DIAGNOSIS OF PRIMARY CILIARY DYSKINESIA

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Introduction

• **Cause**
  – Congenital dysfunction of motile cilia
  – Absence/dysfunction of one of the ciliary (motor) proteins

• **Clinical signs**
  – Chronic upper and lower respiratory tract infections
  – Situs inversus in about 50%
  – Increased incidence of male infertility

Very heterogeneous disorder
Think of PCD when

- Neonatal respiratory problems
- Situs inversus (only <50%) + cardiac anomalies
- Lower airways:
  - Chronic cough
  - Therapy resistant wheeze ‘asthma’
  - Recurrent chest infections
  - **Bronchiectasis**
- **ENT:**
  - Recurrent otitis with otorhea
  - Chronic rhinosinusitus
  - **Nasal polyps**
- Male infertility (not universal)
### All PCD (n = 168)

<table>
<thead>
<tr>
<th></th>
<th>All PCD (n = 168)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis in years (median - IQR)</td>
<td>9.9 (3.7-23.4)</td>
</tr>
<tr>
<td>Male gender %</td>
<td>55</td>
</tr>
<tr>
<td>Consanguinity %</td>
<td>20</td>
</tr>
<tr>
<td>Sibling with PCD %</td>
<td>22</td>
</tr>
<tr>
<td>Situs inversus %</td>
<td>41</td>
</tr>
<tr>
<td>Structural cardiac abnormality %</td>
<td>6</td>
</tr>
<tr>
<td><strong>Lower respiratory tract</strong></td>
<td></td>
</tr>
<tr>
<td>Neonatal respiratory problems %</td>
<td>45</td>
</tr>
<tr>
<td>Wheezing %</td>
<td>47</td>
</tr>
<tr>
<td>Bronchiectasis %</td>
<td>68</td>
</tr>
<tr>
<td>Lobar collaps %</td>
<td>40</td>
</tr>
<tr>
<td>Pulmonary infiltrate %</td>
<td>60</td>
</tr>
<tr>
<td>Lobectomy %</td>
<td>9</td>
</tr>
<tr>
<td><strong>Upper respiratory tract</strong></td>
<td></td>
</tr>
<tr>
<td>Recurrent sinusitis %</td>
<td>66</td>
</tr>
<tr>
<td>Nasal polyps %</td>
<td>32</td>
</tr>
<tr>
<td>Sinus surgery %</td>
<td>40</td>
</tr>
<tr>
<td>Ear discharge %</td>
<td>42</td>
</tr>
<tr>
<td>Hearing loss %</td>
<td>34</td>
</tr>
</tbody>
</table>

PCD is diagnosed too late
Nasal mucociliary transport test

- 99mTc-albumin colloid drop 1 cm past naris
- Motion evaluated cinematographically
- Abnormal if >10 min to nasopharynx
- Feasible in young children
- Sensitivity 100%
- Specificity 53%

De Boeck et al, Thorax 2005
Pulmonary radioaerosol clearance test

- 99mTc-albumin colloid inhalation
- Pulmonary radioactivity at 0, 30, 60, 90 and 120 min
- Compared to reference
- Sens 88%, Spec 100%
- Not widely available
- Not feasible <5y

Marthin et al, Chest 2007
Screening tests

Nasal Nitric Oxide (nNO)

• nNO measurement
  – Direct sampling in the nose
  – Oral exhalation against resistance to close velum
  – Chemiluminescence analyzer

• Good screening test

• BUT
  – Poor standardisation
  – Cut-off values vary
  – Unfeasable in children <6y
  – Sensitivity and specificity not optimal
Screening tests

Nasal Nitric Oxide (nNO)

Cut-off 300 ppb
Sensitivity 89.5%
Specificity 87.3%
Overlap with disease controls

Boon M et al, submitted
Ciliary function analysis

- Brush or punch biopsy
- Direct evaluation of ciliary movement by light microscopy
  - Coordination of ciliary motion
    - Movement of cell debris and RBC
    - Rotation of cell humps
  - Ciliary beat frequency (CBF)
    - High speed videocamera
- **BUT**
  - Ciliary immotility not absolute in PCD
  - Immotility in secondary ciliary dyskinesia (smoke, infection, toxic substances)
Diagnostic tests

