Primary Ciliary Dyskinesia
Clinical Presentation and Diagnosis

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Disclosures

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- None

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- Michael Knowles* (primary slide set)
- Michelle Manion
- PCD Foundation

OVERVIEW

Introduction

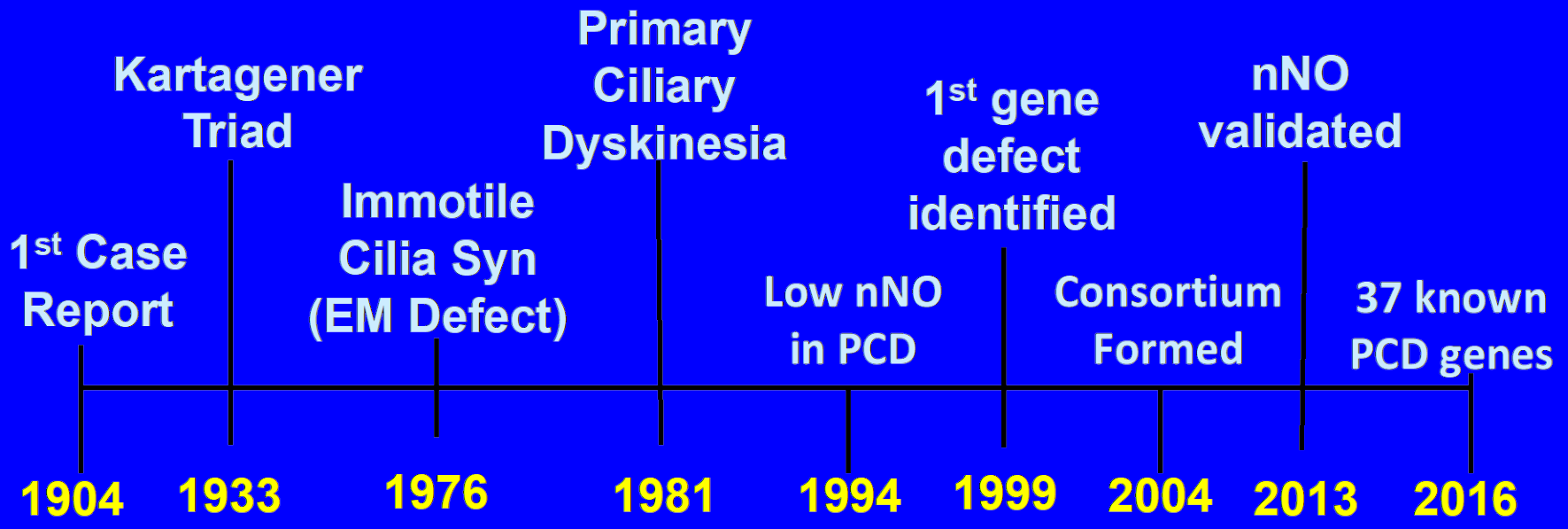
Motile & Non-Motile Cilia

PCD Clinical Presentation

Diagnostic Approaches & Challenges

- Clinical Phenotype
- Electron Microscopy
- Genetic Screening
- Nasal NO (nNO)

Primary Ciliary Dyskinesia Timeline:



Primary Ciliary Dyskinesia

- Rare genetically heterogeneous recessive disorder (1 in 15-30,000 live births; ~15,000 patients in U.S.)

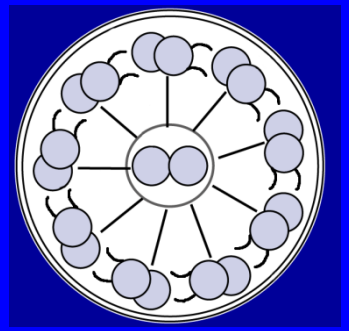
- Disease reflects abnormal ciliary biogenesis, structure, and/or function, and occurs in organs where motile cilia pay an important role:
  - Neonatal respiratory distress (≥ 80%).
  - Chronic daily wet cough from birth (97%).
  - Chronic airway disease with age-dependent bronchiectasis (100%, adults).
  - Chronic nasal congestion; sinusitis.
  - Recurrent otitis media (90%); “glue ear” with frequent hearing loss.
  - Reduced fertility (nearly all males).
  - ~50% have situs inversus or heterotaxy (Kartagener’s).

