the three studies were as follows: accrual for the identification
58 study was started on 2009-01-13, end of accrual was on 2013-08-01. Accrual for the first
59 validation study was started on 2013-09-09 and ended on 2015-09-28. Accrual for the
60 second validation study started on 2002-10-23 and ended on 2010-06-10.

61

62 The general inclusion criteria for all groups included written informed consent prior to
63 any study procedures, age ≥ 18 to 85 years and eight hours fasting prior to blood draw.

64 The general exclusion criteria for all groups included type I diabetes, pregnancy or
65 lactation phase, known viral infections like hepatitis B, hepatitis C, HIV, major surgery within
66 the last 4 weeks before sample collection, acute anemia (Hb < 9 g/dl or < 5.58 mmol/l),
67 malignant tumors within the last 5 years.

68 Chronic pancreatitis patients were included if one or more of the following criteria were
69 met and no other diagnosis was more likely [2, 3, 4]: recurrent bouts of pancreatic pain with
70 documented rise in amylase or lipase activity for a duration of more than one year plus
71 radiological evidence supporting the diagnosis, pancreatic calcifications, histological proof

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72 of chronic pancreatitis, unequivocal changes in pancreatic duct morphology, severely
73 abnormal pancreatic function tests with maldigestion. Calcifications were identified on CT-
74 scan, diabetes was diagnosed as suggested by the WHO definition and exocrine
75 insufficiency was determined by either fecal elastase measurement or concurrent
76 pancreatic enzyme supplementation. Pancreatitis patients were excluded if they had
77 undergone pancreatitis surgery within 6 months before sample collection, bile duct stent
78 placement or surgery, endoscopically assisted pancreatic aspiration <5 days before sample
79 collection or had known liver cirrhosis.

80 Liver cirrhosis patients were included if preexisting liver cirrhosis had been diagnosed
81 based on imaging and clinical chemistry. Liver cirrhosis patients were excluded if
82 concomitant chronic pancreatitis was present.

83 Control patients were included if they were undergoing minor non-pancreatic surgery
84 under general anesthesia. Control patients were excluded if they had chronic pancreatitis
85 or liver cirrhosis or if a hernia was due to solid organ transplantation.

86 For the blood donors, only the standard blood donor inclusion criteria applied, i.e. the
87 donors had to be in good general health, body weight at least 110 pounds. Participants with
88 diabetes type II were excluded from the blood donor group because of the requirement of
89 a fasting period of at least 8 hours which was not considered feasible for diabetics.

90

91 From the patients in the non-pancreatic control group in the identification study, 20
92 patients underwent vascular surgery, 18 patients received a hernia repair, 3 were resected
93 for goiter and 39 received various other small surgical procedures under general
94 anesthesia. From the patients in the non-pancreatic control group in the first validation
95 study, 164 underwent hernia repair surgery and 40 were resected for thyroid goiter. None
96 were operated in metabolically deranged state. Thus, the data in the first validation study
97 differed from the identification study as it was a multicentric study, and in the composition
98 of the non-pancreatic controls. The second validation study differed in the matrix used for
99 analysis (serum instead of plasma), the center where the samples were obtained, the
100 control group (healthy blood donors instead of non-pancreatic controls), and the inclusion
101 of liver cirrhosis patients as an additional control group. Furthermore, 22.5% of the non-
102 pancreatic controls in the identification study were diabetes type II patients, while 13.5% of
103 the patients suffered from diabetes type II in the first validation study, and diabetes patients
104 were excluded as control in the second validation study. As opposed to the identification
105 study, the genesis of pancreatitis, calcifications, exocrine insufficiency, and enzyme
106 supplementation were only partially available in the validation studies.

107

108 *Sample Storage*

109 Before freezing, the EDTA plasma samples and serum samples were aliquoted to avoid
110 freeze-thaw cycles during the measurement period. Samples were stored at the respective
111 center at -80°C until transport to the measurement location, which occurred on dry ice.
112 Samples were stored at the measurement location at -80°C until measurement.

