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Published in:
Ultrastructural Pathology

DOI:
10.1080/01913123.2017.1370050

Publication date:
2017

Document Version
Peer reviewed version

Motile cilia defects in diseases other than primary ciliary dyskinesia: the contemporary diagnostic and research role for transmission electron microscopy

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Running title: Motile cilia defects in diseases other than PCD

Abstract

Ultrastructural studies have underpinned cell biological and clinical investigations of the varied roles of motile cilia in health and disease, with a long history since the 1950s. Recent developments from transmission electron microscopy (cryo–electron microscopy, electron tomography) have yielded higher resolution and fresh insights into the structure and function of these complex organelles. Microscopy in ciliated organisms, disease models and in patients with ciliopathy diseases, has dramatically expanded our understanding of the ubiquity, multisystem involvement and importance of cilia for normal human development. Here, we review the importance of motile cilia ultrastructural studies in understanding the basis of diseases other than primary ciliary dyskinesia.

Introduction

Cilia are small projections, of up to 10 micrometres in length, which protrude from almost every cell within the human body. These ubiquitous organelles play a key role in reproduction, development, cell signalling, tissue homeostasis, and defence against disease **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Cilia have been observed by microscopy techniques for centuries and were first described in 1675 by one of the founders of microscopy, Antonie Van Leuwenhoek, in conjunction with the discovery of micro-organisms **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Cilia are frequently categorised into two groups, 'primary' cilia and 'motile' cilia **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Although there are exceptions the primary cilium is ordinarily solitary – present in single copy on the cell surface - and immotile. Primary cilia are more ubiquitous in the body, sensing the environment around them in many tissues and acting as a coordinator of a diverse number of signalling pathways essential for normal development **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The focus of this review however is the actively beating, motile cilia which are located on a limited number of specialised epithelial surfaces, serving a critical function to direct the motility of body fluids and particles.

Structurally akin to the primary cilium, there are ordinarily several hundred motile cilia per cell, hence they are often also referred to as multicilia. The motile cilia beat in a coordinated fashion to move surrounding and overlying fluids from various epithelia **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The most studied example of ciliary motility is in the respiratory tract where the epithelial cilia form part of the mucociliary escalator, keeping the airways clean by a constant clearing of their overlying mucus that traps potentially harmful particulate matter and microorganisms for expulsion at the throat **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The coordinated planar aspect beating of multicilia **{** ADDIN EN.CITE <EndNote><Cite><Author>Chilvers</Author><Year>2003</Year><RecNum>7</RecNum><DisplayText><

style face="superscript">12</style></DisplayText><record><rec-number>7</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161116">7</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Chilvers, M. A.</author><author>Rutman, A.</author><author>O'Callaghan, C.</author></authors></contributors><auth-address>Department of Child Health, University of Leicester School of Medicine, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX, United Kingdom.</auth-address><titles><title>Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia</title><secondary-title>J Allergy Clin Immunol</secondary-title></titles><periodical><full-title>J Allergy Clin Immunol</full-title></periodical><pages>518-24</pages><volume>112</volume><number>3</number><keywords><keyword>Adolescent</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cilia/*physiology/*ultrastructure</keyword><keyword>Dyneins/physiology/ultrastructure</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>In Vitro Techniques</keyword><keyword>Infant</keyword><keyword>Kartagener Syndrome/diagnosis/*pathology/*physiopathology</keyword><keyword>Male</keyword><keyword>Microscopy, Electron</keyword><keyword>Microscopy, Video</keyword><keyword>Models, Biological</keyword><keyword>Periodicity</keyword></keywords><dates><year>2003</year><pub-dates><date>Sep</date></pub-dates></dates><isbn>0091-6749 (Print)
0091-6749 (Linking)</isbn><accession-num>13679810</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/13679810</url></related-urls></urls></record></Cite></EndNote>} creates a fluid flow that pushes molecules across the surfaces of our respiratory tracts (trachea, lungs, ear canals) but they also affect other systems. Multiciliated ventricles in the brain move the cerebrospinal fluid (CSF), potentially also influencing it's production and

composition although this is not well understood **{** ADDIN EN.CITE <EndNote><Cite><Author>Narita</Author><Year>2015</Year><RecNum>33</RecNum><DisplayText><style face="superscript">13</style></DisplayText><record><rec-number>33</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501256694">33</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Narita, K.</author><author>Takeda, S.</author></authors></contributors><auth-address>Department of Anatomy and Cell Biology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi Chuo, Yamanashi, Japan.</auth-address><titles><title>Cilia in the choroid plexus: their roles in hydrocephalus and beyond</title><secondary-title>Front Cell Neurosci</secondary-title></titles><periodical><full-title>Front Cell Neurosci</full-title></periodical><pages>39</pages><volume>9</volume><keywords><keyword>cerebrospinal fluid</keyword><keyword>cilia</keyword><keyword>diversity</keyword><keyword>hydrocephalus</keyword><keyword>multiciliogenesis</keyword></keywords><dates><year>2015</year></dates><isbn>1662-5102 (Print)
1662-5102 (Linking)</isbn><accession-num>25729351</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/25729351</url></related-urls></urls><custom2>PMC4325912</custom2><electronic-resource-num>10.3389/fncel.2015.00039</electronic-resource-num></record></Cite></EndNote>**}**. Recent studies have also raised discussion about the role of cilia motility in spinal CSF flow and development of correct spine curvature **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. In fertility, multiciliated fallopian tubes working along with muscle peristalsis move the oocyte to the uterus and since cilia are structurally related to motile sperm flagella, it is important to include reference to the fact that these related molecular functions also move the male gametes in fluid **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. An exception to the multicilia rule, 'nodal' cilia are singleton motile cilia that have a different waveform,

beating in a unidirectional manner to create a leftward flow of fluid across the surface of the transiently forming left-right organiser structure of the developing embryo, which is involved in ciliary signalling functions during organ embryogenesis that govern the determination of our left-right asymmetric body axis organ arrangement { ADDIN EN.CITE <EndNote><Cite><Author>Pennekamp</Author><Year>2015</Year><RecNum>57</RecNum><DisplayText><style face="superscript">17</style></DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501271953">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pennekamp, P.</author><author>Menchen, T.</author><author>Dworniczak, B.</author><author>Hamada, H.</author></authors></contributors><auth-address>Department of General Pediatrics, University Children's Hospital Muenster, 48149 Muenster, Germany.
Department of Human Genetics, University Hospital Muenster, 48149 Muenster, Germany.
Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan.</auth-address><titles><title>Situs inversus and ciliary abnormalities: 20 years later, what is the connection?</title><secondary-title>Cilia</secondary-title></titles><periodical><full-title>Cilia</full-title></periodical><pages>1</pages><volume>4</volume><number>1</number><keywords><keyword>Cilia</keyword><keyword>Left-right organizer</keyword><keyword>Motile cilia</keyword><keyword>Nodal</keyword><keyword>Nodal flow</keyword><keyword>Node</keyword><keyword>Planar cell polarity</keyword><keyword>Sensory cilia</keyword><keyword>Situs inversus</keyword><keyword>Two cilia model</keyword></keywords><dates><year>2015</year></dates><isbn>2046-2530 (Print)
2046-2530 (Linking)</isbn><accession-num>25589952</accession-num><urls><related-

urls><url>https://www.ncbi.nlm.nih.gov/pubmed/25589952</url></related-urls></urls><custom2>PMC4292827</custom2><electronic-resource-num>10.1186/s13630-014-0010-9</electronic-resource-num></record></Cite></EndNote>**}**.

Bjorn Afzelius, in work spanning more than 40 years until his death in 2008, was a pioneer in defining the ultrastructural composition of different human motile cilia and of sperm to investigate how their deficiencies cause different aspects of disease. He described along with other colleagues the hypotheses that immotile cilia syndrome now known as primary ciliary dyskinesia (PCD) affects the cilia, sperm and body situs **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. His proposal that 'all symptoms of [PCD] are directly or indirectly a consequence of the inborn inability of cilia to move or perform normal and coordinated movements' still holds true, with now more than 35 different PCD genes isolated that all affect the structure and motility of cilia and more variably also sperm **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The cilium consists broadly of three regions, the ciliary tip, main microtubular core or 'axoneme' and the base. The most proximal region close to the cell body forms a ciliary 'gate' consisting of transition fibres and the transition zone, located above the basal body from which the ciliary microtubules nucleate and extend **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

The ultrastructure of the axoneme scaffold of respiratory motile cilia that Afzelius helped to define is shown in **Figure 1**. The motile cilium axoneme in cross section has a 9-fold helical symmetry consisting of nine outer microtubular doublets containing an A and B tubule, that surround a central microtubular pair in the classical '9+2' array that is preserved amongst cilia across billions of years from the earliest eukaryotes. Attached along the length of the A microtubule of each microtubular doublet of the axoneme is an assembly of multiprotein complexes that repeat every 96-nm, including inner and outer dynein arm complexes containing the ATP-dependent motors that power ciliary beating, with docking

complexes connecting the arms to the microtubules. The dynein arms are the key structures in driving the ciliary beating, but regulation of the beat is conferred by other repeating structures within the 96-nm repeat: nexin-dynein regulatory complexes that connect between the peripheral doublets and radial spoke structures that connect between the central pair and peripheral doublet microtubules to allow a scaffold of signalling from the centre to the peripheral dyneins to regulate the beat **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

