AAIR Charity
ASTHMA, ALLERGY & INFLAMMATION RESEARCH

Research Award:
Generation of PCD gene CRISPR Cas9 knock out cell lines

Awarded to: Dr Claire Jackson Amount: £10,000 (March 2021 – April 2022)

Lay summary

Cilia are moving 'hair-like' structures on the surface of our airway, which sweep mucus, along with inhaled pollution and infectious bugs, from our lungs. Patients with a rare inherited condition called primary ciliary dyskinesia (PCD) have permanently abnormal cilia from birth. They don't clear mucus and suffer regular infections with progressive lung disease. Our laboratory houses a PCD diagnostic service where we look at cilia function using microscopes. Genetic testing is used to determine the gene changes that cause patients' abnormal cilia. Although we know that mutations in over 50 genes cause most PCD, approximately 20% of PCD genes are unaccounted for. For research we grow patients' airway cells in the lab to model their diseased airway, but often don't have enough cells for large-scale experiments. AAIR funding allowed us to work with Cytosurge (a company in Switzerland) to try to develop a CRISPR/Cas9 gene edited 'PCD model', using cells that grow cilia and have been made to live 'forever'.

A commonly mutated gene in PCD (*DNAH5*) was targeted because the patients' cilia are always immotile and easy to characterise using our diagnostic tests. Two different positions of the DNAH5 gene were separately targeted in two batch experiments. Single cells were CRISPR/Cas9 'edited', multiplied and called 'clones'. Four clones had 'frame shift' mutations in *DNAH5* (DNA sequence verified); predicted to cause a gene function 'knock out' and lack of protein expression. All clones required initial culture expansion and storage.

Three *DNAH5* gene edited 'clones' have been compared to two unedited control 'clones'. When cultured at 'air-liquid interface' to simulate the human airway (in a dish), cilia grew after 7 days. We have tested the percentage ciliation, speed and quality of cilia movement by high-speed video microscopy and cell membrane barrier function (weekly). We harvested cellular DNA, RNA and protein and fixed samples for electron microscopy and immunofluorescence at day 28. Two 'clones' edited at exon one became widespread ciliated by day 28 and retained normal membrane barrier function, however their cilia were motile and similar to unedited controls; indicating that *DNAH5* gene expression was retained. Therefore, we are currently testing a third 'clone' edited at exon two (shipped to us in March 2022). Electron microscopy, live cilia imaging and protein analysis is underway to verify loss of DNAH5 protein. We would also aim to carry out whole genome sequencing to test of specific and 'off target' CRISPR/Cas9 editing effects if cilia are proven to be immotile and outer dynein arm structures lost from the ciliary axoneme.

Once we have the data to show that we have created an authentic DNAH5 knock out, we will aim to produce a research paper (acknowledging AAIR funding). We are continuing to collaborate with the lead Cytosurge scientist, and I am exploring whether we can create an industrial partnership to take 'PCD modelling' further. We aim to apply for further substantive funding for a PhD studentship to carry out bacterial co-infection studies. We also would like to generate other PCD models targeting other genes that cause a worse prognosis in patients. PCD patients with specific gene mutations may be more susceptible to infection than others, depending on cilia abnormality. Our project has shown that the host cells continue to differentiate and grow widespread cilia after CRISPR editing and cloning. It may also be possible to 'knock in' specific patient mutations as a means to verifying 'variants of unknown pathogenicity' (VUS); supporting difficult to diagnose PCD cases.

AAIR funding for this ‘PCD modelling’ project will enable me to primary supervise students (MSc, BMedSci and MMedSci) coming (2022-23) to help characterise the exon two clone and develop a co-infection model; with existing resources and stored samples from this project. I have developed a BRC studentship application for the next available funding call in 2023. All of these activities are supporting my recent promotion to level 5.

Ultimately, we will aim to present our ‘model’ within the FOM, the BRC, nationally and internationally at BEAT-PCD, the ERS Congress 2023 (acknowledging AAIR funding).
