

University of Dundee

## Primary ciliary dyskinesia with normal ultrastructure

Shoemark, Amelia; Burgoyne, Thomas; Kwan, Robert; Dixon, Mellisa; Patel, Mitali P.; Rogers, Andrew V.

*Published in:*  
European Respiratory Journal

*DOI:*  
[10.1183/13993003.01809-2017](https://doi.org/10.1183/13993003.01809-2017)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

### *Citation for published version (APA):*

Shoemark, A., Burgoyne, T., Kwan, R., Dixon, M., Patel, M. P., Rogers, A. V., Onoufriadis, A., Scully, J., Daudyohra, F., Cullup, T., Loebinger, M. R., Wilson, R., Chung, E. M. K., Bush, A., Mitchison, H. M., & Hogg, C. (2018). Primary ciliary dyskinesia with normal ultrastructure: three-dimensional tomography detects absence of DNAH11. *European Respiratory Journal*, *51*(2), [1701809]. <https://doi.org/10.1183/13993003.01809-2017>

### **General rights**

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## **Supplementary materials and methods**

### **Immunofluorescence antibody staining**

Immunofluorescence antibody staining was performed on paraformaldehyde fixed nasal brushings to investigate the presence of ciliary proteins. Double labelling was conducted with the following primary antibodies: DNAH5 (HPA037470, Sigma Aldrich), DNAH9 (HPA052641, Sigma Aldrich), DNAH11 (HPA045880, Sigma Aldrich), DNAH11 (sc-16274, Santa Cruz), NME8 (HPA019259, Sigma Aldrich), Acetylated tubulin (T7451, Sigma Aldrich) and appropriate secondary fluorescent antibodies (Alexafluor 488 and 594). Slides were mounted with anti-fade mountant containing DAPI to label the nucleus (Prolong gold, Sigma Aldrich) and images were taken on an inverted confocal microscope (Leica SP5) with 63x oil objective [20].

### **Electron tomography volume assessment and area measurement**

Following construction of a dual axis tomogram, and averaging in PEET, the iMOD file was inverted using the clipcontrast -1 command.

Imaging and quantification of the outer dynein arms was performed using the UCSF Chimera program. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311). The hide dust command was set at <100 pixels and applied. Thresholding was

This is an author-submitted, peer-reviewed version of a manuscript that has been accepted for publication in the *European Respiratory Journal*, prior to copy-editing, formatting and typesetting. This version of the manuscript may not be duplicated or reproduced without prior permission from the copyright owner, the European Respiratory Society. The publisher is not responsible or liable for any errors or omissions in this version of the manuscript or in any version derived from it by any other parties. The final, copy-edited, published article, which is the version of record, is available without a subscription 18 months after the date of issue publication.

applied visually to achieve an image where the microtubular doublets were hollow but the inner dynein arm and radial spoke remained visible. To reduce thresholding bias thresholding was achieved by a single individual blinded to details of the case. The image was quantified with the MTD and ODA at the same threshold, thus the microtubular doublet acts as an internal control. The entire averaged and processed microtubule doublet was positioned within a bounding box (figure 3C) and the volume of the structure was determined using the 'measure volume' command. The outer dynein arm was then manually selected to reside within the bounding box and the volume was also established. The thresholding, position of the boundary boxes, volume of averaged microtubule doublets and ODA was determined by a single individual blinded to details of the case. The percentage of the microtubule doublet that the outer dynein arm occupied was then calculated as a proportion of the total volume i.e. (volume of ODA/ volume MTD) x 100. Surface area was trialled as a secondary outcome but deemed to be a less effective at determining differences in structure. Statistical testing was performed in graphpad prism using a Mann Whitney U test due to the use of non-parametric data and a p value of less than 0.05 was considered statistically significant.

### **Technical failure with longitudinal sections**

The technique to obtain longitudinal axoneme tomograms was successful in 7/14 attempts in *DNAH11* mutation patients and in 5/12 attempts in controls. A high rate of failure of the tomograms was due to difficulty visualising the ODAs in the longitudinal sections, where the bend of the cilia resulted in dynein arms arcing out of the area of the tomogram. This is a drawback that reduces the utility of longitudinal tomography for routine *DNAH11* analysis.