At the embryonic left-right organiser, two populations of cilia have been identified by transmission electron microscopy (TEM) as having either 9+2 or 9+0 microtubule arrangements, the 9+0 cilia lacking the central pair microtubules and having a unidirectional leftward beating pattern for nodal flow **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. This can explain why mutations causing PCD that result in specific central pair loss do not confer laterality defects, because they do not impact upon the nodal flow **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. However the exact ratio, distribution and interplay of the two different node epithelial cilia types in the determination of the left-right body axis is not yet fully established and remains a debate in the literature **{** ADDIN EN.CITE <EndNote><Cite><Author>Vandenberg</Author><Year>2013</Year><RecNum>94</RecNum><DisplayText><style face="superscript">32</style></DisplayText><record><rec-number>94</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501399717">94</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Vandenberg, L. N.</author><author>Levin, M.</author></authors></contributors><auth-address>Center for Regenerative and Developmental Biology, and Biology Department, Tufts University, Medford, MA 02155, USA.</auth-address><titles><title>A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality</title><secondary-title>Dev Biol</secondary-title><alt-

title>Developmental biology</alt-title></titles><periodical><full-title>Dev Biol</full-title><abbr-1>Developmental biology</abbr-1></periodical><alt-periodical><full-title>Dev Biol</full-title><abbr-1>Developmental biology</abbr-1></alt-periodical><pages>1-15</pages><volume>379</volume><number>1</number><keywords><keyword>Animals</keyword><keyword>*Body Patterning</keyword><keyword>*Cell Movement</keyword><keyword>Cell Polarity</keyword><keyword>Chromatids/genetics/metabolism</keyword><keyword>Cilia/genetics/metabolism</keyword><keyword>Cytoplasm/genetics/metabolism</keyword><keyword>Cytoskeleton/genetics/metabolism</keyword><keyword>Embryonic Development</keyword><keyword>*Gene Expression Regulation, Developmental</keyword><keyword>Mice</keyword><keyword>Models, Animal</keyword><keyword>*Models, Biological</keyword><keyword>Penetrance</keyword></keywords><dates><year>2013</year><pub-dates><date>Jul 01</date></pub-dates></dates><isbn>1095-564X (Electronic)
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Technical challenges exist for visualising motile cilia and determining their dysfunction in disease states. Due to the small size of the substructures of the ciliary axoneme (dynein arms are approximately 5 nanometres in size) they cannot be resolved with light microscopy which is limited by the wavelength of light. Consequently, electron microscopy approaches are the only techniques with sufficient resolution to accurately visualise the internal structures of the motile cilium. The development of advancements in the electron microscopy technique such as cryo–electron microscopy and electron tomography in the

last decade, have resulted in exciting new insights into the structure and function of motile cilia, identifying and clarifying many sub-structures **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

An expanding group of human disorders of cilia are known collectively as 'ciliopathies'. Ciliopathies are most often individually rare inherited disorders affecting either the primary or motile cilia, or both, but their collective medical impact is increasingly significant and complex as they become better understood and recognised by clinicians, with identification of numerous disease variants, multi-organ involvement and overlapping disease symptoms **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. **Table 1** describes a selection of the known ciliopathies and their primarily affected organs, with reference to recent reviews **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. In the clinic, TEM of motile cilia is most commonly used as a confirmatory diagnostic investigation for the motile cilia ciliopathy, PCD. PCD is a genetically heterogeneous condition in which defects of motile cilia lead to ineffective mucociliary clearance and consequentially a common set of associated respiratory symptoms including wet cough, rhinosinusitis, otitis media, repeated infections of the respiratory tract, situs inversus and infertility **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Axoneme dysmotility outside the respiratory system causes laterality defects associated with heart malformations, infertility and hydrocephalus **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

Within research, TEM of motile cilia is also frequently used to understand disease processes beyond PCD. In this article we review the use of TEM to visualise motile cilia and ciliated epithelial cells in diseases other than PCD, highlighting the multiple and varied applications of TEM in motile cilia diagnostics and research.

TEM of respiratory motile cilia and the ciliated epithelium

Bacterial and viral infections. Motile multicilia of the respiratory tract form part of the body's first defence against inhaled pathogens and particulate matter. Structural changes occur to the integrity of the ciliated epithelium, the level of multiciliation and the ultrastructure of the cilia themselves in response to acute and chronic infection. TEM of the motile cilia and ciliated epithelium has frequently been used to assess the effects of bacterial and viral infection with an aim to furthering the understanding of the pathogenesis of these organisms. Respiratory syncytial virus (RSV), for example, is the major cause of hospitalisation with respiratory disease in the first 12 months of life **{** ADDIN EN.CITE <EndNote><Cite><Author>Taylor</Author><Year>2016</Year><RecNum>48</RecNum><DisplayText><style face="superscript">41</style></DisplayText><record><rec-number>48</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501267368">48</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Taylor, S.</author><author>Taylor, R. J.</author><author>Lustig, R. L.</author><author>Schuck-Paim, C.</author><author>Haguinet, F.</author><author>Webb, D. J.</author><author>Logie, J.</author><author>Matias, G.</author><author>Fleming, D. M.</author></authors></contributors><auth-address>GSK Vaccines, Wavre, Belgium.
Sage Analytica, Bethesda, Maryland, USA.
GSK Pharmaceuticals, Uxbridge, UK.
Faculty of Health and Medical Sciences University of Surrey, Department of Clinical and Experimental Medicine, University of Surrey, Guildford, UK.</auth-address><titles><title>Modelling estimates of the burden of respiratory syncytial virus infection in children in the UK</title><secondary-title>BMJ Open</secondary-title></titles><periodical><full-title>BMJ Open</full-title></periodical><pages>e009337</pages><volume>6</volume><number>6</number><keywords><keyword>Children</keyword><keyword>Mortality</keyword><keyword>Otitis Media</keyword><keyword>Respiratory syncytial

virus</keyword></keywords><dates><year>2016</year><pub-dates><date>Jun 02</date></pub-dates></dates><isbn>2044-6055 (Electronic)
2044-6055 (Linking)</isbn><accession-num>27256085</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/27256085</url></related-urls></urls><custom2>PMC4893852</custom2><electronic-resource-num>10.1136/bmjopen-2015-009337</electronic-resource-num></record></Cite></EndNote>**}**. The virus is thought to initiate infection by targeting the respiratory ciliated epithelial cells. When infected with RSV, ciliated epithelial cells in a cell culture model show disruption of the tight junctions, gaps develop between cells and there is a reduction in numbers of cilia. These TEM findings helped to define the role of ciliated cells in the establishment of RSV infection **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

Typical changes to the ultrastructure of cilia following acute infection include swollen cilia with excess cytoplasm, compound cilia, disorganized axonemes, addition or deletion of peripheral microtubules or loss of one or both of the central pair microtubules **{** ADDIN EN.CITE <EndNote><Cite><Author>Pizzi</Author><Year>2003</Year><RecNum>6</RecNum><DisplayText><style face="superscript">43</style></DisplayText><record><rec-number>6</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161116">6</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pizzi, S.</author><author>Cazzato, S.</author><author>Bernardi, F.</author><author>Mantovani, W.</author><author>Cenacchi, G.</author></authors></contributors><auth-address>Dipartimento Clinico di Scienze Radiologiche e Istocitopatologiche, Ospedale S. Orsola-Malpighi, Bologna, Italy.</auth-address><titles><title>Clinico-pathological evaluation of ciliary dyskinesia: diagnostic role of electron microscopy</title><secondary-title>Ultrastruct Pathol</secondary-title></titles><periodical><full-title>Ultrastruct Pathol</full-

title></periodical><pages>243-52</pages><volume>27</volume><number>4</number><edition>2003/08/09</edition><keywords><keyword>Child</keyword><keyword>Cilia/*ultrastructure</keyword><keyword>Ciliary Motility Disorders/*diagnosis</keyword><keyword>Cytodiagnosis/methods</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Male</keyword><keyword>*Microscopy, Electron</keyword><keyword>Nasal Lavage Fluid</keyword><keyword>Nasal Mucosa/*ultrastructure</keyword></keywords><dates><year>2003</year><pub-dates><date>Jul-Aug</date></pub-dates></dates><isbn>0191-3123 (Print)
0191-3123 (Linking)</isbn><accession-num>12907369</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/12907369</url></related-urls></urls><electronic-resource-num>FDYFQLHWRD5WDU67 [pii]</electronic-resource-num><language>eng</language></record></Cite></EndNote>**}**. Ciliary ultrastructural changes are correlated with changes in the effectiveness of the function of the cilia through measurement of their ciliary beat frequency, ciliary dyskinesia or effective mucociliary clearance. Unlike in the inherited disease PCD, these changes and the associated slow and disordered ciliary beat pattern are often reversible and therefore termed 'secondary ciliary dyskinesia'. TEM studies have shown that the percentage of secondary ciliary defects naturally increases with age in healthy individuals and interestingly, the number of ciliary ultrastructural defects visualised correlates with susceptibility to respiratory infections with age **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Recovery of the structural integrity of the cilium following an infection can also take a significant amount of time.