Types of Cilia

CILIUM

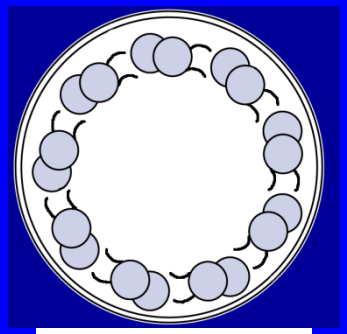
MOTILE

Planar motion (9+2)
Multiple cilia on a cell



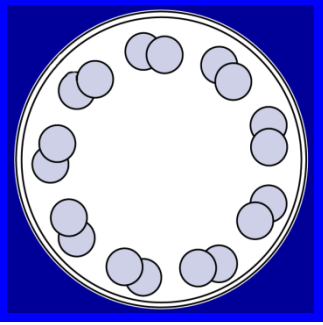
Airways (Respiratory)
Brain (Ependymal)
Reproductive Tract (Fallopian tubes, Sperm Flagellum*)

Rotary motion (9+0)
"Primary" monocilium



Embryo (Nodal cilium)

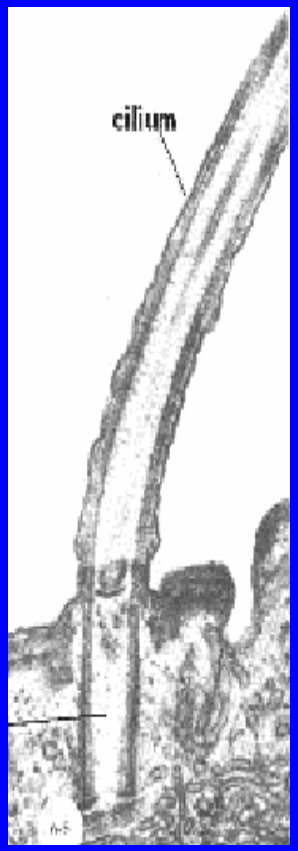
NON-MOTILE (9+0)
"Primary" monocilium (sensory)

