Supplementary Information

Contents

1.0 Delphi Survey results

1.1 Results from 1st survey

Question 1: Please select which of the following you consider to be a hallmark PCD defect.

We unanimously agreed that these three defects are hallmark:

- **Outer dynein arm absence**
- **Combined outer and inner dynein arm absence**
- **Combined inner dynein arm absence and microtubular disorganisation.**

Question 2: Would you consider any of the following TEM defects to be diagnostic of PCD?

We agreed (>80%) that the following defects can be considered to be diagnostic of PCD but not always (i.e they are not hallmark)

Central complex defect
Mislocalisation of basal bodies with few or no cilia
Outer dynein arm absence from 25%-50% cross sections
Combined inner and outer dynein arm absence from 25-50% cross sections

We did not reach consensus regarding the following:
Inner dynein arm defect (2 did not respond): **No** 43% **Yes (any category)** 57%
Isolated microtubular disorganisation (1 did not respond): **No** 29% **Yes (any category)** 71%
Outer dynein arm absence <25% cilia (1 did not respond): **No** 21% **Yes (any category)** 79%
Combined outer and inner dynein arm absence <25% cilia (1 did not respond): **No** 21% **Yes (any category)** 79%

Orientation defect: **No** 43% **Yes (any category)** 57%
Abnormally long cilia: **No** 57% **Yes (any category)** 43%

Question 3: Please describe what you consider to be 'hallmark 'for each of the following e.g. 'outer dynein arm absence': absence of the whole outer dynein arm from the majority of cross sections.

We have many descriptions of the hallmark defects. Highlighting nicely why this consensus is necessary. These have been combined together for example for the ODA with the phrase below for voting in the second round.

Absence of the whole or part of the outer dynein arm structure from the majority of (>5) microtubular doublets in the majority (>50%) of cilia cross sections **OR** Presence of the complete outer dynein arm structure on the minority of (≤ 4) microtubular doublets in the majority (>50%) of cilia cross sections.

Question 4: Which of the following items is it important to include in a TEM cilia report?

We met consensus (>80%) that it is important to include in every TEM cilia report

- Source of the sample (e.g. nasal brushing)
- Adequacy of the sample
- The number of cross sections assessed
- % abnormal cilia
- Presence of the central complex
- The consistency of a defect across several cells
- 1 sentence summary of key findings

We also met consensus that in (all or some) circumstances the following should be included:

- Orientation/ alignment of the basal body or central pair of microtubules
- The number of cells assessed
- Blebs/membrane swelling/membrane condition
- Presence of compound cilia (more than one axoneme within a membrane)
- Preservation of the sample
- % cilia with a hallmark defect
- Presence of shortened or truncated ODA projections
- Microtubular organisation
- Evidence of inflammation
- Evidence of bacteria

We did not meet consensus on

- Estimated location of defect (distal or proximal)
- location of the basal body
- Presence of nexin links/DRC
- Presence of radial spokes
- Fixation and processing protocol
- Section thickness
- Comments on the semi- thin sections
- Evidence of blood
- % ciliated cells
- Length of cilia

1.2 Results from 2nd survey

Question 1: Please state whether you agree or disagree with the definitions in the text above. If you disagree please rephrase the definition in the comments box below

18 respondents:

ODA defect definition 17/18 agreed (94%)

ODA+IDA defect definition 13/18 agreed (72%) DID NOT MEET CONSENSUS

IDA & MTD defect definition 15/18 agreed (83%)

Question 2: Using the definition above what is the minimum number of ciliary axonemes in cross section needed to assess before confirming a hallmark defect

Consensus 80% = 50, median = 50, mode = 50, mean = 58

Question 3: Please list your criteria for inclusion of a ciliary axoneme in cross section in your assessment. Examples may include: an intact membrane, sufficient contrast to see the radial spokes, visualisation of a healthy epithelial cell from which the cross section originates, presence of 9 microtubular doublets and a central pair etc.This question is designed to understand differences in the number of cross sections we assess.
Themes included: Membrane =14, Cell =6, Contrast =5, Visulisation of other structures =10

