Primary Ciliary Dyskinesia
Pathogenesis, diagnosis and treatment

DR CLAIRE HOGG
DEPT. RESPIRATORY PAEDIATRICS
ROYAL BROMPTON HOSPITAL
LONDON.
Autosomal recessive
Heterogeneous
Defect of ciliary ultra structure
Abnormal ciliary beat motion
 Interruption of muco-ciliary clearance
Break down of primary defence mechanism throughout the airway.
PCD statistics

- Incidence – 1 in 15,000-30,000
- Higher in some populations [1:2,500 Pakistani tribe]
- Age of diagnosis is 4.4 years despite presence of situs inversus and other early indicators in > 50% cases
- Approx 3,000 cases in England and Wales

Bush, O’Callaghan 1994
O’Callaghan 2010
Ciliated Epithelium
Nasal brush biopsy
Normal ciliary ultrastructure
1. ODA defect
2. IDA defect
3. IDA and ODA defect
4. Radial spoke and IDA
5. Transposition defect [8+1]
6. Missing central pair
Abnormal ciliary ultrastructure identified in 275 patients with ciliary dyskinesia at the Brompton Hospital 1988-2008

<table>
<thead>
<tr>
<th>Ciliary defect</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer dynein arm defect</td>
<td>105</td>
<td>38</td>
</tr>
<tr>
<td>Outer and Inner dynein arm defect</td>
<td>57</td>
<td>21</td>
</tr>
<tr>
<td>Inner dynein arm defect</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Transposition defect</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>Radial spoke defect</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>No ultrastructural abnormality</td>
<td>33</td>
<td>12</td>
</tr>
</tbody>
</table>
Dynein arm defects

% Arm defects - London 1988-2008

- Inner arms present %
- Outer arms present %

Graph showing the percentage of inner and outer arms present across different patient groups:
- Non-PCD
- IAD
- OAD
- IAD and OAD
- RSD with IAD

The graph illustrates the distribution of dynein arm defects in London from 1988 to 2008.
Genotyping

- >8 known mutations:
  - DNAI1, DNAH5, TXNDC3, DNAH11, DNAI2, C14orf104 (KTU), RSPH4A, and RSPH9
  - Mutations in DNAI1 account for approximately 2%-10% of cases
  - Mutations in DNAH5 account for approximately 15%-28% of cases
  - Clinical testing available in US and Germany

Genotyping

- > 60% patients with PCD do not have an identifiable mutation in any of the eight known genes.
- *DNAH11* mutation identified in patients with typical clinical phenotype but no identifiable EM defect.
- None of the 8 known mutations are associated with non-PCD phenotypes
PCD genes
8 genes causing 17-38% disease

Outer dynein arm (Chlamydomonas)

DNAI2 2008
DNAI1 1999
TXNDC3 2007
DNAH5 2002
DNAH11 2002
RSPH9, RSPH4A 2009

Clinical Features

- **Respiratory**
  - neonatal respiratory symptoms (73%)
  - Wet/ productive cough
  - Recurrent chest infections
  - Bronchiectasis
- **Chronic Rhinitis (100%)/ sinusitis (60-80%)**
- **Recurrent otitis media (95%)**
- **Situs Inversus (55%)**
- **Other features**
  - Hydrocephalus
  - Hepatic/Renal Disease
  - Retinal abnormalities
  - Abnormal sperm flagella

*Figures in brackets from PCD Diagnostic and Phenotypic features Noone et al AJRCCM 2003*
Early diagnostic clues

- History of neonatal respiratory distress - >75%
- Dextrocardia +/- total situs inversus
- Chronic nasal congestion from birth
- Recurrent otitis media
- Hearing loss/speech delay
- Placement of grommets with subsequent complications.
Other features

- Polyps
- Recurrent LRTI
- Bronchiectasis
- Heterotaxy
- Cardiac defects
- Infertility/subfertility
PCD bronchoscopy

Typical bronchoscopic findings in a patient with PCD
CF bronchoscopy
Otorrhoea in a patient with PCD
Chronic rhinosinusitus

