Primary ciliary dyskinesia with normal ultrastructure

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Supplementary materials and methods

Immunofluorescene antibody staining

Immunofluorescene 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

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.