Institution: University of Leicester

Unit of Assessment: UoA1

Title of case study: Redefining ciliary function: improving diagnostic testing and management of ciliary disorders and phenotyping of other respiratory diseases

1. Summary of the impact

The Leicester Cilia Group (LCG) established methods to study ciliary damage and dysfunction, transforming the diagnosis and management of Primary Ciliary Dyskinesia (PCD), a genetic disorder that causes severe permanent lung damage in children. The group developed diagnostic methods, adopted in the UK and internationally, that increased the accuracy and speed of diagnosis, uncovering a number of previously unrecognised phenotypes. The group was instrumental in the establishment of the first nationally funded diagnostic service (three centres, including Leicester) in the world. This has resulted in the group jointly leading a successful bid (2012) to set up the first nationally funded management service for children with PCD.

2. Underpinning research

Cilia are microscopic hair-like projections from the lining of the air conducting tubes which protect our lungs from damage and infection. These cilia, together with the mucus that covers them, constantly remove inhaled foreign particles by coordinated movements. Normally, cilia beat 10 to 15 times per second, and any impairment can result in poor mucociliary clearance, with subsequent upper and lower respiratory infection. Over the past decade, the Leicester Ciliary Group (LCG) group has redefined how cilia from the respiratory tract function, overturning previous theories in this area.

Importance of ciliary analysis in CPD

Work by Stannard, Chilvers, Thomas, Hirst and Rutman in Professor Chris O'Callaghan's laboratory using novel high speed video imaging and cell culture has defined normal ciliary function (papers in 1999, 2000, 2003 & 2009), and the effect of secondary damage on ciliary function (papers in 2001, 2009 & 2010). By applying this research to the study of disease states, they have shown the huge importance of ciliary analysis in PCD. Previous diagnostic testing simply relied on measuring beat frequency as a screening test, however, a major discovery by Chilvers (**1**) that ciliary beat pattern predicted ultrastructural abnormalities in PCD led to the discovery by Stannard and colleagues that beat pattern analysis in addition to frequency measurement significantly increased the diagnostic yield (**2**). The landmark paper of Stannard in 2010 (**3**), using these methods to diagnose patients suspected of PCD, has been adopted internationally as the 'gold standard'. This has been followed by the development of additional methods to allow diagnostic testing to be performed in resource-poor countries.

Discovery of previously unrecognised phenotypes of PCD

The development of high-speed videomicroscopy of ciliary biopsies obtained from brushing or scraping the lining of the nose has not only enabled rapid diagnosis of known phenotypes of PCD but also led to the discovery of previously unrecognised phenotypes of PCD. (The defect is universal throughout the respiratory tract, including the nose, from which it is easier and less painful to remove a specimen.) This has significantly increased the ability to diagnose patients with atypical PCD who are at risk of developing long-term complications. The group has also pioneered the use of ciliated cell culture to identify and confirm these previously unrecognised phenotypes of PCD. This work has contributed to the discovery of the genes of four different types of PCD out of the 21 discovered to date (O'Callaghan 2012 – also Leicester LCG; **4,5**). The group is now involved in developing gene therapy for this disease following on from grant funded work to study gene therapy in cystic fibrosis.

In addition, the research has highlighted the very large numbers of patients with this condition in the Asian community in Bradford, UK, where the group has shown it is more common than cystic

fibrosis, with the prevalence the highest reported at 1 in 2,265 (**6**).

Improving the interpretation of diagnostic tests

The group has also defined the effect of epithelial disruption on ciliary function and identified for the first time that viral infections cause defects in ciliary beat pattern, while beat frequency may be maintained. The appreciation of these effects has significantly improved the interpretation of diagnostic tests. Successfully growing ciliated cells from children with PCD at an air interface culture allowed Hirst (**7**) to confirm suspected new phenotypes of PCD and markedly reduce secondary damage in the original sample, reducing the need for further biopsies. This method has been adopted as part of the national diagnostic service.

Furthermore, this research has also allowed other respiratory diseases to be studied in detail, for example, in adults with severe asthma. The group has shown that these patients have the equivalent of a functional PCD in addition to their asthma. This discovery suggests that both the asthma and the PCD phenotype need to be treated and helps to explain why this group is so refractory to traditional asthma medication and why so many of these patients develop bronchiectasis, a long-term condition where the airways of the lungs become abnormally widened, leading to a build-up of excess mucus.