113 Our own work has shown that there were no significant differences in the metabolome
114 of plasma samples due to storage when stored at -80°C for up to 7 years [5]. Even though
115 the sample collection for one of the studies employed here started in 2003 already, all
116 samples were measured within 7 years after sample collection. The longest sample storage
117 time before measurement was 1637 days (about 4.5 years). Thus, a marked influence of
118 freeze-thaw cycles or sample age on the results of this study can be excluded.

119 *Metabolite profiling details*

120 Briefly, proteins were removed from the samples by precipitation, using three volumes of
121 acetonitrile. Polar and nonpolar fractions were separated by adding water and a mixture of
122 ethanol and dichloromethane (2:1, v/v). For GC-MS analysis, the nonpolar fraction was treated
123 with methanol under acidic conditions to yield the fatty acid methyl esters derived from both
124 free fatty acids and hydrolyzed complex lipids. The polar and nonpolar fractions were further
125 derivatized with O-methyl-hydroxylamine hydrochloride to convert oxo-groups to O-methyl-
126 oximes, and subsequently with N-methyl-N-(trimethylsilyl)trifluoroacetamide prior to analysis.
127 For LC-MS/MS analysis, both fractions were dried and reconstituted in appropriate solvent
128 mixtures. High-performance liquid chromatography was performed by gradient elution using
129 methanol/water/formic acid on reversed phase separation columns. Mass spectrometric
130 detection technology was applied as described in patent WO2003073464 [6] which allows
131 targeted and high-sensitivity multiple reaction monitoring (MRM) profiling in parallel to a full
132 screen analysis. In brief, mass spectrometric detection was performed with repetitive cycles of
133 MRM transitions for pre-selected metabolites followed by a full scan from a mass-to-charge
134 ratio of 100 to 1000. The instrument was operated in positive ionization mode for metabolites
135 in the nonpolar fraction, and in negative ionization mode for metabolites in the polar fraction.
136 Metabolite identification was done by comparing sample data to authentic standards where
137 applicable, as outlined previously [7].

138 MxP® Lipids covered profiling of sphingolipids (ceramides, sphingomyelins, and
139 sphingobases). Total lipids were extracted from the sample by liquid/liquid extraction using
140 chloroform/methanol. The lipid extracts were subsequently fractionated by normal phase liquid
141 chromatography (NPLC) into different lipid groups according to [8, 9]. The fractions were
142 analyzed by LC-MS/MS using electrospray ionization (ESI) and atmospheric pressure
143 chemical ionization (APCI) with detection of specific MRM transitions for preselected
144 sphingolipids.

145 Metabolite profiling generated semi-quantitative data of metabolite concentrations
146 calculated by determining metabolite levels in each study sample relative to metabolite
147 concentrations in reference pool samples that were created from aliquots of all study samples.
148 The normalization to reference pool samples compensates for inter- and intra-instrumental
149 variation, i.e. variability that occurs when different analytical sequences were analyzed by
150 different devices. To allow comparison of data sets between the different studies, the semi-
151 quantitative data were further normalized to the median of MxPool™ samples representing a
152 pool of commercial human EDTA plasma containing more than 2,000 different metabolites of
153 known concentrations. A one-point calibration was used to calculate quantitative absolute
154 concentrations for those metabolites present in the MxPool. Both types of pooled reference
155 samples were run in parallel through the entire process.

156

157 *Biomarker selection*

158 The metabolites for the biomarker panel were nominated based on biomedical expertise.
159 In a first step, features that markedly differentiate CP patients from controls that could have an
160 influence on the metabolome were considered. CP patients frequently suffer from lipid
161 malabsorption and gut microbiome changes due to reduced bile acid secretion, reduced
162 endocrine pancreatic function, pancreatic tissue fibrosis, and pancreatic inflammation. In a
163 second step, metabolite groups that were expected to be different between CP patients and
164 controls based on these physiological differences were collected: nutritional lipids that would
165 be affected from malabsorption, microbiome-derived metabolites that could be affected by gut
166 microbiome changes, carbohydrate metabolites that that would be affected by the reduced
167 endocrine function, metabolites that would be altered in response to fibrosis, and metabolites
168 that would be altered in response to inflammatory processes. In a third step, single
169 representative metabolites from these groups were chosen for the signature panel based on
170 methodical experience (the metabolites needed to allow for robust measurements above the
171 limit of detection), available literature, and experience from previous experiments with CP
172 patients and controls.