Respiratory conditions. Poor mucociliary clearance is a feature of a number of chronic respiratory conditions and TEM studies have highlighted that the ciliary axoneme can be affected. These include the effects of smoking and environmental pollutants as well as disease conditions including chronic

bronchitis, bronchiectasis, cystic fibrosis and asthma { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Ultrastructural defects manifest as a denuding of the epithelium and a significant increase in ciliary disorientation and microtubular defects. Interestingly in asthma, these ultrastructural changes correlate with disease severity { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Likewise in the upper airways patients with chronic rhinosinusitis have increased levels of secondary defects, these most commonly take the form of compound cilia together with peripheral microtubule disarrangements and are associated with respiratory epithelium modifications such as a reduction in the number of ciliated cells { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Following transplantation of the lungs for multiple different pathologies, TEM has shown that ciliary ultrastructure remains abnormal up to 12 months post-transplant, possibly making patients more vulnerable to bronchiolitis obliterans syndrome { ADDIN EN.CITE <EndNote><Cite><Author>Thomas</Author><Year>2012</Year><RecNum>9</RecNum><DisplayText><style face="superscript">51</style></DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161116">9</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Thomas, B.</author><author>Aurora, P.</author><author>Spencer, H.</author><author>Elliott, M.</author><author>Rutman, A.</author><author>Hirst, R. A.</author><author>O'Callaghan, C.</author></authors></contributors><auth-address>Immunity and Inflammation, University of Leicester, Leicester, UK.</auth-address><titles><title>Persistent disruption of ciliated epithelium following paediatric lung transplantation</title><secondary-title>Eur Respir J</secondary-title></titles><periodical><full-title>Eur Respir J</full-title></periodical><pages>1245-52</pages><volume>40</volume><number>5</number><edition>2012/04/24</edition><keywords><keyword>Adolescent</keyword><keyword>Anastomosis, Surgical</keyword><keyword>Bronchi/*physiopathology/surgery/*ultrastructure</keyword><keyword

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10.1183/09031936.00174711</electronic-resource-num><language>eng</language></record></Cite></EndNote>**}**.

Cystic fibrosis (CF) is a lethal inherited condition caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel which affect the regulation of chloride and other ion transport through the epithelium **{** ADDIN EN.CITE <EndNote><Cite><Author>Ehre</Author><Year>2014</Year><RecNum>68</RecNum><DisplayText><style face="superscript">52</style></DisplayText><record><rec-number>68</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501368079">68</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ehre, C.</author><author>Ridley, C.</author><author>Thornton, D. J.</author></authors></contributors><auth-address>CF/Pulmonary Research & Treatment Centre, The University of North Carolina at Chapel Hill, USA. Electronic address: cehre@med.unc.edu.
Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, UK.</auth-address><titles><title>Cystic fibrosis: an inherited disease affecting mucin-producing organs</title><secondary-title>Int J Biochem Cell Biol</secondary-title><alt-title>The international journal of biochemistry & cell biology</alt-

title></titles><periodical><full-title>Int J Biochem Cell Biol</full-title><abbr-1>The international journal of biochemistry & cell biology</abbr-1></periodical><alt-periodical><full-title>Int J Biochem Cell Biol</full-title><abbr-1>The international journal of biochemistry & cell biology</abbr-1></alt-periodical><pages>136-45</pages><volume>52</volume><keywords><keyword>Animals</keyword><keyword>Cystic Fibrosis/*metabolism/*pathology</keyword><keyword>Cystic Fibrosis Transmembrane Conductance Regulator/*metabolism</keyword><keyword>Humans</keyword><keyword>Mucins/*biosynthesis/genetics</keyword></keywords><dates><year>2014</year><pub-dates><date>Jul</date></pub-dates></dates><isbn>1878-5875 (Electronic)
1357-2725 (Linking)</isbn><accession-num>24685676</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/24685676</url></related-urls></urls><custom2>4449140</custom2><electronic-resource-num>10.1016/j.biocel.2014.03.011</electronic-resource-num></record></Cite></EndNote>**}**. CFTR dysfunction causes increased fluid absorption and decreased water content at the epithelial surface which changes the mucus properties and its interaction with the underlying ciliated epithelium of the airways, physically interfering with the movement of cilia and normal mucociliary clearance, which leads to a severe condition of repeated infections and bronchiectasis **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. TEM studies in CF patient lungs show defects of the motile multicilia including compound cilia, excess cytoplasmic matrix, and a disrupted peripheral microtubule arrangement, ciliary dysplasia that is reported to get worse as the disease progresses with more structural defects of the cilia and increased cilia loss **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Motile cilia ultrastructural analysis was recently considered for use as a biomarker in CF, with assessment of the use of TEM-based measurement of the height of the periciliary layer in lower airway biopsies sampled from cystic fibrosis patients. Although

possible, this was not deemed accurate enough to be useful for example in evaluation of clinical trials for CF { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

A recent TEM study has described mis-orientation of the motile cilia in CF patients that disrupts mucociliary clearance and is associated with defects of the planar cell polarity signalling pathway that is required for the planar polarized motility of cilia that drives directional mucociliary clearance { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. This was determined by detection of misalignment of the polarity of the basal foot ciliary appendages. These normally align with the direction of ciliary beating, pointing them all in the same direction which is defined as being perpendicular to a line drawn across the central microtubular pair in TEM images { ADDIN EN.CITE <EndNote><Cite><Author>Vladar</Author><Year>2016</Year><RecNum>67</RecNum><DisplayText><style face="superscript">58</style></DisplayText><record><rec-number>67</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501367284">67</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Vladar, E. K.</author><author>Nayak, J. V.</author><author>Milla, C. E.</author><author>Axelrod, J. D.</author></authors></contributors><auth-address>Department of Pathology, Stanford University School of Medicine, Stanford, California, USA.
Department of Otolaryngology-Head and Neck Surgery, Stanford University School of Medicine, Stanford, California, USA.
Division of Pulmonary Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA.</auth-address><titles><title>Airway epithelial homeostasis and planar cell polarity signaling depend on multiciliated cell differentiation</title><secondary-title>JCI Insight</secondary-title><alt-title>JCI insight</alt-title></titles><periodical><full-title>JCI Insight</full-title><abbr-1>JCI insight</abbr-1></periodical><alt-periodical><full-title>JCI Insight</full-title><abbr-1>JCI insight</abbr-

1></alt-periodical><volume>1</volume><number>13</number><dates><year>2016</year><pub-dates><date>Aug 18</date></pub-dates></dates><isbn>2379-3708 (Print)
2379-3708 (Linking)</isbn><accession-num>27570836</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/27570836</url></related-urls></urls><custom2>4996276</custom2><electronic-resource-num>10.1172/jci.insight.88027</electronic-resource-num></record></Cite></EndNote>**}**. Motile cilia mis-orientation as diagnosed through TEM studies has been described elsewhere in the literature, which may implicate planar cell polarity and multiciliogenesis defects in other diseases, albeit with varying degrees of support through a rigorous quantification of the results. For example, cilia mis-orientation has been described to affect the respiratory cells of PCD patients carrying *RPGR* mutations **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. In studies of early-onset severe and otherwise idiopathic bronchiectasis, cyst-like structures were detected in the axoneme of the cilia from TEM analysis **{** ADDIN EN.CITE

<EndNote><Cite><Author>Tsang</Author><Year>2000</Year><RecNum>12</RecNum><DisplayText><style face="superscript">46</style></DisplayText><record><rec-number>12</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161116">12</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Tsang, K. W.</author><author>Tipoe, G.</author><author>Sun, J.</author><author>Ho, J. C.</author><author>Lam, B.</author><author>Zheng, L.</author><author>Ooi, G. C.</author><author>Ip, M.</author><author>Lam, W. K.</author></authors></contributors><auth-address>University Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong SAR, China. kwttsang@hkucc.hku.hk</auth-address><titles><title>Severe bronchiectasis in patients with "cystlike" structures within the ciliary shafts</title><secondary-title>Am J Respir Crit Care Med</secondary-

title></titles><periodical><full-title>Am J Respir Crit Care Med</full-title></periodical><pages>1300-5</pages><volume>161</volume><number>4 Pt 1</number><edition>2000/04/14</edition><keywords><keyword>Adult</keyword><keyword>Bronchiectasis/diagnostic imaging/*pathology</keyword><keyword>Cilia/ultrastructure</keyword><keyword>Cysts</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Male</keyword><keyword>Microscopy, Electron</keyword><keyword>Microtubules/ultrastructure</keyword><keyword>Tomography, X-Ray Computed</keyword></keywords><dates><year>2000</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>1073-449X (Print)
1073-449X (Linking)</isbn><accession-num>10764327</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/10764327</url></related-urls></urls><electronic-resource-num>10.1164/ajrccm.161.4.9904088</electronic-resource-num><language>eng</language></record></Cite></EndNote>**}**. Cilia disorientation was also reported for otherwise unexplained bronchiectasis cases, but the results were not deemed significantly different from disorientation seen in controls and were reversible following culture of the cells in vitro **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

Severe impaired respiratory ciliary function has been reported in Wegener granulomatosis, a rare autoimmune disease that affects the respiratory system with a necrotising granulomatous inflammation and vasculitis. Interestingly, it also affects other parts of the body such as the kidneys which lack multicilia **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. However, although ciliary beat frequency was affected in this disease, TEM showed no apparent defects of the multicilia ultrastructure in nasal epithelial cell samples from patients.