Leicester Cilia Group

1. Professor Chris O'Callaghan, Professor of Paediatrics, 1991-2012
2. Dr Wendy A Stannard, Clinical Research Fellow, 2000-2004
3. Dr Biju Thomas, Clinical Research Fellow, 2003
4. Dr Mark A Chilvers, Clinical Research Fellow, 1999 – present
5. Dr Prtiti Kenia, Clinical Research Fellow, 2007 – present
6. Dr Mina Fahdee-Shoad, PhD student
7. Dr Robert A Hirst, Post-doctoral Scientist, 2001-present
8. Dr Andrew Rutman 1999-present
9. CD Williams, 2006-present
10. G Williams 2006 - present

3. References to the research

1. **Chilvers MA, Rutman A, O'Callaghan C.** Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. J Allergy Clin Immunol 2003;112(3):518-24.
2. **Thomas B, Rutman A, O'Callaghan C.** Disrupted ciliated epithelium shows slower ciliary beat frequency and increased dyskinesia. Eur Respir J 2009;34:401-4.
3. **Stannard W, Chilvers M, Rutman A, Williams CD, O'Callaghan C.** Diagnostic testing of patients suspected of primary ciliary dyskinesia. Am J Respir Crit Care Med 2010;181(4):307-314.
4. Mitchison H, Schmidts M, Loges N, Freshour J, Dritsoula A, **Hirst R**, **O'Callaghan C**, Blau H, Al Dabbagh M, Olbrich H, Beales PL, Yagi T, Mussaffi H, Chung E, Omran H, Mitchell DR. Mutations in axonemal dynein assembly factor DNAAF3 cause primary ciliary dyskinesia. Nat Genet. 2012 Mar 4;44(4):381-9, S1-2
5. Panizzi JR, Becker-Heck A, Castleman VH, Al-Mutairi D, Liu Y, Loges NT, Pathak N, Austin-Tse C, Sheridan E, Schmidts M, Olbrich H, Werner C, Haffner K, Hellman N, Chodhari R, Gupta A, **O'Callaghan C** et al. Schmalhans / CCDC103 encodes a novel cilia dynein arm assembly factor that is mutated in primary ciliary dyskinesia. Nat Genet. 2012 May 13;44(6):714-9
6. **O'Callaghan C**, Chetcuti P, Moya E. High prevalence of primary ciliary dyskinesia in a British Asian population. Arch Dis Child. 2010 Jan;95(1):51-2. doi: 10.1136/adc.2009.158493. Epub 2009 Aug 30.
7. **Hirst RA, Rutman A, Williams G, O'Callaghan C**. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia (PCD). Chest 2010 138(6):1441-7.

Selected grant income over the past decade

2003: The effect of RSV infection on pneumococcal adherence and invasion of the ciliated

respiratory epithelium. Dr Wendy Stannard. Action Medical Research: £129,000
2005: Pneumococcal infection in primary ciliary dyskinesia. Liverpool Children's Charity. O'Callaghan C: £60,000
2005: Primary ciliary dyskinesia and bacterial infection: SPARKS: O'Callaghan C, Andrew PW: £129,000
2007: Investigation of the synergy between RSV and Pneumococcal infection. Action Medical Research. O'Callaghan C, Andrew PW. £87,000
2008: Investigating the molecular process of ciliogenesis in normal and disease states. MRC/Faculty funded Translational Studentship. O'Callaghan C, Fry A. £52,620
2009: A new approach to the treatment of pneumococcal induced toxaemia using drugs that selectively inhibit the activity of the pneumococcal toxin, pneumolysin. Wellcome Trust. Andrew PW, O'Callaghan C, El-Rachkidy Lonnen R. £3,498,098
2009: Why is invasive pneumococcal disease more common following viral infection? Action Medical Research. O'Callaghan C, Andrew PW, Easton A. £119,218
2010: Can naturally occurring stress hormones and prescribed catecholamines increase the risk of serious infections in newborns? SPARKS. O'Callaghan C, Freestone P, Field DJ. £140,098.

4. Details of the impact

It is estimated that as many as 400,000 people worldwide suffer from Primary Ciliary Dyskinesia (PCD). Most affected children and all adults will develop chronic severe lung infection (bronchiectasis), with 25% of adults going on to develop respiratory failure leading to premature death. Patients also suffer from recurrent nasal symptoms and sinusitis, and approximately half will ultimately require hearing aids due to the development of conductive hearing loss.

Benefits of early diagnosis
It is now accepted that analysis of ciliary function is the most important test in the diagnosis of PCD. These methods have been adopted in the UK and worldwide for the diagnosis of patients suspected of PCD.