173

174 *Prediction model*

175 One prediction model was employed for all three study cohorts, i.e. the beta coefficients
176 obtained from the first cohort were then applied to the individuals from the other 2 cohorts.
177 Our prediction model, consisting of the biomarker signature, the corresponding algorithm,
178 and the established cut-off, predicts whether a patient suffers from chronic pancreatitis. The
179 biomarker enables a clinical diagnosis, supporting the standard diagnostic means for

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180 diagnosis of chronic pancreatitis (see above). The biomarker is not designed to be applied
181 for screening of the general population.

182 To avoid any bias when analyzing the concentrations of the metabolites present in the
183 biomarker signature, the diagnosis was blinded to the scientists measuring the samples
184 using mass spectrometry. The concentration values in the plasma samples of the 8
185 metabolites present in the biomarker signature are the only predictors used in the prediction
186 model. Furthermore, the calculation of the biomarker score by the algorithm and selection
187 of the cut-off was done fully automated, without human interference. After the initial
188 calculation based on the identification study results, there were no subsequent interventions
189 like patient exclusions, cut-off optimization, or re-training of the algorithm. Vice versa, the
190 clinical diagnosis was established in the participating clinical centers according to the
191 criteria mentioned above before the plasma samples were taken and analyzed in this study.
192 Thus, the outcome obtained with the prediction model did not have any effect on the clinical
193 diagnosis.

194 *Statistical analysis details*

195 Power analysis was performed to estimate an adequate sample size using
196 representative metabolite profiling standard deviations that were determined by
197 metanomics GmbH in earlier studies. Primary goal of the study was to determine a 20%
198 metabolic difference on a 5% significance level with approximately 72-99% power on the
199 basis of the patient samples. Metabolic difference was defined as absolute or relative
200 difference in concentrations of individual metabolites. Power estimates were based on t-test
201 statistics.

202 Missing data were handled differently depending on the analysis. For the Naive Bayes
203 algorithm and the principal component analysis, missing values were imputed with the
204 NIPALS (Non-linear Iterative Partial Least Squares) algorithm [10]. In the second validation
205 study, BMI values were not available for all participants. For the inclusion of the BMI as a
206 confounding factor in the MANOVA, the missing BMI values were imputed using K-means
207 clustering [11] for this purpose.

208 All R packages used, sorted by analysis step and including the utilized functions, are
209 listed in supplemental **Table S1**.

210

211 **Suppl. Results**

212 Metabolomic analyses details

213 The metabolomics data underwent a strict quality control after which 505 known and
214 115 unknown metabolites remained for statistical analysis in the datasets based on plasma
215 samples. Most of these metabolites could also be detected in the study conducted with
216 serum samples. In this dataset, 498 known and 118 unknown metabolites remained for
217 statistical analysis that met the quality control criteria.

218 Concentration data were missing for beta-carotin from 2 samples in the second
219 validation study, for cryptoxanthin in 7 samples from the second validation study, for N-
220 acetylcytidine in 2 samples from the first and 1 sample from the second validation study, for
221 behenic acid in 1 sample from the identification study, 6 samples from the first validation
222 study, and 1 sample in the second validation study, for mannose in 16 samples from the
223 first validation study and 5 samples from the second validation study, for indole-3-acetic
224 acid for 1 sample in the identification study, for 28 samples in the first validation study, and
225 for 7 samples in the second validation study, for hippuric acid for 1 sample in the second
226 validation study, and for ceramide (d18:1,C24:1) for 1 sample in the first validation study
227 and 17 samples in the second validation study. Normalized to the number of samples in
228 each study, this means that a maximum of 1% of the values for a given metabolite were
229 missing in the identification study, maximally 8% in the first validation study and maximally
230 10% in the second validation study. There were no cases where the outcome (diagnosis)
231 was unknown or missing.