Nasal Cycle

Mucociliary clearance of frontal sinus

Ostiomeatal complex

Mucociliary clearance of maxillary sinus

Fluid collected in sinus

Cilia drain sinuses by propelling mucus toward natural ostia (mucociliary clearance)
Imaging of sinusitis
Situs anomalies in PCD

- Situs solitus 46%
- Heterotaxy 6.3%
- Situs inversus 47.7%
- Left isomerism 3.3%
- Right isomerism cardiac defects 0.3%
- Other 2.7%

Heterotaxy has much higher incidence of other situs and organ anomalies such as asplenia or polysplenia
Screening and diagnosis

**Aim:** Identification of abnormal ciliary motility and/or **ultrastructural defects**

**Screening – non-invasive**
- Nasal NO
- Imaging – situs, cardiac and abdo.

**Diagnostics – invasive**
- Nasal brush biopsy
- Light microscopy:
  - CBF and beat pattern
- Transmission electron microscopy:
  - Ultrastructure
- Primary cell culture
  - Eliminate secondary defects
- Immunofluorescent staining of ciliary biopsies
- Molecular genetic testing
Implications of missed diagnosis

- Bronchiectasis
- Progressive reduction in lung function
- Mismanagement of ENT issues
- Inappropriate ENT surgery
- Developmental and speech delay

- Cardiac, splenic and gut anomalies
- Reduced fertility/ectopic pregnancy
Nitric Oxide

- Low nasal NO [<200ppb] unique to PCD
- Nitric oxide has an important host defence role
- Antibacterial/antiviral effect
- Modulates ciliary function with up regulation of motility
- Replaced historic screening techniques in specialist centres
Nasal Nitric Oxide in respiratory diseases

Nasal NO in patients with PCD compared to disease controls and normal subjects. Wodehouse et al; ERJ 2003;21:43-47
From nose to EM

Post screening:
- Nasal brushing taken
- Examined under LM and assessed for:
  - appearance
  - movement
  - clearing of debris
  - ciliary beat frequency
- If abnormal sample sent for EM
Light Microscopy

Nasal brushing is taken from the inferior turbinate.
High magnification photograph
Normal ciliary cross sections with 9 + 2 arrangement, visible radial spokes and presence of both outer and inner dynein arms
Outer dynein arm defect

Outer dynein arms are missing or if present appear shortened
Inner dynein arm defect

Inner dynein arms are missing
Outer and Inner dynein arm defect

Both inner and outer dynein arms are missing.
Inner dynein arm and radial spoke defect

Inner dynein arms are missing and a large proportion of cross sections appear disarranged.
Transposition defect

A transposed cross section with an 8+1 arrangement

The central pair disappears and is replaced by an outer doublet
Disarrangement in tip

Transposed cross section showing 8+1 arrangement

Missing central pair 9+0 arrangement

Normal ciliary ultrastructure
Secondary defects

1. Compound cilia
2. Extra microtubule
3. Missing central pair
4. Missing one of the central pair
Transposition Defect
Pitfalls

- Nasal obstruction = falsely low NO
- Inflamed/infected noses = nude cilia, secondary defects
- Painful procedure – traumatic for patients/parents
- 15-20% patients with classical phenotype have ‘normal’ EM
Effects of commissioning

Number of referrals for diagnosis

- Southampton
- Leicester
- RBH
Difficult cases

- **Cell culture**
  - Primary from nasal brushings

- **Tomography**
  - 3D EM tomograms

- **Immunofluorescence**
  - Specific Abs to axonemal proteins

- **Immuno-EM**

- **Radio-labelled mucociliary clearance**
Immunofluorescence microscopy

- Analysis of protein expression throughout the axoneme related to specific gene mutations
- Visualization of proteins along the length of the ciliary axoneme, the transition zone and the basal body
Immunoflouresence

- $DNAH5$ – absence of ODA protein throughout axoneme, accumulation at base of cilium. No interruption of IDA proteins.
- $DNAI2$ – affects assembly of both proximal and distal ODA complexes
- $DNAI1$ – proximal disruption of ODA assembly
- Isolated IDA defects – no disruption of $DNAH5$
- $DNAH9$ ?