Early diagnosis makes a very significant impact on both short-term and long-term morbidity and mortality. The diagnostic methods and diagnostic algorithm developed by the group have led to a number of phenotypes of PCD being recognised that would have previously been missed using the old diagnostic tests, which resulted in an under-diagnosis of around 15%. The old tests only identified the phenotypes of PCD which have a normal beat frequency, whereas all cilia, being dyskinetic, are picked up by high-speed videomicroscopy analysis. As well as faster screening of patient samples this has increased the number of people being diagnosed by around 20% (**3**).

First nationally funded diagnostic service for patients with PCD
The group's research was the basis for its leading the successful application to the National Commissioning Group of the NHS to set up a National Diagnostic Service for patients with PCD. In 2006, three highly specialised diagnostic centres were established in Leicester, London and Southampton. This was the first nationally funded diagnostic service for patients with PCD worldwide. Around 30 scientists and support staff are involved in this service, with funding at £2.26 million per annum (**1**).

Prior to the establishment of these centres, there were problems with the diagnostic process. This was due to several factors, including the landscape of isolated units with a special interest, limited access to diagnostic equipment, and long waiting times for diagnostic test results.

Since the centres were implemented in 2006, the following improvements have been made to the diagnosis of PCD patients:

- Reduced waiting times for PCD diagnostic testing
- More equal access for patients (i.e. not such a postcode lottery to the diagnostic service)
- Development of standardised processes (with equal access to equipment) for the diagnostic testing which are audited across the three centres
- Coordination and sharing of best practice - the three centres meet on a regular basis to

compare and refine the PCD Diagnostic Service
- Establishment of a database used for capturing data of patients referred to the diagnostic centres (authored by the group).

Fiona Copeland, chair of the PCD Family Support Group, says: "The diagnostic service has led to research, development and audit programmes, which have resulted in a big increase in the understanding of the cell biology involved in ciliopathy diseases, as well as improving clinical testing." (**2**).

Development of services in other countries
The model has led directly to the development of services for PCD in other countries. The diagnostic algorithm has been adopted by the European Taskforce Recommendations (**3**) that have been published in the European Respiratory Journal and also by the PCD groups in the US (**4**). Over the last 5 years the group has hosted and trained scientists and clinicians from China, Singapore, Canada, Australia, Spain, Denmark, Holland and Italy in high-speed videomicroscopy of ciliary biopsies to diagnose PCD and to establish national diagnostic centres.

Establishment of a Patient Management Service for children with PCD
Following establishment of the UK National Diagnostic Service many more patients than suspected were diagnosed with PCD (around 300 since 2006). Their condition is very different from other chronic diseases such as cystic fibrosis, and it became obvious that their care was substandard in many cases. The group and colleagues from the other National Diagnostic Centres jointly led a bid to establish a nationally commissioned Patient Management Service for children with PCD, concentrated at four centres (Leicester, London, Southampton and Leeds) (**5**).This again is the first of its kind worldwide and was signed off by the Secretary of State in February 2012. The new clinical management service, with funding of £1,708,843 per annum on a long-term basis, will transform the management of children with PCD and reduce the degree of bronchiectasis and respiratory failure they experience later in life.

Other impact
The group sits on and has co-chaired the medical advisory board of the Primary Ciliary Dyskinesia Family Support Group and has been instrumental in the development of their website which offers support for children and adults with PCD (**6**).

5. Sources to corroborate the impact

1. National Commissioning Group: NHS Specialised Services – Primary Ciliary Dyskinesia http://www.specialisedservices.nhs.uk/service/primary-ciliary-dyskinesia

2. Letter from the Chairman of the PCD Family Support Group, 22 April 2013

3. Barbato A, Frischer T, Kuehni CE, Snijders D, Azevedo I, Baktai G, Bartoloni L, Eber E, Escribano A, Haarman E, Hesselmar B, Jaspers M, Lucas J, Nielsen KG, **O'Callaghan C**, Omran H, Pohunek P, Strippoli MPF, Bush A. Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches and future perspectives. Eur Respir J 2009;34:1264-1276.

4. Leigh MW, O'Callaghan C, Knowles MR. The challenges of diagnosing primary ciliary dyskinesia. Proc Am Thorac Soc 2011;8(5):434-7.

5. National Commissioning Group: Primary Ciliary Dyskinesia (PCD) A National Management Service for Children
http://www.specialisedservices.nhs.uk/library/36/Service_Specification_and_Standards__Primary_Cilliary_Dyskinesia_Service_1.pdf

6. Primary Ciliary Dyskinesia Family Support Group: http://www.pcdsupport.org.uk/forum/