232 In total, 60 metabolites were not significantly different ($p > 0.05$ or $q > 0.2$) between CP
233 and control groups in any of the three studies. 516 metabolites were significantly different
234 in some of the studies and 39 metabolites were significantly ($p < 0.05$ and $q < 0.2$) different
235 in all of the three studies. 6 of the 8 metabolites from the signature metabolite panel had a
236 p value below the significance threshold ($p < 0.05$) in all three studies (see **Table 4**). The
237 other 2 of the 8 metabolites from the panel (behenic acid and indole-3-acetic acid) were
238 significantly different between the groups in the identification and the first validation study,
239 but not in the second validation study that had serum as a sample matrix.

240 The panel metabolites were not the best 8 discriminators between CP patients and
241 controls. Beta-carotene and cryptoxanthin were among the top 3 discriminators in the
242 plasma-based studies (identification and first validation study), with lycopene being the best
243 discriminator in the plasma studies. Looking at all three studies together, beta-carotene,
244 cryptoxanthin, and mannose were among the top 5 discriminators, with 3-hydroxybutyrate
245 being the best discriminator.

246 The distribution of age and BMI over the biomarker signature score is shown in
247 supplemental **Figure S1**. The age gap between CP patients and non-pancreatic controls is
248 markedly higher in the identification study than in the validation. In the first validation study,
249 the age of the patients follows an even Gaussian distribution for both CP patients and non-

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250 pancreatic controls. In both studies, the score is markedly higher for CP patients compared
251 to non-pancreatic controls irrespective of the age. As the BMI was calculated with a decimal,
252 there are a lot of potential sublevels, which results in more data being needed for Gaussian
253 curves. Nevertheless, the BMI of non-pancreatic controls in the validation study also follows
254 a Gaussian distribution, while the BMI of CP patient is clearly skewed due to the increased
255 frequency of patients with low BMI. This is an inherent feature of the disease concomitant
256 with the malnutrition caused by CP. These trends can also be observed in the identification
257 study. Despite the uneven BMI distribution, the graphs show that the biomarker score is
258 markedly higher for CP patients compared to non-pancreatic controls irrespective of the
259 BMI.

260 The full prediction model can be used universally. The weighting of the metabolites as
261 shown in supplemental **Table S2** can be used as coefficients to be multiplied with the
262 respective concentrations of the eight metabolites in the biomarker signature (in $\mu\text{mol} / \text{L}$)
263 to calculate the biomarker signature score. Whether the score is above or below the cut-off
264 value of 0.479 determines whether the patient is evaluated as positive or negative for the
265 diagnosis “chronic pancreatitis”.

266

267 *Effect of exocrine insufficiency and enzyme supplementation on carotenoid levels*

268 Because the identification of beta-carotene and cryptoxanthin suggested a
269 pathophysiological mechanism of malabsorption, it was analyzed whether pancreatic
270 exocrine insufficiency and enzyme supplementation had an effect on plasma carotenoid
271 levels. This analysis was limited to the identification study because the full information was
272 available for this cohort only. Almost all patients with exocrine insufficiency also received
273 enzyme supplementation so that a separate comparison of the effect of exocrine
274 insufficiency alone was not possible. As obvious from **Figure S2**, there was no significant
275 increase of carotenoid levels in plasma of chronic pancreatitis patients supplemented with
276 enzymes to treat exocrine insufficiency.

