Title: Novel multi-omic insight into evolution of antibiotic resistance in P. aeruginosa in cystic fibrosis and relationship to clinical outcomes.

Pseudomonas aeruginosa is the bacterium most strongly associated with the pathogenesis of lung disease in cystic fibrosis (CF). Antibiotic (ABX) multi-drug resistant (MDR) strains of *P. aeruginosa* are common in CF which presents a significant problem with disease management. Despite advances in the understanding of ABX resistance mechanisms, there has been no change in the way that clinicians prescribe ABX in CF, either during in-hospital management of pulmonary exacerbations (PEx) or when the individual is clinically stable. We urgently need new methods at the clinical coalface that will inform on the most appropriate ABX to employ in an individual patient. We also need to improve understanding of how ABX resistance develops in CF and how this impacts on clinical outcomes, which will direct new treatment strategies.
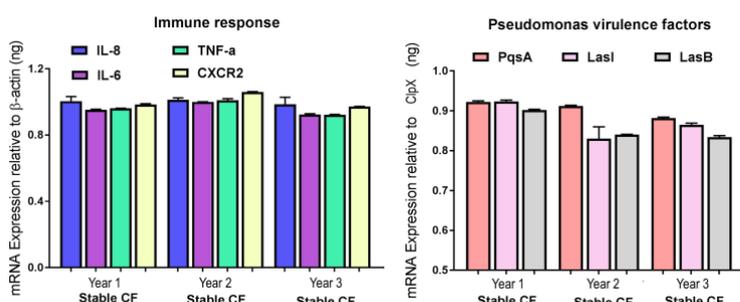
Our aims for this project were to profile the CF patient immune response, *P. aeruginosa* virulence factors, microbiome and clinical outcomes with ABX resistance of *P. aeruginosa* during treatment, comparing exacerbation with stable disease states. Secondly, to use whole genome sequencing (WGS) to study ABX resistance in CF patients infected with an epidemic versus non-epidemic strains of *P. aeruginosa*.

In the last 6 months we have been able to:
1. Profile patient immune responses over a 3-year period from sputum samples (about 70 samples)
2. Profile *P. aeruginosa* virulence factors in the same time period (also about 70 samples)
3. Isolate gDNA from these same sampling timepoints for microbiome analysis.

The figures below are an example of what some of our data looks like when we compare a patient with stable disease (A) to a patient that has experienced an exacerbation then been treated (B).

(**A**) Stable disease responses   (**B**) Responses after exacerbation (PEx) & treatment
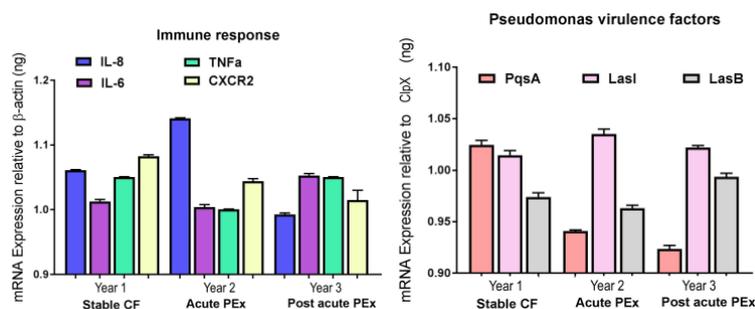
We also have data to compare patients colonised with epidemic vs non-epidemic strains of *P. aeruginosa*. In the next 6 months of the project,
- we will be analysing the microbiome data that we have collected,
- continuing with the antibiotic testing of the isolated *P. aeruginosa* strains
- collecting and analysing *P. aeruginosa* DNA samples by WGS to examine the change in ABX resistance.

I would again like to thank the donors and CF Australia for funding this project. We believe that this work will make a real difference to how CF patients are administered treatments, and help us understand more about *P. aeruginosa* infection and how to stop its progression in CF.