Project number: 6574
Funding source: EU - FP7
Date: June 2017
Project dates: 2013-2017

Microbiota of Cystic Fibrosis and implications for patient treatment

Key external stakeholders:
Functional and medical foods companies, medical profession.
Practical implications for stakeholders:

- We investigated the influence of the GI microbiota in the treatment of CF disease.
- The gastrointestinal microbiota composition of persons with CF were investigated during periods of stability, infective exacerbation and maintenance antibiotic therapy
- We investigated if antibiotic treatment alters CF gut microbiota composition and the development and function of T cell pathways
- We determined whether the CF gut is a reservoir for antibiotic resistance genes
- We isolated, characterised, assessed and evaluated strains of probiotics isolated from persons with CF in ex vivo model.

Main results:
The microbiota composition of the gut of CF patients during periods of stability, on maintenance antibiotic therapy-treatment was determined. *Firmicutes* are the most abundant phylum in CF faecal samples during stability (~60% of the phylum reads), with *Bacteroidetes* the second most abundant phylum. We have shown that in some patients, their gut microbiota shows significant fluctuations in bacterial profile during periods of pulmonary stability. This highlights the importance of studying longitudinal data in this cohort, to help determine the cause of such changes, independent of pulmonary exacerbation. Furthermore, when we compared the gut microbiota of the CF and control participants, they were very distinct. Alpha diversity indexes (which measure the diversity within samples) found the highest diversity in the controls (higher diversity is seen as a positive trait). Diversity decreased in the CF patients at exacerbation and recovered to near stable levels three months post exacerbation. Beta diversity (a measure of the diversity between groups) showed that CF and control samples cluster independently.

For each patient that has been recruited a portion (1g) of the fresh faecal sample has been collected and used for culture based analysis to determine the prevalence of antibiotic resistant isolates in CF samples. Each sample was serially diluted and plated on 1) Wilkens Chalgrens Agar (WCA; total bacteria counts), 2) WCA with vancomycin (40mg/L), 3) WCA with ciprofloxacin (40mg/L) and 4) WCA with metronidazole (40mg/L). Colonies are enumerated on antibiotic containing plates and compared to plates without antibiotics. In addition, gDNA was quantitatively assessed for antibiotic resistance genes associated with vancomycin, ciprofloxacin and metronidazole using quantitative PCR. The data suggest that the majority of total anaerobes cultured were resistant to vancomycin, metronidazole and ciprofloxacin.

Strains of *Lactobacillus* from the gut of stable CF subjects who are receiving chronic macrolide therapy were isolated and characterized with a view to determining their potential usefulness as probiotics. Fresh stool was serially diluted in maximum recovery diluent (MRD) under anaerobic conditions and plated on *Lactobacillus* (LBS) selective media. Colonies were selected and biobanked at -80^{o}C. Six hundred isolates (500 lactobacilli) from 58 samples were stocked. From this we identified several potential probiotics and chose one isolate (*L. plantarum*) for further analysis. Further studies confirmed the ability of *L. plantarum* strain to survive in the model of the gastrointestinal tract in CF patients.

Contact Catherine Stanton Email: Catherine.stanton@teagasc.ie.

Opportunity / Benefit:

1. We have shown that the gut microbiota of stable CF patients shows temporal variability, demonstrating the importance of longitudinal analysis.
2. The gut microbiota of CF patients can be significantly altered at exacerbation- with those showing the greatest change in gut microbiota having significant increases in Enterococcus.
3. Bifidobacterium levels significantly decrease at exacerbation, with recovery to pre-exacerbation levels incomplete in several patients 3 months post exacerbation.
4. CF samples have a reduced alpha diversity compared to controls.
5. Gut microbiota diversity is lowest in CF patients during pulmonary exacerbation.
6. The gut microbiota of CF patients is distinct from sibling and non-sibling controls.
7. The gut microbiota of CF patients contains a large percentage of antibiotic resistant bacteria, with resistance to common CF antibiotics including vancomycin, metronidazole and ciprofloxacin.