CBF distribution in biopsies

- Normal
- 5-15% SCD
- >15% SCD
- PCD
Ciliary structure analysis: TEM
Ciliary structure analysis: TEM

- Normal
- ODA deficiency
- Partial ODA deficiency
- ODA+IDA deficiency
- Absence of central pair
- Displacement of peripheral pair
- Absence of peripheral pair
- Ciliary aplasia

Boon M et al, Eur J Ped 2013
Diagnostic problems with biopsies

- Functional heterogeneity
  - Ciliary immotility not absolute in PCD
  - Immotility in SCD

- Ultrastructural heterogeneity
  - normal ultrastructure in PCD
  - Overlap SCD - PCD
  - SCD + PCD
Diagnostic tests

Signs of SCD

- Compound cilia
- Naked cilia
- Membrane blebs
- Disorganisation of microtubular structure

Boon M et al, Eur J Ped 2013
Jorissen et al, Acta otorhinolaryngol Belg 2000
Culture and ciliogenesis

- Biopsy
- Culture
- TEM
- CBF
- Coordinated activity
- Ciliogenesis
- Monolayer (3 weeks)
- Suspension (3 weeks)
Monolayer → suspension
PCD - Diagnostic solution

functional evaluation after ciliogenesis in culture

1. normal = normal
2. SCD is absent
3. PCD is expressed
NORMAL: coordinated ciliary activity
Diagnostic tests

PCD: non-coordinated ciliary activity
## Coordinated Activity

<table>
<thead>
<tr>
<th></th>
<th>Coordinated activity</th>
<th>No Coordinated Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biopsies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-PCD</td>
<td>289</td>
<td>73</td>
</tr>
<tr>
<td>PCD</td>
<td>14</td>
<td>70</td>
</tr>
<tr>
<td><strong>Ciliogenesis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-PCD</td>
<td>780</td>
<td>0</td>
</tr>
<tr>
<td>PCD</td>
<td>0</td>
<td>104</td>
</tr>
</tbody>
</table>
PCD diagnosis after ciliogenesis in culture

14 of 84 PCD patients with “normal” ultrastructure:

- **Biopsy:**
  - Coordinated activity
  - Normal CBF values

- **After ciliogenesis**
  - Non-coordinated activity
  - Immotile or low CBF values

- **Mutations in DNAH11 in >50%**
CBF after ciliogenesis

Diagnostic tests

number of patients

CBF (Hz)

PCD (N=67)
non-PCD (N=505)
Diagnostic tests

Dynein arms

biopsy

ciliogenesis

outer dynein

inner dynein

number of patients

0.0 0.6 1.2 1.8 2.4 3.0 3.6 4.2 4.8 5.4 6.0 6.6 7.2 7.8 8.4 9.0

outer dynein

inner dynein

number of patients

0 10 20 30 40 50 60 70

0.0 0.6 1.2 1.8 2.4 3.0 3.6 4.2 4.8 5.4 6.0 6.6 7.2 7.8 8.4 9.0

outer dynein

inner dynein

number of patients

0 50 100 150 200 250

0.0 0.6 1.2 1.8 2.4 3.0 3.6 4.2 4.8 5.4 6.0 6.6 7.2 7.8 8.4 9.0
50 cross sections near base

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal values: mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer dynein arms</td>
<td>8.4 (0.8)</td>
</tr>
<tr>
<td>Inner dynein arms</td>
<td>3.7 (1.4)</td>
</tr>
<tr>
<td>Radial spokes</td>
<td>5.5 (1.5)</td>
</tr>
<tr>
<td>Absent central pair</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Abnormalities peripheral microtubules</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>Membrane abnormalities</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>
PCD: Ultrastructural classification (N=208)