An example of TEM results showing the compound cilia commonly arising as a secondary effect of infection or caused by a chronic respiratory condition is shown in **Figure 2**. These kinds of cases and the overall difficulty of accurately quantifying changes to cilia structures identified using the TEM method, raises the point that in addition to studying respiratory conditions by assessment of primary samples from the nose or lungs of affected patients, it is also useful to develop in-vitro model systems. This allows assessment of disease processes, genetic modification and of treatments in-vitro in a more controlled environment and can also overcome secondary effects on ciliary motility arising from damage during sampling, local inflammation, recent infection or another external insult. These changes may be normalised after in-vitro maintenance of the ciliated cells over an extended period and a number of methods now exist for culturing multiciliated cells in suspension or by maintenance at an air-liquid interface, as recently reviewed { ADDIN EN.CITE <EndNote><Cite><Author>Marthin</Author><Year>2017</Year><RecNum>76</RecNum><DisplayText><style face="superscript">61</style></DisplayText><record><rec-number>76</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501393551">76</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Marthin, J. K.</author><author>Stevens, E. M.</author><author>Larsen, L. A.</author><author>Christensen, S. T.</author><author>Nielsen, K. G.</author></authors></contributors><auth-address>Danish PCD & chILD Centre, CF Centre Copenhagen, Paediatric Pulmonary Service, Department of Paediatrics and Adolescent Medicine, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, Copenhagen, DK-2100 Denmark.
Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark.
Department of Biology, Section of Cell Biology and Physiology, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark.</auth-

address><titles><title>Patient-specific three-dimensional explant spheroids derived from human nasal airway epithelium: a simple methodological approach for ex vivo studies of primary ciliary dyskinesia</title><secondary-title>Cilia</secondary-title><alt-title>Cilia</alt-title></titles><periodical><full-title>Cilia</full-title></periodical><alt-periodical><full-title>Cilia</full-title></alt-periodical><pages>3</pages><volume>6</volume><dates><year>2017</year></dates><isbn>2046-2530 (Print)
2046-2530 (Linking)</isbn><accession-num>28344781</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/28344781</url></related-urls></urls><custom2>5364668</custom2><electronic-resource-num>10.1186/s13630-017-0049-5</electronic-resource-num></record></Cite></EndNote>**}**. To validate the authenticity of these models, TEM-based assessment of ciliary ultrastructure can be used **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

TEM of motile cilia at non-respiratory sites

Ultrastructural imaging of motile multicilia can also have utility outside of the respiratory system but this tends to be shown in individual reports rather than by use of systematic, large-scale data so the full potential and quantification of this method still needs to be realised. In fertility, the ultrastructure and function of fallopian tube cilia has been shown to be highly similar to that of the respiratory cilia, as was recently reviewed **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Reports on damage to the fallopian tube cilia in PCD shows that this contributes to infertility, highlighting PCD-characteristic ultrastructural defects and reduced cilia numbers **{** ADDIN EN.CITE <EndNote><Cite><Author>Halbert</Author><Year>1997</Year><RecNum>80</RecNum><DisplayText><style face="superscript">66</style></DisplayText><record><rec-number>80</rec-number><foreign-

keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501395583">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Halbert, S. A.</author><author>Patton, D. L.</author><author>Zarutskie, P. W.</author><author>Soules, M. R.</author></authors></contributors><auth-address>Department of Obstetrics and Gynecology, University of Washington, Seattle 98195-6460, USA.</auth-address><titles><title>Function and structure of cilia in the fallopian tube of an infertile woman with Kartagener's syndrome</title><secondary-title>Hum Reprod</secondary-title><alt-title>Human reproduction</alt-title></titles><periodical><full-title>Hum Reprod</full-title></periodical><pages>55-8</pages><volume>12</volume><number>1</number><keywords><keyword>Adult</keyword><keyword>Cilia/*physiology/*ultrastructure</keyword><keyword>Fallopian Tubes/*ultrastructure</keyword><keyword>Female</keyword><keyword>Fertilization in Vitro</keyword><keyword>Humans</keyword><keyword>Infertility, Female/etiology/*pathology/physiopathology</keyword><keyword>Kartagener Syndrome/*complications</keyword><keyword>Microscopy, Electron</keyword><keyword>Microscopy, Electron, Scanning</keyword><keyword>Mucous Membrane/ultrastructure</keyword><keyword>Nasal Mucosa/ultrastructure</keyword></keywords><dates><year>1997</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>0268-1161 (Print)
0268-1161 (Linking)</isbn><accession-num>9043902</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/9043902</url></related-urls></urls></record></Cite></EndNote>**}**. TEM was used in a study of the effects of a female progesterone-based contraceptive, showing no ultrastructural changes but an effect on ciliary beat frequency that might explain its mode of action **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The effects

on fallopian multicilia of cigarette smoking and various pathological states linked to infertility, including endometriosis and microbial infections, has also been investigated by TEM and scanning electron microscopy { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. TEM is also widely used in studies of sperm axoneme structural defects in infertile male patients and has also been used in mouse models for investigations of the molecular basis of spermiogenesis { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

Of relevance for studies of disease, the effects of meningitis have been assessed using TEM. The brain motile cilia line the ventricular ependyma providing a local mechanism for movement of cerebrospinal fluid and the absence or dysfunction of this process is reported to make mammals more vulnerable to hydrocephalus { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Ependymal cilia in mammals are longer than those of the respiratory tract and beat at almost double the speed { ADDIN EN.CITE <EndNote><Cite><Author>O'Callaghan</Author><Year>2012</Year><RecNum>89</RecNum><DisplayText><style face="superscript">74</style></DisplayText><record><rec-number>89</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501398396">89</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>O'Callaghan, C.</author><author>Sikand, K.</author><author>Chilvers, M. A.</author></authors></contributors><auth-address>Department of Infection, Immunity and Inflammation, Division of Child Health, CSB, University of Leicester, Leicester, LE2 7LX, UK. co54@le.ac.uk.</auth-address><titles><title>Analysis of ependymal ciliary beat pattern and beat frequency using high speed imaging: comparison with the photomultiplier and photodiode methods</title><secondary-title>Cilia</secondary-title><alt-title>Cilia</alt-title></titles><periodical><full-title>Cilia</full-title></periodical><alt-periodical><full-title>Cilia</full-title></alt-periodical><pages>8</pages><volume>1</volume><number>1</number><dates><year>2012</year><

pub-dates><date>Jun 07</date></pub-dates></dates><isbn>2046-2530 (Print)
2046-2530 (Linking)</isbn><accession-num>23351965</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/23351965</url></related-urls></urls><custom2>3555703</custom2><electronic-resource-num>10.1186/2046-2530-1-8</electronic-resource-num></record></Cite></EndNote>**}**. It is thought that they are important for protection of the central nervous system against pathogens such streptococcus pneumonia and TEM ultrastructural studies in rats show disruption of ciliated epithelial cells and reduction of cilia number in response to streptococcus pneumonia infection **{** ADDIN EN.CITE <EndNote><Cite><Author>Hirst</Author><Year>2003</Year><RecNum>90</RecNum><DisplayText><style face="superscript">75</style></DisplayText><record><rec-number>90</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501398738">90</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hirst, R. A.</author><author>Gosai, B.</author><author>Rutman, A.</author><author>Andrew, P. W.</author><author>O'Callaghan, C.</author></authors></contributors><auth-address>Department of Child Health, Robert Kilpatrick Clinical Sciences Building, University of Leicester, Leicestert Royal Infirmary, PO Box 65, Leicester LE2 7LX, U.K. rah9@le.ac.uk</auth-address><titles><title>Streptococcus pneumoniae damages the ciliated ependyma of the brain during meningitis</title><secondary-title>Infect Immun</secondary-title><alt-title>Infection and immunity</alt-title></titles><periodical><full-title>Infect Immun</full-title><abbr-1>Infection and immunity</abbr-1></periodical><alt-periodical><full-title>Infect Immun</full-title><abbr-1>Infection and immunity</abbr-1></alt-periodical><pages>6095-100</pages><volume>71</volume><number>10</number><keywords><keyword>Animals</keyword><keyword>Cilia/physiology/*ultrastructure</keyword><keyword>Ependyma/physiopathology/*ultrastructure</keyword><keyword>Male</keyword><keyword>Meningitis,

Pneumococcal/etiology/microbiology/*pathology</keyword><keyword>Microscopy, Electron</keyword><keyword>Microscopy, Electron, Scanning</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword><keyword>Streptococcus pneumoniae/*pathogenicity</keyword></keywords><dates><year>2003</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>0019-9567 (Print)
0019-9567 (Linking)</isbn><accession-num>14500537</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/14500537</url></related-urls></urls><custom2>201093</custom2></record></Cite></EndNote>**}**. It is hypothesised that this epithelial damage allows access of the bacteria to the endothelium thus facilitating the development of bacterial meningitis.