Collaborating Institutions:
UCC, Teagasc, and FP7 CFMATTERS EU Partners

Teagasc project team: Prof Catherine Stanton (Teagasc Project Leader)

External collaborators: Prof. Barry Plant, UCC (Coordinator)
Prof. Paul Ross, UCC
Dr. Mary Rea, Teagasc.

1. Project background:

Cystic Fibrosis (CF) is an autosomal recessive disease affecting over 70,000 individuals globally. CF is caused by a mutation to the cystic fibrosis transmembrane conductance regulator gene, which encodes a chloride channel located on the apical layer of epithelial cells throughout the body. Thus the effects of this disease, while most severe in the lungs, occur throughout the body. The composition of the adult CF gut microbiota has not been extensively studied. Due to the lifelong exposure of the gut microbiota of CF patients to antibiotics, in addition to an altered diet, pancreatic insufficiency and frequent hospitalisation, it is likely the gut microbiota will be unique in CF patients. The aim of the work was to investigate the gut microbiota of CF patients during periods of pulmonary stability, exacerbation and 3 months post exacerbation.

Furthermore, the prevalence of antimicrobial resistance in the gut microbiota of CF patients was investigated. Recent studies have demonstrated that probiotic interventions in CF patients beneficially impact on the frequency of pulmonary exacerbations. We created a biobank of isolates from the CF gut that are antibiotic tolerant (to antibiotics frequently used in CF). These have potential benefits and the most promising probiotic was taken forward and tested *in-vitro* for ability to survive the gastrointestinal tract and to beneficially modulate the gut microbiota of CF patients.

Finally, *Clostridium difficile* carriage has previously been shown to be higher amongst CF patients compared to non-CF individuals. Additionally, CF patients generally are asymptomatic carriers of *C. difficile*. This work investigated the carriage rates of *C. difficile* in CF patients and investigated their ribotypes and toxin production.

2. Questions addressed by the project:

Cystic Fibrosis (CF) is an autosomal recessive disease affecting over 70,000 individuals globally. CF is caused by a mutation to the cystic fibrosis transmembrane conductance regulator gene, which encodes a chloride channel located on the apical layer of epithelial cells throughout the body. Thus the effects of this disease, while most severe in the lungs, occur throughout the body. The composition of the adult CF gut microbiota has not been extensively studied. Due to the lifelong exposure of the gut microbiota of CF patients to antibiotics, in addition to an altered diet, pancreatic insufficiency and frequent hospitalisation, it is likely the gut microbiota will be unique in CF patients. This work investigated the gut microbiota of CF patients during periods of pulmonary stability, exacerbation and 3 months post exacerbation.

3. The experimental studies:

This project involved studies to assess the composition of the adult CF gut microbiota, during periods of pulmonary stability, exacerbation and 3 months post exacerbation. We have provided the largest longitudinal

Contact Catherine Stanton Email: Catherine.stanton@teagasc.ie.

investigation into the gut microbiota of adult CF patients.

4. Main results:

This work addressed the significant alterations in the lung and gut microbiota across time and disease state in CF patients. Additionally, we have demonstated that there are significant correlations between the lung and gut microbiota. We have demonstrated the impact that pulmonary exacerbation and its associated treatment has on the CF gut microbiota.

5. Opportunity/Benefit:

This is invaluable information to help advance our understanding of how the CF gut is altered during different stages of pulmonary disease. Furthermore, we can use such information to advance our understanding of the lung-gut axis and investigate how changes in the gut microbiota impact on the lung microbiota. Furthermore, we have significantly advanced our understanding of the carriage rate of C. difficile within the CF community, which will enable us to further investigate why this cohort has such high asymptomatic carriage rates. Additionally, we have developed a CF specific probiotic, with tolerance to antibiotics commonly used in CF patients and an ability to survive in the gastrointestinal tract. We successfully isolated an antibiotic tolerant probiotic with potential to be administered as an adjunct therapy to common antibiotic regimes in CF. This potential probiotic was then tested *in vitro* for its ability to survive in the gastrointestinal tract (as tested using a distal colon model) and to exert modulatory effects on the gut microbiota.

