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METABOLOMIC PROFILING FOR IBD DIAGNOSIS: SEARCH OF OPTIMAL SETS OF METABOLOMIC IBD MARKER

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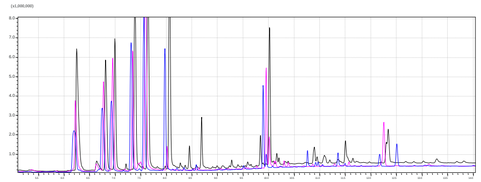
INRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the most common types of IBD. CD and UC are practically indistinguishable at early stages, which makes its diagnostics difficult. Various molecular methods are designed to carry out for global search of the most informative markers for IBD types verification and CD and UC differential diagnosis. Among omics technologies, metabolomics, as one of the promising tools that can contribute to the search and rapid implementation of informative markers. Today, various options are available for the assessment of low molecular weight compounds, such as HPLC-MS/MS, GC-MS/MS, etc. In this study, we combined the search for metabolomic markers using HPLC-MS/MS and HS GC-MS/MS methods for analyzing such groups of metabolites as lipids, volatile metabolites and hydrophilic metabolites in serum and stool samples from Crohn's disease and ulcerative colitis patients.

Shimadzu QP2010 Ultra GC / MS
with a Shimadzu HS-20 headspace extractor

MATERIAL & METHODS

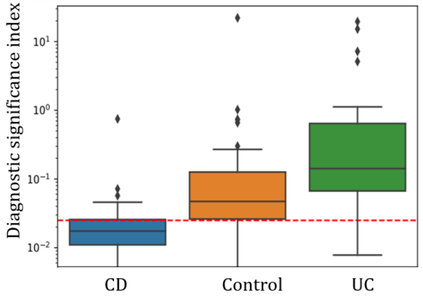
The study was conducted for groups of patients with a verified diagnosis of Crohn's disease and ulcerative colitis. Serum and stool samples were obtained from patients in a hospital. Informed consent for the study was obtained from all patients. The total number of serum and stool samples was 160 samples (CD and UC combined). The samples were provided by the Department of Gastroenterology of the M.F. Vladimirskiy Moscow Regional Research and Clinical Institute, Moscow, Russia., as well as the Department of Gastroenterology of the LOPUKHIN FRCC PCM. Lipid and hydrophilic fractions of metabolites extracted from serum was carried out using HPLC-MS TripleTOF 6600+ System. Stool samples were analyzed using a Shimadzu QP2010 Ultra GC/MS with a Shimadzu HS-20 headspace extractor.



SCFA distribution in fecal samples of Crohn's (CD - pink), Ulcerative colitis (UC - blue) and norm (black) measured by HS GC-MS

RESULTS

Stool samples analysis shown that isovaleric acid is one of the markers for the IBD and the ratio of valeric and isovaleric acids using the logistic regression method allows us to obtain a risk coefficient for the IBD development. Analysis of the serum hydrophilic fraction demonstrated quantitative difference for the following compounds: Sphingosine, Urocanic Acid, N-Acetylcytidine, 5'-S-Methylthioadenosine, Adenine, -Hydroxyanthranilic acid, Acetylcarnitine, O-Acetyl-L-carnitine, Nicotinamide, Phenylalanine, Isoleucine, Choline chloride, Proline, Cholic acid_CA, Chenodeoxycholic acid, Inosine, Glycoursodeoxycholic acid. Mentioned markers contributed to the IBD verification, while O-Acetyl-L-carnitine contribute to the differentiation between Crohn's disease and ulcerative colitis. When analyzing the lipid fraction, differences were identified for a limited range of lipids, however, such lipids as Cer 37:0;3O|Cer 20:0;2O/17:0;O, PE P-36:2|PE P-18:0_18 :2 and PC 36:2|PC 18:1_18:1 were detected only in CD samples.



COMPOUND	IBD P val ≤ 0.005
Phenylalanine	0.000284
Isoleucine	4.38E-08
Choline chloride	1.34E-05
Proline	4.83E-05
Cholic acid_CA	0.0002
Chenodeoxycholic acid_CDCA	2.52E-12
Inosine	1.21E-10
Glycoursodeoxycholic acid_GUDCA	0.000278
COMPOUND	CD P val ≤ 0.005
o-acetyl-l-carnitine	2.07E-05

COMPOUND	CD P val ≤ 0.005	UC P val ≤ 0.005
Sphingosine	0.0148	0.0066
Urocanic Acid	0.00018	0.0051
N-Acetylcytidine	1.48E-12	4.24E-10
5'-S-Methylthioadenosine	1.95E-08	1.58E-05
Adenine	4.86E-12	1.24E-09
3-Hydroxyanthranilic acid	0.00123	0.004
Acetylcarnitine	0.1395	0.004
O-Acetyl-L-carnitine	0.0481	0.0015
Nicotinamide	6.23E-06	0.0785

COMPOUND	CD P val ≤ 0.005	UC P val ≤ 0.005
Butanoic acid	0.004462696	0.064402354
Pentanoic acid	0.013579858	0.003341907
Pentanoic acid 4-methyl-	0.000321018	5.34E-05
Phenol	0.0032843	9.22E-05
Hexadecanal	0.003604542	0.01381602
Indole	0.000694253	0.019997108
Hydrocinnamic acid	0.000750808	0.00062105

SUMMARY / CONCLUSION

Conclusions: The obtained metabolomic differences in the hydrophilic fraction and volatile components make it possible to reliably verify inflammatory bowel diseases, since separate lipid markers and O-Acetyl-L-carnitine contribute to the CD detection.

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All authors declare that they have no conflicts of interest related to this work.