- Normal: 33.7%
- Partial dyn def: 17.8%
- Outer dyn def: 21.6%
- Outer + inner dyn def: 10.6%
- Central ecc + inner dyn def: 10.6%
- Central absent: 8.7%
- Peripheral microt abn: 0.5%
- Aplasia: 3.4%
Genetics of PCD

- Mainly autosomal recessive inheritance

- Very heterogeneous
  - Cilium consists of >250 proteins

- >20 genes identified as disease causing

- In most case series, gene defect found in 50%
To some degree specific genes cluster to specific ultrastructural defects

- **ODA deficiency**
  - DNAH5 (33-50%), DNAI1 (2-13%), DNAI2 (4%), DNAL1, TXNDC3, CCDC114, ARMC4 (rare)

- **ODA + IDA deficiency**
  - DNAH5 (24%), DNAAF1 (17%), DNAAF2 (12%), DNAAF3, CCDC103, HEATR2, LRRC6 (10,6%), RPGR, ZMYND10 (rare), SPAG1 (rare), DNAAH4 (rare), C21orf59 (rare)

- **Isolated IDA deficiency**

- **Normal ultrastructure**
  - DNAH11 (22%), HYDIN (rare), CCDC65 (rare)

- **Central pair absent**
  - RSPH4A

- **Dislocation central pair (+IDA deficiency)**
  - RSPH9, CCDC39 (38-64%), CCDC40 (23-57%), RSPH1 (rare)

- **Absent peripheral pair**

- **Ciliary aplasia**
  
  *Boon et al, Eur J Ped 2013*
Conclusion: PCD diagnosis

• High index of suspicion

• Screening test: nasal NO or Tc scan

• Coordinated ciliary activity after ciliogenesis in culture

• Genetic diagnosis in the future
Acknowledgements

• Mieke Boon, fellow pediatric pulmonology
• Mark Jorissen, Department of Otorhinolaryngology, Head and Neck Surgery
• Martine Jaspers and Valerie Vlaeminck, Experimental Lab Otorhinolaryngology
• Harry Cuppens, Genetics
## Diagnostic tests

### Outer and inner dynein arms in PCD with dynein deficiency

<table>
<thead>
<tr>
<th></th>
<th>biopsy</th>
<th>ciliogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>outer</td>
<td>inner</td>
</tr>
<tr>
<td>IODD (N=6)</td>
<td>1.0 ± 0.5</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>ODD (N=27)</td>
<td>1.9 ± 1.0</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>pDD (N=8)</td>
<td>3.1 ± 1.3</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td>non-PCD</td>
<td>8.4 ± 0.5</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>“normal” PCD</td>
<td>8.3 ± 1.0</td>
<td>3.1 ± 1.0</td>
</tr>
</tbody>
</table>
# Diagnostic tests

## Ultrastructural parameters in PCD with absent central pair

<table>
<thead>
<tr>
<th>parameter</th>
<th>biopsy</th>
<th>ciliogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>outer dynein arms</td>
<td>8.4 ± 0.3</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>inner dynein arms</td>
<td>3.4 ± 0.6</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>spokes</td>
<td>4.7 ± 1.1</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td>absent central pair</td>
<td>15 ± 16%</td>
<td>21 ± 19%</td>
</tr>
<tr>
<td>(3 – 43)</td>
<td>(4 – 53)</td>
<td></td>
</tr>
<tr>
<td>SCD</td>
<td>11.4 ± 7.3%</td>
<td>0.5 ± 1.3%</td>
</tr>
<tr>
<td>ciliary orientation</td>
<td>38 ± 11°</td>
<td>38 ± 9°</td>
</tr>
</tbody>
</table>
## CBF in PCD in biopsies

<table>
<thead>
<tr>
<th>Ultrastructure</th>
<th>mean (Hz)</th>
<th>CBF=0</th>
<th>CBF=nl</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>dynein deficiency</td>
<td>0.5 ± 1.4</td>
<td>26</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>ECP+IDD</td>
<td>0.5 ± 1.4</td>
<td>6</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>central absent</td>
<td>4.7 ± 2.7</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>“normal”</td>
<td>5.8 ± 5.1</td>
<td>4</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>
Diagnostic tests