277

278 *Biomarker signature score increases with disease severity*

279 In clinical daily routine, patients that will be tested for chronic pancreatitis are not
280 always as healthy as the control groups used in this study. We therefore wanted to
281 investigate whether the biomarker signature score was more accurate in patients with
282 advanced disease than in less severe cases. Because other clinical data regarding disease
283 severity was elusive, we used the information whether the patients suffered from pancreatic
284 endocrine or exocrine insufficiency, which is a good surrogate marker for severity and time

285 since disease onset. 199 chronic pancreatitis patients from the identification and the first
286 validation study for whom this information was available together were categorized in three
287 groups: those without insufficiencies, those with either endo- or exocrine insufficiency, and
288 those with both endo- and exocrine insufficiencies. The distribution of biomarker signature
289 score values in the three groups is shown in **Figure S3**. The average biomarker signature
290 score was 0.68 in patients without insufficiencies, 0.78 in patients with either endo-or
291 exocrine insufficiency, and 0.90 in patients with both endo- and exocrine insufficiency. An
292 ANOVA was employed to test whether the differences in the biomarker signature score
293 were significant. While the group with one pancreatic insufficiency did not have a
294 significantly different score compared to the other groups, the scores of the groups without
295 pancreatic insufficiencies and with both endo- and exocrine insufficiencies were significantly
296 different ($p = 0.0018$). This indicates that the biomarker signature score is higher in patients
297 with more severe pancreatic disease.

298 Using the established cut-off of 0.479, we concluded for each patient in the three groups
299 whether the diagnosis based on the signature score was correct or a false negative. A
300 chi-squared test was employed to investigate whether the diagnosis and the severity were
301 co-dependent. The result of $p = 0.056$ shows a clear trend towards a higher fraction of
302 correct diagnoses in more severe cases, although there was no significant dependency
303 between the group affiliation and the diagnosis.

304

305 References

- 306 1 Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent Reporting of a multivariable prediction
307 model for Individual Prognosis Or Diagnosis (TRIPOD): the TRIPOD Statement. *Br J Surg* 2015;**102**:148-58.
- 308 2 Mayerle J, Hoffmeister A, Werner J, Witt H, Lerch MM, Mossner J. Chronic pancreatitis--definition,
309 etiology, investigation and treatment. *Dtsch Arztebl Int* 2013;**110**:387-93.
- 310 3 Ito T, Ishiguro H, Ohara H, Kamisawa T, Sakagami J, Sata N, *et al*. Evidence-based clinical practice
311 guidelines for chronic pancreatitis 2015. *J Gastroenterol* 2016;**51**:85-92.
- 312 4 Whitcomb DC, Shimosegawa T, Chari ST, Forsmark CE, Frulloni L, Garg P, *et al*. International consensus
313 statements on early chronic Pancreatitis. Recommendations from the working group for the international consensus
314 guidelines for chronic pancreatitis in collaboration with The International Association of Pancreatology, American
315 Pancreatic Association, Japan Pancreas Society, PancreasFest Working Group and European Pancreatic Club.
316 *Pancreatology* 2018.
- 317 5 Wagner-Golbs A, Neuber S, Kamlage B, Christiansen N, Bethan B, Rennefahrt U, *et al*. Effects of Long-
318 Term Storage at -80 °C on the Human Plasma Metabolome. *Metabolites* 2019;**9**.
- 319 6 Walk TB, Dostler M. Mass Spectrometry Method for Analysing Mixtures of Substances.
320 2003:PCT/EP2003/001274.
- 321 7 Kamlage B, Neuber S, Bethan B, González Maldonado S, Wagner-Golbs A, Peter E, *et al*. Impact of
322 Prolonged Blood Incubation and Extended Serum Storage at Room Temperature on the Human Serum
323 Metabolome. *Metabolites* 2018;**8**.
- 324 8 Christie WW. Rapid separation and quantification of lipid classes by high performance liquid
325 chromatography and mass (light-scattering) detection. *J Lipid Res* 1985;**26**:507-12.
- 326 9 Schmidt H, Schmidt R, Geisslinger G. LC-MS/MS-analysis of sphingosine-1-phosphate and related
327 compounds in plasma samples. *Prostaglandins Other Lipid Mediat* 2006;**81**:162-70.