Congenital cardiac conditions and conditions of defective situs are known to arise from deficiencies of the embryonic nodal cilia and this has been reinforced in large scale mouse mutational analysis and PCD patient studies that identified motile cilia genes (in addition to genes involved in cilia-transduced cell signalling and trafficking functions) as a major cause of heterotaxy and cardiac defects **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The use of TEM for imaging of the embryonic node cilia and their structure is challenging but has been achieved in model organisms, with identification of cilia at the node that have a normal 9+2 ultrastructure seen together with others of 9+0 ultrastructure that lack central pair microtubules, in relative proportions that remain unknown and with the nodal cilia composition of humans also not defined at present **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Defects in PCD patients that result in loss of the central pairs, radial spoke head proteins and nexin-dynein regulatory complexes - as confirmed by TEM in the respiratory multicilia only - have never been reported to be associated with situs inversus, deficient laterality or cardiac defects, suggesting these cilia sub-structures are all

dispensable for correct functioning of the motile nodal cilia in left-right determination **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

TEM in motile cilia of patients with ciliopathies

More than 35 ciliopathy diseases arising from mutations in more than 180 different genes are all caused by cilia dysfunction (**Table 1**) **{** ADDIN EN.CITE <EndNote><Cite><Author>Reiter</Author><Year>2017</Year><RecNum>23</RecNum><DisplayText><style face="superscript">1</style></DisplayText><record><rec-number>23</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501167612">23</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Reiter, J. F.</author><author>Leroux, M. R.</author></authors></contributors><auth-address>Department of Biochemistry and Biophysics and Cardiovascular Research Institute, University of California, San Francisco, San Francisco CA 94158, USA.
Department of Molecular Biology and Biochemistry and Centre for Cell Biology, Development and Disease, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada.</auth-address><titles><title>Genes and molecular pathways underpinning ciliopathies</title><secondary-title>Nat Rev Mol Cell Biol</secondary-title></titles><periodical><full-title>Nat Rev Mol Cell Biol</full-title></periodical><dates><year>2017</year><pub-dates><date>Jul 12</date></pub-dates></dates><isbn>1471-0080 (Electronic)
1471-0072 (Linking)</isbn><accession-num>28698599</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/28698599</url></related-urls></urls><electronic-resource-num>10.1038/nrm.2017.60</electronic-resource-num></record></Cite></EndNote>**}**.
Collectively between PCD and the ciliopathy diseases that are considered to affect the primary cilia, this

is therefore an increasingly medically important disease grouping associated with a significant disease burden for health services. Although still poorly understood with few precise diagnostic clinical tests available, the ciliopathies are now increasingly recognised, more accurately and efficiently diagnosed and as new variants continue to emerge they are thought to affect up to 1 in 2,000 individuals globally **{** ADDIN EN.CITE <EndNote><Cite><Author>Quinlan</Author><Year>2008</Year><RecNum>50</RecNum><DisplayText><style face="superscript">80</style></DisplayText><record><rec-number>50</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501268488">50</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Quinlan, R. J.</author><author>Tobin, J. L.</author><author>Beales, P. L.</author></authors></contributors><auth-address>Institute of Child Health, University College London, London, WC1N1EH, UK</auth-address><titles><title>Modeling ciliopathies: Primary cilia in development and disease</title><secondary-title>Curr Top Dev Biol</secondary-title></titles><periodical><full-title>Curr Top Dev Biol</full-title></periodical><pages>249-310</pages><volume>84</volume><keywords><keyword>Abnormalities, Multiple/genetics/*pathology</keyword><keyword>Animals</keyword><keyword>Bardet-Biedl Syndrome/genetics/pathology</keyword><keyword>Body Patterning/genetics</keyword><keyword>Cilia/*pathology/*physiology</keyword><keyword>Cysts/genetics/pathology/therapy</keyword><keyword>Disease/etiology</keyword><keyword>*Disease Models, Animal</keyword><keyword>Embryonic Development/genetics/physiology</keyword><keyword>Humans</keyword><keyword>*Mice</keyword><keyword>Mice, Transgenic</keyword><keyword>Models, Biological</keyword><keyword>Polycystic Kidney

Diseases/genetics/pathology</keyword><keyword>Syndrome</keyword></keywords><dates><year>2008</year></dates><isbn>0070-2153 (Print)
0070-2153 (Linking)</isbn><accession-num>19186246</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/19186246</url></related-urls></urls><electronic-resource-num>10.1016/S0070-2153(08)00605-4</electronic-resource-num></record></Cite></EndNote>}.

TEM studies have assisted in molecular definition of a new, more severe, subtype of PCD that has been termed RGMC (reduced generation of multiple motile cilia) which arises from multiciliogenesis defects caused by mutations in two co-regulated genes expressed only in multiciliated cells, *MCIDAS* and *CCNO* { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. They are both involved in the multiciliogenesis program { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}, rather than being structural or assembly proteins of the motile cilia like all the other known PCD-mutated genes { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. RGMC disease is linked to higher levels of hydrocephalus and worse lung function but avoids laterality or spermiogenesis defects and it can be diagnosed by TEM of the respiratory epithelium showing a mislocalisation of the basal bodies and the absence or reduction in the numbers of multiple motile cilia in nasal biopsy samples. Furthermore, TEM in these studies reveals an intriguing distinction between mutations caused by the two genes, whereby *MCIDAS* mutations cause in the rare cilium still found on the patient's cells, a loss of the outer and inner dynein arms which is accompanied by a static lack of movement as recorded in high speed video imaging; whilst *CCNO* mutations are associated with a retained internal motile cilia structure and dyneins, and preserved ciliary beating of the few cilia still visible on the cells (usually more cilia retained with *CCNO* compared to *MCIDAS* mutations). This was concluded to result from *CCNO* functioning further downstream from *MCIDAS* within the multiciliogenesis program { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

TEM studies of two unusual X-inked disease variants of PCD have also indicated the potential for involvement of motile cilia-associated proteins in functions normally considered to be governed by primary cilia, since they cause symptoms outside of the classical PCD phenotypic spectrum. In one family with several affected individuals, all had severe neurological dysfunction with macrocephaly accompanied by motile cilia dysfunction, recorded as reduced cilia motility in high speed video imaging and also indicated by recurrent respiratory tract infections. These traits all co-segregated with a frameshift mutation in the X-linked *OFD1* gene, and this cilia motility-related variant of orofaciodigital syndrome is now termed type 2 Simpson-Golabi-Behmel syndrome **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. This link to respiratory defects appears to be highly variable in males carrying *OFD1* mutations, but it was also been seen in a larger patient cohort where TEM of a bronchial epithelia ciliated cell sample from one patient showed sparse, disorganized and disorientated cilia at the cell surface, indicating a possible ciliary docking and/or ciliogenesis defect **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The *RPGR* gene encoding a photoreceptor connecting cilium protein was found to be mutated in males with a complex X-linked phenotype combining PCD with multicilia structural defects and retinitis pigmentosa **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. *RPGR* mutations are a relatively commonly caused of retinitis pigmentosa, but this association with PCD appears to be rare and specific since several cases are published. There is TEM data to suggest mis-orientation of the respiratory cilia in some PCD-RP cases with *RPGR* mutations, in addition to reported cases of X-linked retinitis pigmentosa, recurrent infections and hearing loss that are not yet genetically solved **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. An example of TEM results showing *RPGR*-mutation associated motile cilia disorientation is shown in **Figure 2**.

PCD is regarded as the archetypal, classical motile cilia disease but there have long been other indications that motile cilia dysfunction could also affect the primary cilia-associated ciliopathies. For the ciliopathies manifesting features of motile cilia dysfunction such as situs inversus and other heterotaxies or cardiac involvement, respiratory complications, infertility and enlarged brain ventricles, TEM is a central technique. **Table 1** indicates the ciliopathies where motile cilia might be involved. This area of research remains poorly investigated or understood but we can find examples in the literature of primary ciliopathy patients in whom there is suggested motile cilia involvement beyond simple secondary dyskinesia features or co-inherited morbidities. However it is, confusingly, furthermore clear that situs inversus is an association that is connected to dysfunction both of motile cilia and of the immotile primary cilia. The cilia-associated proteins polycystin-2 (PKD2) **{** ADDIN EN.CITE <EndNote><Cite><Author>Pennekamp</Author><Year>2002</Year><RecNum>68</RecNum><DisplayText><style face="superscript">89</style></DisplayText><record><rec-number>68</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1502809753">68</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pennekamp, P.</author><author>Karcher, C.</author><author>Fischer, A.</author><author>Schweickert, A.</author><author>Skryabin, B.</author><author>Horst, J.</author><author>Blum, M.</author><author>Dworniczak, B.</author></authors></contributors><auth-address>Universitatsklinikum Munster, Institut fur Humangenetik, Vesaliusweg 12-14, Germany.</auth-address><titles><title>The ion channel polycystin-2 is required for left-right axis determination in mice</title><secondary-title>Curr Biol</secondary-title></titles><periodical><full-title>Curr Biol</full-title></periodical><pages>938-43</pages><volume>12</volume><number>11</number><keywords><keyword>Animals</keyword><keyword>Functional Laterality/*physiology</keyword><keyword>Gene Expression Regulation</keyword><keyword>Membrane