Question 4: The following defects can be considered to be suggestive of PCD if reproducible on a second sample or culture AND consistent with supporting evidence from other investigations

We met consensus on
Central complex defect (83%)
Outer dynein arm absence from 25-50% cilia (83%)
Combined outer dynein arm and inner dynein arm absence from 25-50% cilia (82%)

We did not meet consensus

Microtubular disorganisation with IDA present (78%)

Mislocalisation of basal bodies with few or no cilia (77%)

Outer dynein arm absence from <25% cilia (50%)

Outer and inner dynein amr absence <25% cilia (50%)

1.3 Results from final survey

Q1: Please state whether you agree or disagree with the definitions in the text box above.

Inner and outer dynein arm defect:
Absence of the whole or larger part of the outer dynein arm structure from the majority of (> 5) microtubular doublets in the

majority (>50%) of cilia cross sections coupled with absence of the whole or larger part of the inner dynein arm structure from the majority of (> 7) microtubular doublets in the majority (>50%) of ciliary axonemes visualised in cross section

Agree 82%

Q2: In the first round of the survey we agreed '% defects' should be included in the report. In the second round discussions we realised this is a vague definition. Do you agree or disagree the following should be included in the final TEM report

% Hallmark defects Agree 94%

% Hallmark and class 2 defects Agree 82%

% All defects Agree 65%

2.0 Summary of consensus validation analysis

2.1 1st validation summary - grid swap

Diagnosis	Expected guideline result	Returned results					
		1	2	3	4	5	6
DNAI2	Class 1: ODA	ODA+IDA	ODA	Insuff	ODA		
DNAAF1	Class 1: ODA+IDA	ODA+IDA	ODA+IDA	ODA+IDA	ODA+IDA		
Cystic fibrosis	Normal Ultrastructure	Normal Ultrastructure	Normal Ultrastructure	Normal Ultrastructure (chronic bronchitis)	Normal Ultrastructure	Insuff	Normal Ultrastructure
DRC1	Normal Ultrastructure (MTD)	Normal Ultrastructure (MTD)	Normal Ultrastructure (MTD)	Normal Ultrastructure	Normal Ultrastructure (MTD)	Insuff	Normal Ultrastructure
CCNO	Class 2: mislocalised BB + few or no cilia	Insuff	Insuff	Insuff	Insuff		
CCDC40	Class 1: IDA + MTD	IDA + MTD	Insuff	IDA + MTD	IDA + MTD		
CCDC114	Class 1: ODA	ODA+IDA	ODA+IDA	ODA	Mixed (predominant ODA)		
Inadequate - non pcd	Inadequate	Insuff	Insuff	Class 2: mislocalised BB + few or no cilia	Insuff		
RSPH4A	Class2: CC	IDA + MTD	CC	CC	CC	CC	CC
DNAAF3	Class 1: ODA+IDA	ODA+IDA	ODA+IDA	ODA	ODA+IDA		
CCDC39	Class 1: IDA + MTD	insuff (IDA+MTD)	ODA	Insuff	Insuff		
Healthy Volunteer	Normal Ultrastructure	Insuff	class 2	Normal Ultrastructure	Normal Ultrastructure		
DNAH11	Normal Ultrastructure	ODA	Insuff	Normal Ultrastructure	Normal Ultrastructure		
DNAH5	Class 1: ODA	ODA	ODA+IDA	ODA+IDA	ODA+IDA	ODA	ODA
Inadequate - Unaffected sibling	Inadequate	class 2 lack cilia	Insuff	Insuff	CC		

Main results summarised

1. **100% participation**
2. **There were no false positive diagnoses** (i.e No non-PCD sample was classed as having a Class1 Hallmark PCD defect)
3. **17/68 (25%) sections were described as insufficient or inadequate for assessment**
    a. These included 100% returns on a CCNO case and 75% returns for a sample included as an inadequate sample. The 4th operator defined this inadequate sample as class 2: basal body mislocalisation with few or no cilia.
    b. In 2 further samples in which75% returns recorded mostly as inadequate the 4th operator recorded the incorrect defect.