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328 10 Noonan R, Wold H. NIPALS Path Modelling with Latent Variables. Scandinavian Journal of Educational
329 Research 1977;21:33-61.
330 11 Gajawada S, Toshniwal D. Missing Value Imputation Method Based on Clustering and Nearest
331 Neighbours. International Journal of Future Computer and Communication 2012;1:pp. 206-8.
332

333 **Suppl. Figures and Tables:**

334 **Figure S1.** Distribution of age (A,C) and BMI (B,D) over biomarker signature score in the
335 identification study (A,B) and the first validation study (C,D). Demographics are shown on the
336 x axes, the biomarker signature score on the y axes. Stacked columns for age and side-by-
337 side columns for BMI are colored according to outcome (diagnosis). Horizontal lines / functions
338 represent Gaussian approximation of the data.

339

340 **Figure S2.** Boxplots of effect of enzyme supplementation on plasma carotenoid levels. Shown
341 are data for chronic pancreatitis patients of the identification study (plasma). There was no
342 significant increase of carotenoid levels in plasma of patients supplemented with enzymes to
343 treat exocrine insufficiency.

344

345 **Figure S3.** Boxplots of biomarker signature scores in chronic pancreatitis patients from the
346 identification study and the first validation study, which were categorized depending on
347 whether they suffer from endo- and/or exocrine insufficiencies as a measure of disease stage.
348 The average biomarker signature score increased with disease severity and this increase was
349 significant comparing patients without insufficiency and patients with both endo- and exocrine
350 insufficiency.

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352 **Table S1:** R packages and functions used

Analysis	Packages	Functions
PCA	Stats (included in R core pkge)	prcomp()
Biomarker training and test	E1071 ROCR ROCR pROC	naivebayes() performance() prediction() roc()
ANOVA/MANOVA	slme stats (included in R core pkge)	lme() p.adjust()
Visualizations	ggplot2	

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356 **Table S2:** Weightings of all metabolites in the biomarker signature

Metabolite	Weight	Mean	SD ±
Beta-carotene	1.1749422	-0.9281	0.4641
Cryptoxanthin	1.2061438	-1.1714	0.4558
Mannose	0.7865473	1.6548	0.1678
Behenic acid (C22:0)	0.6728898	1.5537	0.2095
Ceramide (d18:1/ C24:1)	0.6066595	0.1294	0.1427
Indole-3-acetic acid	0.5846360	-0.0213	0.3273
Hippuric acid	0.5009786	-0.3020	0.4408
N-Acetylcytidine	0.1068720	0.0807	0.1897

357 SD: standard deviation

358 **Table S3.** Statistical analysis results (linear model) of the signature metabolites (CP vs.
359 control) with FDR corrected p-values (q values).

Dataset (matrix)	Identification study (plasma)	First validation study (plasma)	Second validation study (serum)
Metabolite	Fold change (q-value)	Fold change (q-value)	Fold change (q-value)
Beta-carotene	0.37 (9.8 x 10 ⁻⁶)	0.38 (1.8 x 10 ⁻¹⁹)	0.47 (0.00039)
Cryptoxanthin	0.40 (9.9 x 10 ⁻⁶)	0.38 (4.5 x 10 ⁻¹⁸)	0.48 (0.00090)
Mannose	1.47 (9.9 x 10 ⁻⁶)	1.52 (6.7 x 10 ⁻²⁷)	2.80 (8.4 x 10 ⁻¹³)
Behenic acid (C22:0)	0.72 (0.0080)	0.67 (1.0 x 10 ⁻¹⁵)	1.14 (0.14)
Ceramide (d18:1, C24:1)	1.27 (0.0059)	1.28 (1.0 x 10 ⁻¹⁰)	1.96 (5.9 x 10 ⁻¹²)
Indole-3-acetic acid	0.63 (0.013)	0.62 (2.4 x 10 ⁻⁸)	1.15 (0.40)
Hippuric acid	0.68 (0.15)	0.49 (1.2 x 10 ⁻⁷)	0.51 (0.0034)
N-Acetylcytidine	1.22 (0.058)	1.21 (8.1 x 10 ⁻⁵)	2.36 (3.2 x 10 ⁻¹²)

360 CP: Chronic pancreatitis

361