Proteins/genetics/*physiology</keyword><keyword>Mice</keyword><keyword>Mice, Knockout</keyword><keyword>TRPP Cation Channels</keyword></keywords><dates><year>2002</year><pub-dates><date>Jun 04</date></pub-dates></dates><isbn>0960-9822 (Print)
0960-9822 (Linking)</isbn><accession-num>12062060</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/12062060</url></related-urls></urls></record></Cite></EndNote>}, polaris (IFT88) { ADDIN EN.CITE <EndNote><Cite><Author>Murcia</Author><Year>2000</Year><RecNum>69</RecNum><DisplayText><style face="superscript">90</style></DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1502809873">69</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Murcia, N. S.</author><author>Richards, W. G.</author><author>Yoder, B. K.</author><author>Mucenski, M. L.</author><author>Dunlap, J. R.</author><author>Woychik, R. P.</author></authors></contributors><auth-address>Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH 44106, USA.</auth-address><titles><title>The Oak Ridge Polycystic Kidney (orpk) disease gene is required for left-right axis determination</title><secondary-title>Development</secondary-title></titles><periodical><full-title>Development</full-title></periodical><pages>2347-55</pages><volume>127</volume><number>11</number><keywords><keyword>Alleles</keyword><keyword>Animals</keyword><keyword>Axis, Cervical Vertebra/*physiology</keyword><keyword>Body Patterning/*physiology</keyword><keyword>Embryonic Development/physiology</keyword><keyword>Embryonic and Fetal Development</keyword><keyword>Female</keyword><keyword>Gene Targeting</keyword><keyword>Homozygote</keyword><keyword>Mice</keyword><keyword>Mutage

nesis, Insertional</keyword><keyword>*Polycystic Kidney, Autosomal Recessive</keyword><keyword>Pregnancy</keyword><keyword>Proteins/genetics/*physiology</keyword><keyword>*Tumor Suppressor Proteins</keyword></keywords><dates><year>2000</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0950-1991 (Print)
0950-1991 (Linking)</isbn><accession-num>10804177</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/10804177</url></related-urls></urls></record></Cite></EndNote>} and inversin (NPHP2) { ADDIN EN.CITE <EndNote><Cite><Author>Morgan</Author><Year>1998</Year><RecNum>70</RecNum><DisplayText><style face="superscript">91</style></DisplayText><record><rec-number>70</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1502809928">70</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Morgan, D.</author><author>Turnpenny, L.</author><author>Goodship, J.</author><author>Dai, W.</author><author>Majumder, K.</author><author>Matthews, L.</author><author>Gardner, A.</author><author>Schuster, G.</author><author>Vien, L.</author><author>Harrison, W.</author><author>Elder, F. F.</author><author>Penman-Splitt, M.</author><author>Overbeek, P.</author><author>Strachan, T.</author></authors></contributors><auth-address>Department of Human Genetics, University of Newcastle upon Tyne, UK.</auth-address><titles><title>Inversin, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the inv mouse</title><secondary-title>Nat Genet</secondary-title></titles><periodical><full-title>Nat Genet</full-title></periodical><pages>149-56</pages><volume>20</volume><number>2</number><keywords><keyword>Amino Acid Sequence</keyword><keyword>Animals</keyword><keyword>Base Sequence</keyword><keyword>Chromosomes, Artificial, Yeast</keyword><keyword>In Situ Hybridization, Fluorescence</keyword><keyword>Mice</keyword><keyword>Mice, Mutant

Strains</keyword><keyword>Mice, Transgenic</keyword><keyword>Molecular Sequence Data</keyword><keyword>Phenotype</keyword><keyword>Proteins/*genetics</keyword><keyword>Sequence Deletion</keyword><keyword>*Transcription Factors</keyword></keywords><dates><year>1998</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1061-4036 (Print)
1061-4036 (Linking)</isbn><accession-num>9771707</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/9771707</url></related-urls></urls><electronic-resource-num>10.1038/2450</electronic-resource-num></record></Cite></EndNote>**}** are important both for nodal cilia to function in embryonic development and for tubulogenesis in the kidney, because their mutation causes both situs inversus and polycystic kidneys **{** ADDIN EN.CITE <EndNote><Cite><Author>Pennekamp</Author><Year>2015</Year><RecNum>57</RecNum><DisplayText><style face="superscript">17</style></DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501271953">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pennekamp, P.</author><author>Menchen, T.</author><author>Dworniczak, B.</author><author>Hamada, H.</author></authors></contributors><auth-address>Department of General Pediatrics, University Children's Hospital Muenster, 48149 Muenster, Germany.
Department of Human Genetics, University Hospital Muenster, 48149 Muenster, Germany.
Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan.</auth-address><titles><title>Situs inversus and ciliary abnormalities: 20 years later, what is the connection?</title><secondary-title>Cilia</secondary-title></titles><periodical><full-title>Cilia</full-title></periodical><pages>1</pages><volume>4</volume><number>1</number><keywords><keyword>Cilia</keyword><keyword>Left-right organizer</keyword><keyword>Motile

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2046-2530 (Linking)</isbn><accession-num>25589952</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/25589952</url></related-urls></urls><custom2>PMC4292827</custom2><electronic-resource-num>10.1186/s13630-014-0010-9</electronic-resource-num></record></Cite></EndNote>**}**; polycystic kidneys are not a feature of classic motile multicilia disease.

Motile cilia dysfunction has been directly investigated in a number of primary ciliopathy diseases and although infrequently recorded, there have been a number of reports where a likely primary, rather than secondary, defect of cilia motility was observed in primary ciliopathy patients i.e. those carrying mutations affecting primary cilia signalling functions. Suspicion existed about this for the primary ciliopathy nephronophthisis type 2 (NPHP2) because in addition to the NPHP features of kidney disease with very early involvement and end-stage renal failure around 3 years of age, situs inversus is reported in addition to respiratory involvement although this features pulmonary hypoplasia which is not a specific characteristic of PCD **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. Mutations in the *NPHP2* gene also called *inversin* causing NPHP2 were first discovered because roles for the protein were apparent in both kidney development and left-right laterality determination **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. NPHP2 is a component of and implicated in the function of the embryonic node cilia, several animal models have shown this **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**, and in cilia it is a ciliary transition zone component, considered to function in ciliary gatekeeping and control of delivery and exit of

proteins to and from the primary cilium, interacting with nephrocystin (NPHP1) and beta-tubulin in kidney primary cilia **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

In one report, 24 week prenatal ultrasound of a male foetus with *NPHP2* mutations detected anhydramnios, large and echogenic kidneys and situs inversus totalis i.e. a complete mirror image reversal of the internal organs, a phenotype normally connected to motile cilia dysfunction at the embryonic nodal affecting asymmetry of the body **{** ADDIN EN.CITE <EndNote><Cite><Author>Pennekamp</Author><Year>2015</Year><RecNum>57</RecNum><DisplayText><style face="superscript">17</style></DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501271953">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pennekamp, P.</author><author>Menchen, T.</author><author>Dworniczak, B.</author><author>Hamada, H.</author></authors></contributors><auth-address>Department of General Pediatrics, University Children's Hospital Muenster, 48149 Muenster, Germany.
Department of Human Genetics, University Hospital Muenster, 48149 Muenster, Germany.
Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan.</auth-address><titles><title>Situs inversus and ciliary abnormalities: 20 years later, what is the connection?</title><secondary-title>Cilia</secondary-title></titles><periodical><full-title>Cilia</full-title></periodical><pages>1</pages><volume>4</volume><number>1</number><keywords><keyword>Cilia</keyword><keyword>Left-right organizer</keyword><keyword>Motile cilia</keyword><keyword>Nodal</keyword><keyword>Nodal flow</keyword><keyword>Node</keyword><keyword>Planar cell polarity</keyword><keyword>Sensory cilia</keyword><keyword>Situs

inversus</keyword><keyword>Two cilia model</keyword></keywords><dates><year>2015</year></dates><isbn>2046-2530 (Print)
2046-2530 (Linking)</isbn><accession-num>25589952</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/25589952</url></related-urls></urls><custom2>PMC4292827</custom2><electronic-resource-num>10.1186/s13630-014-0010-9</electronic-resource-num></record></Cite></EndNote>**}**. Dysgenesis of the tracheal motile multicilia was recorded in TEM studies, with a specific defect of loss of the central microtubules detected by TEM, although the frequency of this ultrastructural change in the affected cilia was not recorded **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**.