Green – alpha tubulin [axoneme-specific control] Red – anti-DNAH5 Abs

A. Normal control; B. PCD typical DNAH5; C. PCD DNAH5 affecting splicing
Patient with ODA defect
The Basis of Electron Tomography

Microscope → Sample → Tilt Series → Tomogram → Modelling & Surface Reconstruction
Tomography
1. Averaged reconstruction of human cilia central pair structure

2. Rendered tomogram of human cilia central pair structure

3. Averaged reconstruction of human cilia outer microtubule doublet

4. Rendered tomogram of human cilia outer microtubule doublet
Averaging tomographic data

[a] Tomogram reconstruction from normal human cilium
[b] Tomogram reconstruction from Chlamydomonas
Key aims of management

- Stabilisation of lung function
- Reduce progression of lung disease
- Specific management of middle ear disease
- MDT approach
Clinical care pathway

- Multidisciplinary team approach
  - Respiratory specialist
  - ENT/Audiology
  - Physiotherapy
  - Clinical nurse specialist
  - Respiratory technician/scientists [diagnostics]
  - Associated specialists – cardiologists
- Regular specialist centre review
  - Minimum of annual review
- Well developed shared care links
Management – not specific to PCD

- Chest physiotherapy
- Serial sputum samples
- Prompt antibiotic treatment
- Serial lung function testing
  Management similar to any form of chronic suppurative lung disease.
- Late drop in lung function
- Minimal systemic symptoms
  Clinical experience in PCD needed to assess possible decline.
Preventative measures

- Vaccination against influenza, \textit{s.pneumoniae, h.influenzae and b.pertussis.}
- Avoidance of smoke inhalation
- Relative segregation [in-patients]
- Early diagnosis
Management specific to PCD

ENT:
  - Avoid tympanostomy tube insertion:
    - prolonged offensive otorrhoea
    - rarely improves hearing
  - Audiology
  - Hearing aids
  - Speech therapy
  - Educational support
Audiology and hearing aids
Chronic rhinosinusitis

Major criteria:
- Nasal congestion or obstruction
- Nasal discharge
- Facial pain or pressure
- Headache
- Olfactory disturbance
- Duration >12 weeks
Management of chronic rhinosinusitus

- Treatment is primarily medical:
  - saline drops/sprays/douching
  - corticosteroids
  - antibiotics
  - anti-leukotrienes
  - anti-histamines
- Endoscopic sinus surgery should be considered for complications
Nasal toilet

“Usability” Problem
Prognosis

- **Progressive lung disease**
- With adequate treatment extensive lung damage is avoidable
- Normal life span is possible
- Resolution of middle ear disease by late childhood
- Sinusitus may become predominant cause of upper airway morbidity
Room for improvement

- 15-20% patients have no EM diagnosis
  - R & D ongoing in each centre
- Post diagnostic care pathway
  - Current bid for National Funding
- Better access to ethnic pockets
  - Patient advocacy
  - Access to specialist care
- Database – UK registry → EU registry
What next?

Fill the clinical care gap:
- Early diagnosis and aggressive management
- Access to specialised care for all
- Build up shared care pathways to improve local care
- Home visits/outreach services
- National guidelines
Conclusions

- PCD is uncommon
- Spectrum of severity and ‘familiar’ symptoms often delay diagnosis
- **Cardinal clues are present in 75% cases**
- Array of diagnostic tests needed
- Specialist care with MDT approach
- **Early diagnosis** to be strived for
- Normal life span is possible
‘Cilia in Development and Disease’

CILIA 2012 - 1st International Conference
16-18 May, 2012 | Institute of Child Health/UCL | London

Scientific sessions (17 & 18 May):
- Clinical Aspects of Ciliopathies
- Structure and Function of Cilia
- Cilia and Development
- Cilia and Disease
- Translational Therapy and Ciliotherapeutics

Confirmed speakers:
- Kathryn Anderson
- Rachel Giles
- Friedhelm Hildebrandt
- Peter Jackson
- Heymut Omran
- Greg Pazour
- Jeremy Reiter
- Enza Maria Valente
- John Wallingford
- Brad Yoder

Welcome & VIP reception (16 May):
- Keynote: Joseph Gleeson
- Ciliopathy Alliance Patient Groups

Initiated by
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Registration & abstract submission: cilia2012.org

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