CBF in PCD after ciliogenesis

<table>
<thead>
<tr>
<th>Ultrastructure</th>
<th>mean (Hz)</th>
<th>CBF=0</th>
<th>CBF=nl</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>dynein deficiency</td>
<td>0.1 ± 0.5</td>
<td>34</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>ECP+IDD</td>
<td>0.4 ± 1.2</td>
<td>7</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>central absent</td>
<td>4.4 ± 0.9</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>“normal”</td>
<td>2.6 ± 4.0</td>
<td>10</td>
<td>3</td>
<td>17</td>
</tr>
</tbody>
</table>
Ciliogenesis in culture for diagnosis of Primary Ciliary Dyskinesia

overview of >3244 pts in 23 years
PCD diagnosis after ciliogenesis in culture

- Culture characteristics for diagnosis of PCD
  - absence of secondary abnormalities
  - expression of inherited abnormalities

- Ciliary function
  - CBF: immotility = diagnostic
  - coordination: 100% reliable

- Ciliary ultrastructure
  - normal ultrastructure > 30%
evaluation of ciliary function (CBF and coordination) in PCD with normal ultrastructure

- cilia present
  - n = 38
- no cilia present
  - n = 29

- Normal evaluation
  - n = 12
- Abnormal evaluation
  - n = 26

- Abnormal evaluation
  - N = 12
- Abnormal evaluation
  - N = 26
- Abnormal evaluation
  - N = 29
PCD diagnosis after ciliogenesis in culture ultrastructural subgroup advantages

- **Dynein deficiency**
  - 45%

- **Central complex**
  - + (↔ SCD) 15%

- **Rare abnl.**
  - +++ (↔ SCD) 5%
    - Ciliary aplasia
    - Peripheral microtubular abnl.

- **Normal ultrastructure**
  - +++ (↔ normal) 35%
Saccharine test

- Microtablet of saccharine placed on the inferior nasal turbinate
- Time to ‘sweet taste’ is recorded
- Abnormal if > 1h
- Low specificity and poor feasibility
Screening tests

Nasal Nitric Oxide (nNO)
- Nitric oxide high in healthy paranasal sinuses
- nNO is low in PCD, FENO is low too \( (Lundberg et al \ 1994) \)
- Proposed pathophysiologic mechanisms
  A. Increased breakdown of NO \( (Jones et al \ 1998) \)
  B. Decreased biosynthesis: NO synthase expression ↓ \( (Pifferi et al \ 2011) \)
  C. Obstruction of osteomeatal complex (sinuses are main site of NO production) or hypoplasia of sinuses \( (Pifferi et al \ 2011) \)

BUT nNO also low in CF, nasal polyps

Walker et al, ERJ 2012
Diagnostic problems in biopsies

- **lack of sensitivity**
  - functional: “coordination” - normal CBF
  - ultrastructure: normal (TEM)

- **lack of specificity**
  - functional: immotility in SCD
  - ultrastructure: “primary” being secondary
PCD + SCD

SCD in PCD pts: 13.4 ± 11.5%
Diagnostic tests

Distribution of CBF in normal controls, SCD and PCD after ciliogenesis

![Graph showing the distribution of CBF (Hz) in different categories for controls, SCD, and PCD.](image)

- **CBF (Hz)**: 0-1, 1-3, 3-5, 5-7, 7-9, 9-11, 11-13, 13-15
- **% of patients**
- **Legend**:
  - Controls
  - SCD
  - PCD
PCD - Diagnostic solutions

(functional) evaluation after ciliogenesis in culture

1. normal = normal

2. SCD is absent

3. PCD is expressed
SCD after ciliogenesis

- Controls: 0.5 ± 0.9%
- SCD pts: 0.8 ± 1.7%
- PCD pts: 1.4 ± 1.9%
PCD - Diagnostic solutions

(functional) evaluation after ciliogenesis in culture

1. normal = normal

2. SCD is absent

3. PCD is expressed
Diagnostic tests

Expression of PCD

Central pair eccentric + inner dynein deficiency

Outer dynein deficiency