4. **25/25 (100%) correct identification as a class 1 hallmark defect**
    a. **However 8/25 (32%) returns recorded an incorrect name of the class 1 defect**
        i. ODA defect: recorded as ODA+IDA defect (n=6)
        ii. ODA and IDA defect: recorded as ODA defect (n=1)
        iii. MTD and IDA defect: recorded as ODA defect (n=1)
    b. **2 cases were described as having a class1 defect when they had a class 2 defect or normal ultrastructure**
        i. 1 DNAH11 case described as ODA defect
        ii. 1 RSPH4a case descried as IDA + MTD

5. **5/6 correct identification of class 2 defects**
6. **4 normal ultrastructure cases identified as a class 2 defect**
    a. 2 central complex

b. 1 mislocalisation of basal bodies

Comments, feedback and suggested actions for discussion

All centres have received feedback on individual results and asked for feedback on the process and guideline. If you have not please let me know

Inadequate or insufficient samples

- There were some patterns as to which were deemed inadequate but not all can be explained by poor quality grids. Reasons listed in feedback included: Poor contrast, poor orientation, insufficient cilia, different grid types, different types of sample e.g. culture vs biopsy vs brushing. Operators may find it more difficult to assess grids which were not prepared at their own centre.

*Action: Following update of the guideline re-assess using TEM photographs of cross sections

- Some reports described samples with microtubular defects as inadequate because microtubules could not clearly be seen.

*Action: Update guideline to stress the importance of assessment of arms in perfect cross sections but microtubular organisation in all cross sections.

Miscoding of ODA vs ODA and IDA defects

Missing IDAs in ODA defects

*Action. Reduce the number of arms required to say IDA is present and discuss in the text OR as previous proposed by HO, that the term: class 1 hallmark defect of the ODA +/- IDA should be used if at all unsure (e.g. a poor sample or unfamiliar sample preparation)

Class 2 defects

3 centres were able to identify the DRC defect

*Action: include MTD in the class 2 defect list

Use of class 2: basal body mislocalisation with few or no cilia –used to describe inadequate samples

*Action: Extend the text around this defect

2.2 2nd validation summary - photograph swap

- **100% participation**
- **There were no false positive diagnoses** (i.e No non-PCD sample was classed as having a Class1 Hallmark PCD defect)
    - Normal ultrastructure identified by 18/18 centres
- **100% correct identification as a class 1 hallmark defect**
    - 18/18 correctly identified MTD + IDA
    - 17/18 ODA (1 judged as ODA+IDA)
    - 17/18 ODA+IDA (1 judged ODA- same centre as above (respiratory clinician participant not microscopist or pathologist)
- **100% correct identification as a class 2 defect**
    - 15/18 central complex defect (3 judged as MTD)