Leber congenital amaurosis (LCA) is a ciliopathy presenting as the earliest and most severe inherited retinal degeneration and mutations in the *CEP290* gene are a frequent cause of non-syndromic LCA as well as syndromic LCA, including Joubert syndrome. *CEP290* is one of the most pleiotropic primary ciliopathy genes, with mutations causing many primary ciliopathy diseases and a wide spectrum of defects impacting on functions of the brain, kidneys and liver as well as the retina **{** ADDIN EN.CITE <EndNote><Cite><Author>Coppieters</Author><Year>2010</Year><RecNum>59</RecNum><DisplayText><style face="superscript">95</style></DisplayText><record><rec-number>59</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501273009">59</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Coppieters, F.</author><author>Lefever, S.</author><author>Leroy, B. P.</author><author>De Baere, E.</author></authors></contributors><auth-address>Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium.</auth-address><titles><title>CEP290, a gene with many faces: mutation overview and presentation of CEP290base</title><secondary-title>Hum Mutat</secondary-

title></titles><periodical><full-title>Hum Mutat</full-title></periodical><pages>1097-108</pages><volume>31</volume><number>10</number><keywords><keyword>*Abnormalities, Multiple/genetics/pathology</keyword><keyword>Animals</keyword><keyword>Antigens, Neoplasm/*genetics/metabolism</keyword><keyword>Cilia/*genetics/*pathology</keyword><keyword>*Databases, Genetic</keyword><keyword>Genotype</keyword><keyword>Humans</keyword><keyword>Mice</keyword><keyword>*Mutation</keyword><keyword>Neoplasm Proteins/*genetics/metabolism</keyword><keyword>Phenotype</keyword><keyword>Syndrome</keyword></keywords><dates><year>2010</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1098-1004 (Electronic)
1059-7794 (Linking)</isbn><accession-num>20690115</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/20690115</url></related-urls></urls><electronic-resource-num>10.1002/humu.21337</electronic-resource-num></record></Cite></EndNote>**}**. In a screen of seven LCA patients with rod-cone dystrophy and carrying *CEP290* mutations, the respiratory cilia were studied by TEM and high speed video imaging of nasal biopsies. This showed a high level of respiratory cilia defects amongst the patients, mainly involving reduction and loss of the motile cilia dynein arm motors, the central pair microtubules and/or peripheral microtubules **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. TEM showed that all patients had reduced motile cilia numbers and a variable proportion of short cilia, which was associated with heterogeneous ciliary beat abnormalities. *CEP290* is highly expressed in both the neural retina and nasal epithelial cells, compared with other tissues **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The frequency of these findings in LCA patients suggests a common function for CEP290 protein in the development of respiratory and photoreceptor ciliary structures. The presence of respiratory symptoms in LCA patients could allow a clinically useful criterion

to direct specific *CEP290* genotyping of the patients, as well as indicate additional clinical tests of patient benefit.

Bardet Biedl Syndrome (BBS) is a ciliopathy caused by mutations in over 20 different genes that exemplifies the multi-system involvement of primary ciliopathies and is thought to be caused by a diverse range of inherited defects affecting a multi-protein complex at the base of the cilium called the BBSome. The BBSome is thought to function in transport of protein cargos to the cilia and their transport within cilia **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The hallmark features of BBS consist of cone–rod dystrophy, postaxial polydactyly, truncal obesity and cognitive impairment, with hypogonadism/hypogenitalism in males or genital abnormalities in females. More variable features include speech disorder, developmental delay, ataxia, diabetes, heart defects, anosmia and liver involvement **{** ADDIN EN.CITE <EndNote><Cite><Author>Baker</Author><Year>2009</Year><RecNum>46</RecNum><DisplayText><style face="superscript">39</style></DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501261884">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Baker, K.</author><author>Beales, P. L.</author></authors></contributors><auth-address>UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.</auth-address><titles><title>Making sense of cilia in disease: the human ciliopathies</title><secondary-title>Am J Med Genet C Semin Med Genet</secondary-title></titles><periodical><full-title>Am J Med Genet C Semin Med Genet</full-title></periodical><pages>281-95</pages><volume>151C</volume><number>4</number><keywords><keyword>Animals</keyword><keyword>Bardet-Biedl Syndrome/*diagnosis/physiopathology</keyword><keyword>Biological

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1552-4868 (Linking)</isbn><accession-num>19876933</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/19876933</url></related-urls></urls><electronic-resource-num>10.1002/ajmg.c.30231</electronic-resource-num></record></Cite></EndNote>**}**.

In BBS, predominantly the primary cilia are affected, but TEM studies in *Bbs1, Bbs2, Bbs4*, and *Bbs6* mutant mouse models of BBS showed that a motile cilia ultrastructural phenotype is also present **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. This included disrupted axoneme microtubular structure and the presence of electron-dense vesicular-like material along the ciliary shaft and at the tips of cilia, in both the respiratory tract and the brain where this was associated with ventriculomegaly/hydrocephalus **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The most common abnormality observed in both tissues were bulges filled with vesicles near the tips of cilia, an appearance seen in common in airway motile cilia from all the mutant mice. The structural abnormalities were accompanied by functional defects since the ciliary beat frequency was also reduced **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The clinical significance of these inclusions is unknown, as BBS patients do not in general have symptoms like those seen in PCD, however these findings may represent a wider defect of intraflagellar transport in BBS and a wider phenotypic spectrum. A study in patients with BBS showed a low level of unique inclusions within the ciliary membrane, along with some reported cellular damage, ciliary depletion and goblet cell

hyperplasia in the respiratory epithelia as sampled by nasal biopsy { ADDIN EN.CITE <EndNote><Cite><Author>Shoemark</Author><Year>2015</Year><RecNum>16</RecNum><DisplayText><style face="superscript">99</style></DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161118">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shoemark, A.</author><author>Dixon, M.</author><author>Beales, P. L.</author><author>Hogg, C. L.</author></authors></contributors><titles><title>Bardet Biedl syndrome: motile ciliary phenotype</title><secondary-title>Chest</secondary-title></titles><periodical><full-title>Chest</full-title></periodical><pages>764-70</pages><volume>147</volume><number>3</number><keywords><keyword>Adolescent</keyword><keyword>Adult</keyword><keyword>Asthma/epidemiology</keyword><keyword>Bardet-Biedl Syndrome/*pathology</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cilia/*pathology/ultrastructure</keyword><keyword>Cohort Studies</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Infant</keyword><keyword>Kartagener Syndrome/pathology</keyword><keyword>Male</keyword><keyword>Middle Aged</keyword><keyword>Otitis Media/epidemiology</keyword><keyword>*Phenotype</keyword><keyword>Prevalence</keyword><keyword>Respiratory Mucosa/*pathology</keyword><keyword>Retrospective Studies</keyword><keyword>Rhinitis/epidemiology</keyword><keyword>Young Adult</keyword></keywords><dates><year>2015</year><pub-dates><date>Mar</date></pub-dates></dates><isbn>1931-3543 (Electronic)
0012-3692 (Linking)</isbn><accession-num>25317630</accession-num><urls><related-

urls><url>http://www.ncbi.nlm.nih.gov/pubmed/25317630</url></related-urls></urls><electronic-resource-num>10.1378/chest.13-2913</electronic-resource-num></record></Cite></EndNote>}. This study reported a 12% increased prevalence of neonatal respiratory distress at birth along with instances amongst the BBS patients of asthma, otitis media and rhinitis, which are symptoms of PCD; however, these features occurred at a significantly reduced prevalence compared with PCD patients { ADDIN EN.CITE

<EndNote><Cite><Author>Shoemark</Author><Year>2015</Year><RecNum>16</RecNum><DisplayText><style face="superscript">99</style></DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501161118">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shoemark, A.</author><author>Dixon, M.</author><author>Beales, P. L.</author><author>Hogg, C. L.</author></authors></contributors><titles><title>Bardet Biedl syndrome: motile ciliary phenotype</title><secondary-title>Chest</secondary-title></titles><periodical><full-title>Chest</full-title></periodical><pages>764-70</pages><volume>147</volume><number>3</number><keywords><keyword>Adolescent</keyword><keyword>Adult</keyword><keyword>Asthma/epidemiology</keyword><keyword>Bardet-Biedl Syndrome/*pathology</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cilia/*pathology/ultrastructure</keyword><keyword>Cohort Studies</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Infant</keyword><keyword>Kartagener Syndrome/pathology</keyword><keyword>Male</keyword><keyword>Middle Aged</keyword><keyword>Otitis Media/epidemiology</keyword><keyword>*Phenotype</keyword><keyword>Prevalence</keyword><

keyword>Respiratory Mucosa/*pathology</keyword><keyword>Retrospective Studies</keyword><keyword>Rhinitis/epidemiology</keyword><keyword>Young Adult</keyword></keywords><dates><year>2015</year><pub-dates><date>Mar</date></pub-dates></dates><isbn>1931-3543 (Electronic)
0012-3692 (Linking)</isbn><accession-num>25317630</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/25317630</url></related-urls></urls><electronic-resource-num>10.1378/chest.13-2913</electronic-resource-num></record></Cite></EndNote>**}**. An example of TEM results showing these BBS-mutation associated electron-dense structures in the motile cilia is shown in **Figure 2**.

