Journal of Cystic Fibrosis 15 (2016) 242–250

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Faecal proteomics: A tool to investigate dysbiosis and inflammation in patients with cystic fibrosis

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Received 16 April 2015; revised 2 July 2015; accepted 13 August 2015
Available online 30 August 2015

Abstract

Background: Several microbial studies reported gut microbiota dysbiosis in patients with cystic fibrosis (CF). The functional consequences of this phenomenon are poorly understood. Faecal metaproteomics allows the quantitative analysis of host and microbial proteins to address functional changes resulting from this dysbiosis.
Methods: We analysed faecal protein extracts from fifteen patients with CF that have pancreatic insufficiency and from their unaffected siblings by shotgun proteomics. Novel computational and statistical tools were introduced to evaluate changes in taxonomic composition and protein abundance.
Results: Faecal protein extracts from patients with CF were dominated by host proteins involved in inflammation and mucus formation. Taxonomic analysis of the microbial proteins confirmed the strong reduction of butyrate reducers such as *Faecalibacterium prausnitzii* and increase of Enterobacteriaceae, *Ruminococcus gnavus* and Clostridia species.
Conclusion: Faecal metaproteomics provides insights in intestinal dysbiosis, inflammation in patients with CF and can be used to monitor different disease markers in parallel.

Keywords: Cystic fibrosis; Metaproteomics; Dysbiosis; Unipept

1. Introduction

Patients with cystic fibrosis (CF) suffer from persistent lung infections and undergo repeated antibiotic treatment, disturbing the beneficial host–microbiota relationship [1]. The disease also affects pancreatic and intestinal functions: the thick mucus in the small intestine decreases the absorption of nutrients and bile acids. Moreover, fat-rich diets combined with a decreased release of digestive enzymes by the pancreatic duct lead to a different alimentary environment wherein the gut microbiota resides. These anomalies have major consequences for gut microbiota composition, cause intestinal inflammation and affect well-being of patients with CF [2].

Abbreviations: ACE, angiotensin converting enzyme; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DIOS, distal intestinal obstruction syndrome; DMBT1, deleted in malignant brain tumours 1; GI, gastro-intestinal; GO, gene ontology; LC-MS, liquid chromatography-mass spectrometry; MS-MS, tandem mass spectrometry; NGAL, lipocalin-2; ORM, orosomucoid 1; PERT, pancreatic enzyme replacement therapy; SDS-PAGE, sodium dodecyl sulphate – polyacrylamide gel electrophoresis; TFF, trefoil factor peptide; ZAG, zinc-alpha-2-glycoprotein; ZINB, zero inflated negative binomial.

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http://dx.doi.org/10.1016/j.jcf.2015.08.003
1569-1993/

Previous studies describing the faecal microbiota composition pointed to a decrease in the abundance and diversity of intestinal bacteria of the phylum Firmicutes, specifically of butyrate-producing bacteria of the *Clostridium* clusters IV and XIVa [3]. Furthermore, metagenomic phylogenetic analysis showed that *Escherichia coli* was more abundant in patients with CF, suggesting that it contributes to CF gastrointestinal (GI) disease [4]. However, such taxonomic analyses poorly address the functional consequences in the digestive system. In the present study we introduce faecal metaproteomics as a tool to provide insights in the host and microbial protein composition of the GI tract. We prepared total protein extracts of faecal samples from 15 children with CF and their unaffected siblings. Proteins were separated by SDS-PAGE, digested by trypsin and analysed by LC-MS for identification. The Unipept platform was used to analyse the taxonomic origin of the tryptic peptides [5]. A novel statistical method was implemented to reveal significant differences in both the microbial diversity and the abundance of host and microbe proteins. This cross-sectional analysis provided new insights in the gut dysbiosis and the associated local inflammatory state in patients with CF.