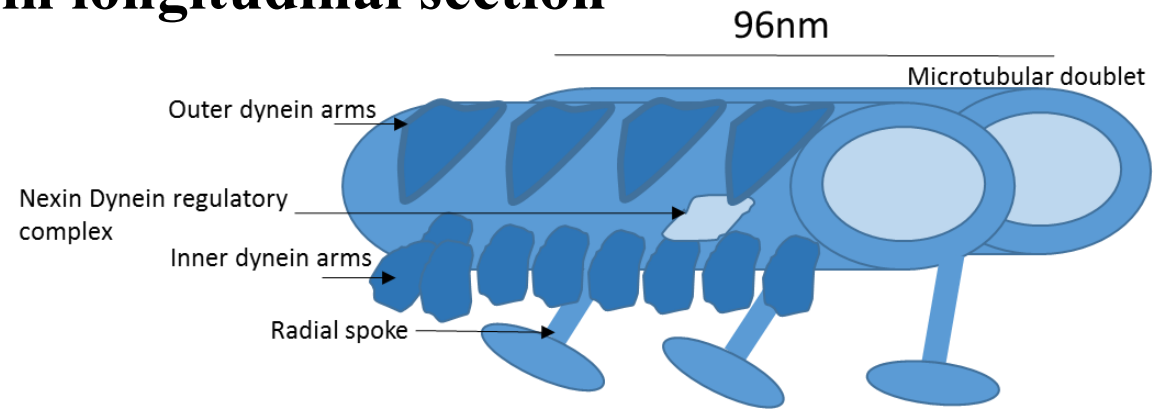
3.0 Link to example images and reports

https://uod.box.com/s/3isd4vk26qj2ac738krm2qlnj2gqf6wa

4.0 Table of Genotype by TEM phenotype

Class 1 defects	Gene	Comments
Outer dynein arm defect	*DNAH5*	Can be subtle with some missense mutations
	DNAI1	
	DNAI2	
	DNAL1	
	NME8	
	DNAH9	Distal cross sections only
	CCDC114	
	ARMC4	
	CCDC151	
	TTC25	
	MNS1	
Outer and inner dynein arm	*DNAAF1*	
	DNAAF2	
	DNAAF3	
	DNAAF4	
	DNAAF5	
	LRRC6	
	ZMYND10	
	SPAG1	
	C21ORF59	
	PIH1D3	
	CCDC103	
Inner dynein arm and microtubular disorganisation	*CCDC39*	
	CCDC40	
Class 2 defects		
Microtubular disorganisation	*CCDC164*	
	CCDC65	
	GAS8	
Central complex defect	*RSPH1*	
	RSPH4A	
	RSPH9	
	RSPH3	
	DNAJB13	
Not diagnostic		Some central pair abnormalities and absence of c2b
	HYDIN	absence
	STK36	
Mislocalisation of basal bodies	*CCNO*	
	MCIDAS	
Not-diagnostic	*CCDC11*	
	ENKUR	
	GAS2L2	
	LRRC56	Can be identified with electron tomography
	DNAH11	

5.0 Supplementary Figure 1: Normal ultrastructure in longitudinal section



5.0 Example reports

THECYPRUSINSTITUTEOFNEUROLOGYAND GENETICS

P.O.Box 23462
1683 Nicosia, CYPRUS
Tel.: 22392631 (Office)
22392792 (Lab)
Fax.: 22358237

Department of Electron Microscopy

EM No.: R-72 (C1)

Specimen Request Form

Patient Surname:	Name:	
ID Number:	Date of Birth:	Sex:
Patient Address:		
Referring Doctor:	Hospital File No.:	
Site of Biopsy: Nasal Brushing	Histology No.:	

ELECTRON MICROSCOPY REPORT

The specimen obtained was of very good quality and appeared well preserved. Electron microscopy was performed on 68 ciliary cross-sections. Ultrastructural analysis revealed abnormal ultrastructure across the sample (100%) with low numbers of outer and inner dynein arms in all ciliary cross-sections. Among the cross-sections examined there was limited evidence of tubular disorganization (7%) and central pair disorientation (9%). Few cross-sections presented with some membrane swelling (6%). Moderate evidence of inflammation and no evidence of bacteria was observed. 3% compound cilia were detected.

Summary of key findings: These results are consistent with the diagnosis of Primary Ciliary Dyskinesia, with a class 1 defect. A combined outer and inner dynein arm defect.

Date reported: 31/07/2018

Name: Example	**EM No. 18/269**	**CRN:**
Date of Birth:	**Reason for referral**	
Sample: Nasal brushing		

Ciliary profile counts:

Microtubular arrangement

Normal microtubular arrangement	Dis-arranged	Extra Tubule	Single Tubule	Other Defect	One of Pair Missing	Both of Pair Missing	Com-pound	Total Cilia
83	5	7	5	0	0	0	0	100
89	3	3	4	0	1	0	0	100
86%	4%	5%	4.5%	-	<1%	-	-	

Dynein arms

Both Arms present	Outer Arms absent	Inner Arms absent	No arms	Total Cilia
29	0	0	0	29
44	0	0	0	44
100%	-	-	-	

Comments: Unhealthy but adequate sample. Some distorted ciliary membranes. Some ciliary disorientation seen. Normal longitudinal profile.

Summary: Predominantly normal ciliary ultrastructure with both dynein arms present. Normal ultrastructure does not exclude a diagnosis of PCD