Title: Faecal (meta)proteomics : A tool to investigate dysbiosis and inflammation in patients with cystic fibrosis

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Cystic fibrosis is a genetic disease caused by mutation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein. The disease is mostly associated with severe lung pathogenesis typically caused by infection by *Pseudomonas aeruginosa*. However, many patients also suffer from intestinal complications. The primary cause is pancreas insufficiency resulting in decreased release of digestive enzymes which is typically cured by enzyme replacement therapy. However, sticky mucus formation and repetitive antibiotic treatment have a severe affect on the gut microcbiota. Several microbial studies indeed reported gut microbiota dysbiosis in patients with cystic fibrosis (CF). However, the functional consequences of this phenomenon are poorly understood.

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, among which the novel Unipept tool were introduced to evaluate changes in taxonomic composition and protein abundance. 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. Our work showed that faecal metaproteomics provides insights in intestinal dysbiosis, inflammation in patients with CF and can be used to monitor different disease markers in parallel.

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