2. Material and methods

2.1. Participants and ethics statement

Faecal samples of fifteen children with CF and their unaffected siblings were collected and frozen at −80 °C. Parents/guidance and participating children received written information containing the details of the study and gave oral informed consent, approved by the Ethics Committee of the University of Leuven (no. ML4698). The CF genotypes were: 10 homozygotes and 5 compound heterozygotes for the ΔF508 mutation. The nutritional and clinical statuses were assessed (Table 1). Patients and siblings were aged between 1.6 and 15.6 years and the average age difference within a patient/sibling pair was 3.9 years. 11 patient–sibling pairs were sex-matched. All patients were pancreatic insufficient.

2.2. Sample preparation

0.5 g fresh stool was homogenised in 9 ml 50 mM Tris-HCl / 10 mM $CaCl_2$ (pH 7.8) and protease inhibitor cocktail (Roche, Indianapolis, IN). This was followed by low speed centrifugation

Table 1
Clinical characteristics of the participants in this cross sectional study. The gender, age, CF genotype, % ideal weight for height (IWH) referring to Flemish Curve (FC), lung function (FEV1: forced expiratory volume in one second), exocrine pancreatic function and antibiotic administration were assessed.

Pat ID	Sample number	CF/ sibling	Gender		Age (Y)	% of IWH	# Days between antibiotics and sampling	Patient info				
			M	F		VC %		FEV1 (%)	IgG (g/l)	Mutation	Mutation class	No functional CFTR
3	3.8	CF	X		11.8	117.7	84	91	3.11	ΔF508/ΔF508	II/II	X
4	4.8	Sibling	X		5.1	106.3	105	–	–	–	–	
5	5.2	CF		X	13.0	106.3	0	90	8.18	ΔF508/C276X	II/I	X
6	6.2	Sibling		X	9.8	96.7	1095	–	–	–	–	
7	7.2	CF	X		6.9	90.9	68	110	8.96	ΔF508/ΔF508	II/II	X
8	8.2	Sibling	X		5.6	93.6	433	–				
9	9.6	CF		X	10.1	101.7	70	100	9.92	N1303K/ΔF508	II/II	X
10	10.6	Sibling		X	2.3	103.5	141	–	–			
11	11.1	CF		X	1.6	101.7	0	NA	3.88	ΔF508/ΔF508	II/II	X
13	13.1	Sibling		X	3.4	89.8	1095	–	–			
14	14.7	CF	X		8.3	91.0	77	88	10.90	ΔF508/ΔF508	II/II	X
15	15.7	Sibling		X	10.4	81.0	427	–	–			
21	21.4	CF	X		12.3	137.4	96	96	5.75	ΔF508/G542X	II/I	X
22	22.4	Sibling	X		13.8	113.6	165	–	–			
23	23.1	CF		X	2.4	105.1	112	NA	4.79	ΔF508/ΔF508	II/II	X
24	24.1	Sibling		X	5.9	133.4	365	–	–	–	–	
25	25.1	CF	X		10.9	96.9	218	87	8.25	ΔF508/S1251N	II/III	
26	26.1	Sibling		X	6.4	88.8	730	–	–			
27	27.1	CF	X		9.0	100.7	215	100	5.16	ΔF508/ΔF508	II/II	X
28	28.1	Sibling	X		6.8	118.2	730	–	–			
29	29.1	CF		X	10.6	87.0	77	93	10.70	ΔF508/ΔF508	II/II	X
30	30.1	Sibling		X	12.5	102.2	1752	–	–			
31	31.8	CF		X	12.5	94.9	84	105	12.60	ΔF508/ΔF508	II/II	X
32	32.8	Sibling		X	4.7	106.8	1096	–				
33	33.1	CF		X	3.9	95.1	0	NA	4.51	ΔF508/2183AA > G	II/I	X
34	34.1	Sibling		X	8.8	76.2	730	–	–			
39	39.1	CF	X		9.4	103.0	0	94	11.70	ΔF508/ΔF508	II/II	X
40	40.1	Sibling		X	15.6	130.4	1095	–	–			
43	43.1	CF	X		7.7	105.0	91	102	7.51	ΔF508/ΔF508	II/II	X
44	44.1	Sibling		X	10.6	118.6	730	–	–			