Sensenbrenner syndrome also known as cranioectodermal dysplasia is a multi-organ ciliopathy resulting from bi-allelic pathogenic variants in genes associated with intraflagellar transport *(IFT122, WDR35, WDR19, IFT43*). TEM of nasal biopsy material from a patient with Sensenbrenner syndrome was also shown to have an unusual, unique motile cilia defect consisting of elongated cilia with swollen racket-shaped tips containing accumulations of electron-dense material **{** ADDIN EN.CITE <EndNote><Cite><Author>Hirst</Author><Year>2015</Year><RecNum>62</RecNum><DisplayText><style face="superscript">101</style></DisplayText><record><rec-number>62</rec-number><foreign-keys><key app="EN" db-id="z0wpezrxjppse1e0tf2pxft3w05ra2r090dp" timestamp="1501275813">62</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hirst, R., Rutman, A., Williams, G., Kulkarni, N., O'Callaghan, C.</author></authors></contributors><titles><title>Ciliary and epithelial abnormalities are features of primary ciliopathies</title><secondary-title>Eur Resp J</secondary-title></titles><periodical><full-title>Eur Resp J</full-title></periodical><pages>OA1992</pages><volume>46</volume><dates><year>2015</year></dates>

<urls></urls></record></Cite></EndNote>}. These inclusions could represent the accumulation of IFT complex B proteins at the ciliary tip resulting in dysfunction of retrograde intraflagellar transport.

Conclusions

Transmission electron microscopy has long held its place as a vital tool in the research of disease pathogenesis at the level of the cilium, offering the axoneme-level resolution for imaging of ciliary health and ciliary defects. It can be used to investigate disease processes, assess the effect of various biological and environmental insults as well as the success of different therapies. Lesions of the motile cilia can be detected in cases of infection and in common and rare respiratory conditions and there are examples of these ciliary symptoms worsening according to the severity of the disease course. TEM has been useful to look at motile cilia in a number of different tissues including the airways and to assess their involvement in diseases of the respiratory system. TEM is starting to help ascertain the extent of involvement of motile cilia dysfunction in diseases other than PCD especially in the phenotypic spectrum of primary ciliopathy diseases where there may be overlapping disease features.

However, the TEM technique is complex and requires significant experience and optimisation: it can lack sensitivity, be non-quantitative and can potentially miss important features of disease due to the scale of analysis that is possible. It remains difficult to distinguish a primary defect from secondary dyskinesia which can have many possible aetiologies. Motile cilia cell culture systems can be used to address this problem, especially since studies can often be observational reports analysing few cases in total so these will require further investigations and more comprehensive analysis.

Although it is not the optimal technique to produce a global survey of an affected tissue, TEM remains unparalleled as a major tool of cilia and flagella structural analysis. It is furthermore of increased current interest because of new technical advances that have extended its utility. In future, still-developing extensions of the transmission electron microscopy technique as applied in correlative light and electron microscopy (CLEM), cryo–electron microscopy and electron tomography, will hopefully become more widely used in less specialised laboratories in order to be increasingly useful in this research, to yield further understanding of these complex, ubiquitous and medically important organelles.

Acknowledgements

The authors acknowledge support from the BEAT-PCD: Better Evidence to Advance Therapeutic options for PCD network (COST Action 1407). Work by A.S. is independent research funded by a postdoctoral research fellowship from the National Institute of Health Research and Health Education England. H.M.M. is supported by the Great Ormond Street Hospital Children's Charity and the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute of Health Research or the Department of Health. We are grateful to Dr Mellisa Dixon for assistance in figure preparation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

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Figure 1.

Major structures of the 9+2 motile cilium axoneme shown in cross-section

Figure 2.

Examples of motile cilia dysfunction defined by transmission electron microscopy

Transmission electron micrographs of cilia from the nasal epithelium obtained by nasal brushing demonstrate a number of different disease states. (A) Ciliary cross sections showing poor orientation, indicated by white lines, from an individual with primary ciliary dyskinesia symptoms carrying an X-linked mutation in the *RPGR* gene. (B) Ciliary cross sections from an individual with Bardet-Biedl syndrome (BBS) showing electron dense inclusions (black arrows) similar to those seen in murine models of BBS. (C) An example of compound cilia (black arrows) where one or more axonemes are seen within one membrane, as are often seen secondary to infection or due to a chronic inflammatory condition such as asthma.

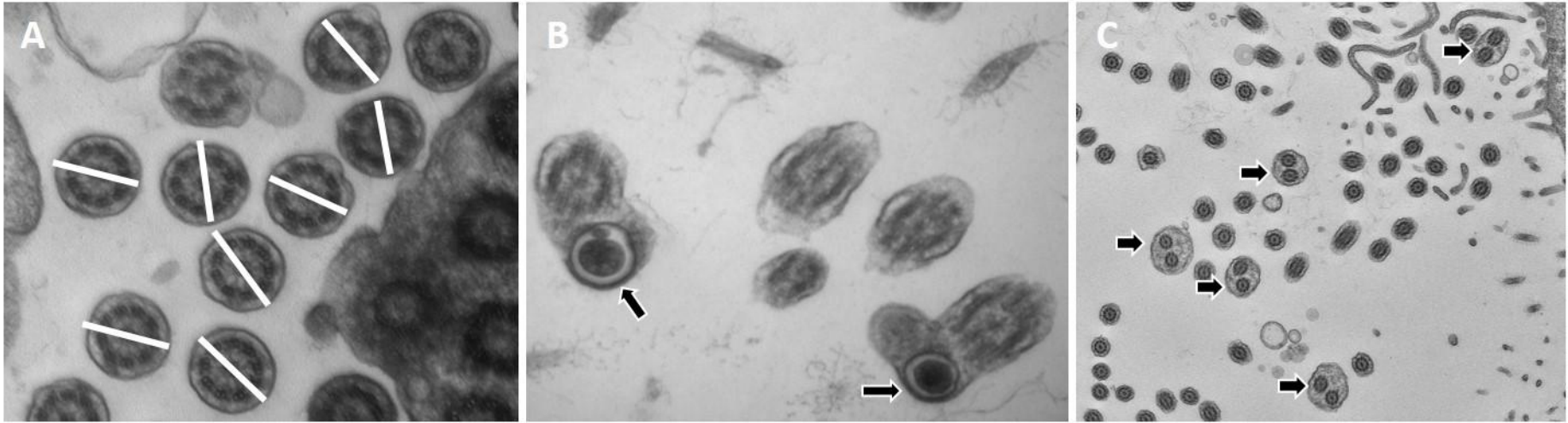
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Table 1. Organ involvement in selected ciliopathies. Ciliary dyskinesia, indicates motile cilia structure/function defects. PCD, primary ciliary dyskinesia, RGMC, reduced generation of multiple motile cilia.

Ciliopathy	Primary affected organs	Symptoms associated with motile cilia and/or nodal cilia involvement (see text for gene details)
Primary ciliary dyskinesia	Respiratory, brain, heart, organ placement, sperm	PCD, RGMC
MORM syndrome	Brain, kidney, eyes	
Meckel-Gruber syndrome	Brain, kidney, skeleton	Situs inversus
Bardet-Biedl syndrome	Brain, kidney, skeleton, eyes	Situs inversus, ciliary dyskinesia reports
Oculocerebrorenal (Lowe) syndrome	Brain, kidney, skeleton, eyes	
Orofaciodigital syndrome	Brain, kidney, skeleton, liver	OFD1 – situs inversus, heart defects
Simpson-Golabi-Behmel syndrome, type 2	Various, respiratory	OFD1 – brain malformation, ciliary dyskinesia
COACH syndrome	Brain, renal	Situs inversus
Hydrolethalus syndrome	Brain, skeleton	Situs inversus
Pallister-Hall syndrome	Brain, skeleton	
Joubert syndrome	Brain, various	Situs inversus
Acrocallosal syndrome	Brain	
Holoprosencephaly	Brain	Situs inversus
Juvenile myoclonic epilepsy	Brain	
Medulloblastoma	Brain	
Stromme syndrome	Brain	
Cone-rod dystrophy	Eyes	Situs inversus, heart defects
Leber congenital amaurosis	Eyes	CEP290 – ciliary dyskinesia
Retinitis pigmentosa	Eyes	RPGR - respiratory defects, PCD
Senior-Løken syndrome	Kidney, eyes	Situs inversus
Nephronophthisis	Kidney	NPHP2 - situs inversus, ciliary dyskinesia
Polycystic kidney disease	Kidney	PKD2, IFT88 - situs inversus
Alström syndrome	Obesity, endrocrine, eyes	Situs inversus
Cranioectodermal dysplasia (Sensenbrenner)	Skeleton, kidney, hepatic	Ciliary dyskinesia
Axial spondylometaphyseal dysplasia	Skeleton	
Carpenter syndrome	Skeleton	Situs inversus
Ellis-van Creveld syndrome	Skeleton	Situs inversus
Grieg cephalopolysyndactyly syndrome	Skeleton	
Jeune asphyxiating thoracic dystrophy	Skeleton	Situs inversus
McKusick-Kaufman syndrome	Skeleton	
Short-rib thoracic dystrophy	Skeleton	Situs inversus
Birt-Hogg-Dubé syndrome	Various	
Lethal congential contracture syndrome	Various	
Spinocerebellar ataxia	Various	