6. Dissemination:

Deane, J. Ph.D. Thesis, UCC: Cystic Fibrosis and the Gut Microbiota, (2017).

Fouhy F, Ronan NJ, O'Sullivan O, McCarthy Y, Walsh AM, Murphy DM, Daly M, Flanagan ET, Fleming C, McCarthy M, Shortt C, Eustace JA, Shanahan F, Rea MC, Ross RP, Stanton C, Plant BJ. (2017). A pilot study demonstrating the altered gut microbiota functionality in stable adults with Cystic Fibrosis. Sci Rep, 7:6685.

Burke DG, Fouhy F, Harrison MJ, Rea MC, Cotter PD, O' Sullivan O, Stanton C, Hill C, Shanahan F, Plant BJ, Ross RP. (2017). The altered gut microbiota in adults with cystic fibrosis. BMC Microbiol, 17: 58.

Burke DG, Harrison MJ, Fleming C, McCarthy M, Shortt C, Sulaiman I, Murphy DM, Eustace JA, Shanahan F, Hill C, Stanton C, Rea MC, Ross RP, Plant BJ. (2017). *Clostridium difficile* carriage in adult cystic fibrosis (CF); implications for patients with CF and the potential for transmission of nosocomial infection. J Cyst Fibros, 16(2): 291-298.

Burke D, Fouhy F, Rea M, Harrison M, Stanton C, O'Sullivan O, Murphy D, O'Callaghan G, Eustace J, Shanahan F: Altered gut microbiota in stable patients with cystic fibrosis (CF) compared to controls and its relationship with intravenous (IV) antibiotic usage and lung function. *Journal of Cystic Fibrosis* 2015, 14:S69.

7. Popular publications:

Intellectual Property on probiotic for CF gut health (Invention Disclosure Form)

Plant, B. et al. A longitudinal, multi-centre investigation into the gut microbiota of adult CF patients-the CFMATTERS perspective, 40th ECFS Conference, 8/6/2017.

Fouhy, F et al. An investigation into the gut microbiota of people with CF and the effects of an altered gut microbiota on functionality and faecal metabolites as determined by the CFMATTERS study, 39th ECFS Conference, 11/6/2016.

Fouhy, F. Gut microbiota in Cystic Fibrosis-current knowledge and future opportunities, 3rd Microbiome R&D and Business Collaboration Forum: Europe 11/4/2016.

Plant, B APC Microbiome Institute Symposium, Cork, 2016.

Contact Catherine Stanton Email: Catherine.stanton@teagasc.ie.

Fouhy, F. et al., Investigating the gut microbiota of patients with CF (PWCF) and the effects of an altered gut microbiota on functionality and faecal metabolites –the CFMATTERS experience. 16th KillarneyNational Cystic Fibrosis Clinical Meeting, 4-5/02/2016

Fouhy, F. et al., Altered gut microbiota in stable patients with cystic fibrosis (CF) compared to controls and its relationship with intravenous (IV) antibiotic usage and lung function at 15th Killarney National Cystic Fibrosis Clinical Meeting, 05/02/2015.

Deane, J. et al., Clinical outcomes of Real-world Kalydeco (CORK) study- Investigating the impact of CFTR potentiation on the intestinal microbiota, pancreatic function and intestinal inflammation prospectively over 12 months. 15th Killarney National Cystic Fibrosis Clinical Meeting, 05/02/2015

8. **Compiled by:** Catherine Stanton

Contact Catherine Stanton Email: Catherine.stanton@teagasc.ie.