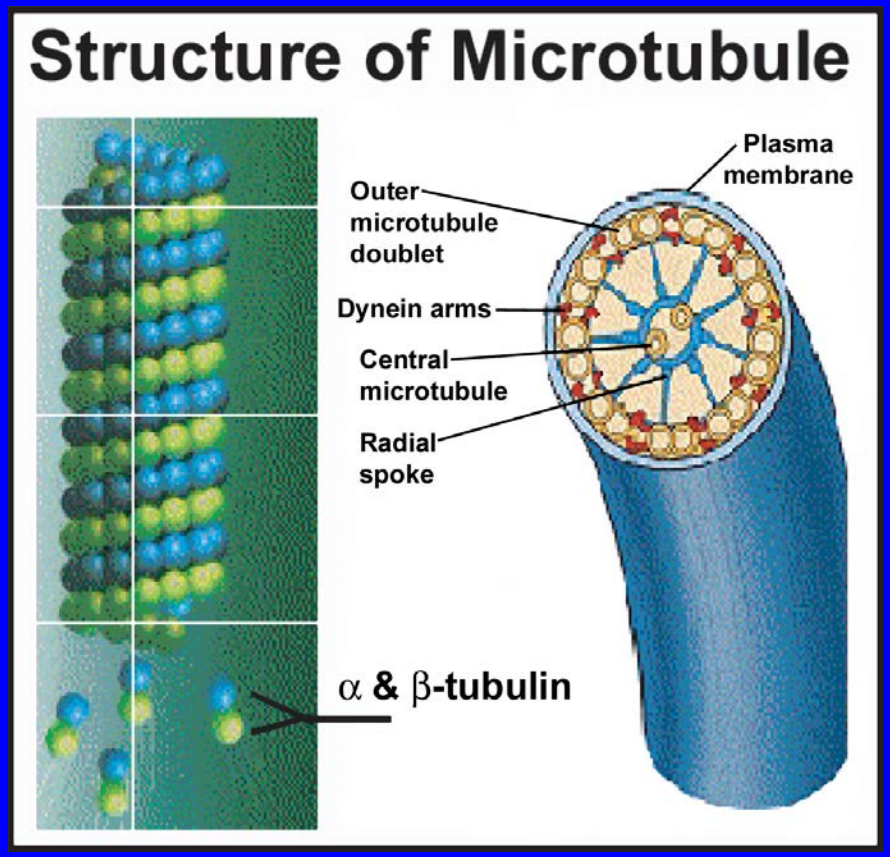


Kidney tubule
Bile duct
Pancreatic duct
Bone, Cartilage
Eye (Photoreceptor-connecting)
Fibroblasts
Stem cells

Axoneme (Cilia / Flagella) Structure and Function

- **Ciliary Beat:**
  - **Coordinated waves beat in simple backward and forward motion**
  - **Frequency 8-15 Hz**

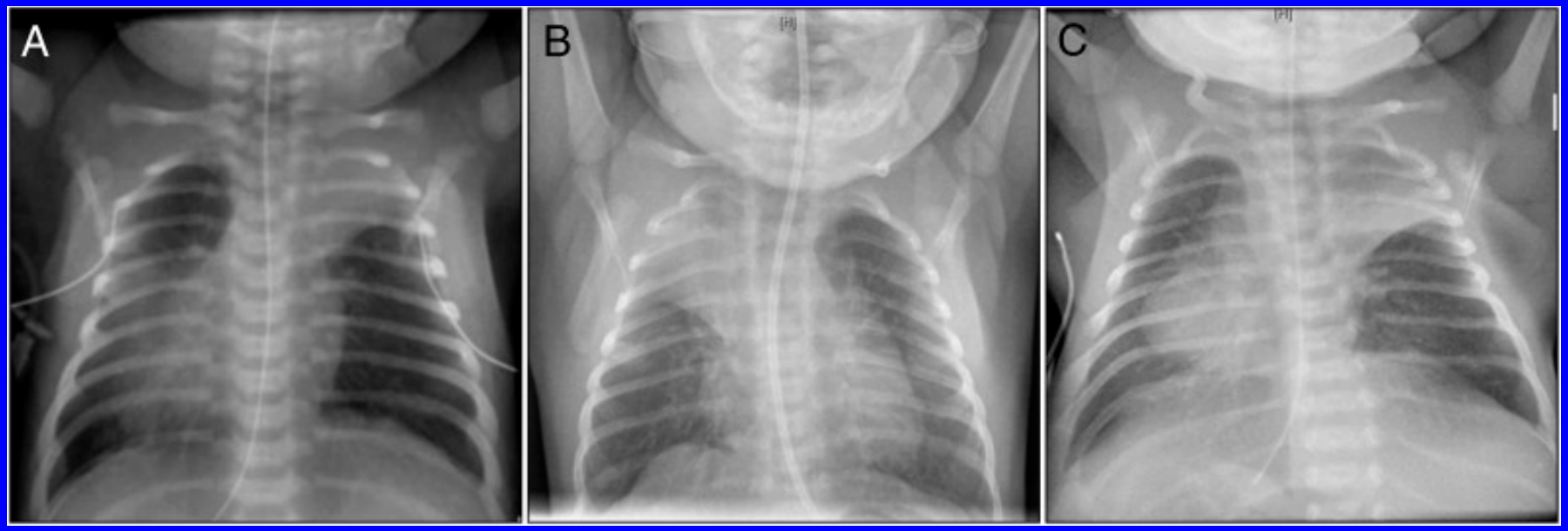




- **Ciliary Axoneme**
  - **Highly conserved structure and function across the species**
  - **Composed of >250 proteins**
  - **9+2 configuration (9 peripheral MT doublet surrounding central pair)**
  - **Each MT doublet consists of several dynein HC, IC and LC**
  - **ATPase of dynein arms provide energy of ciliary beat**

Clinical Presentation and Criteria

Lobar Collapse Occurred in 70% of PCD Cases but No Controls



Mullowney, T., *et. al*., Pediatrics 2014; 134(6):1160-1166

Specificity and Sensitivity of Rigorous Clinical Criteria for PCD in Children and Adolescents

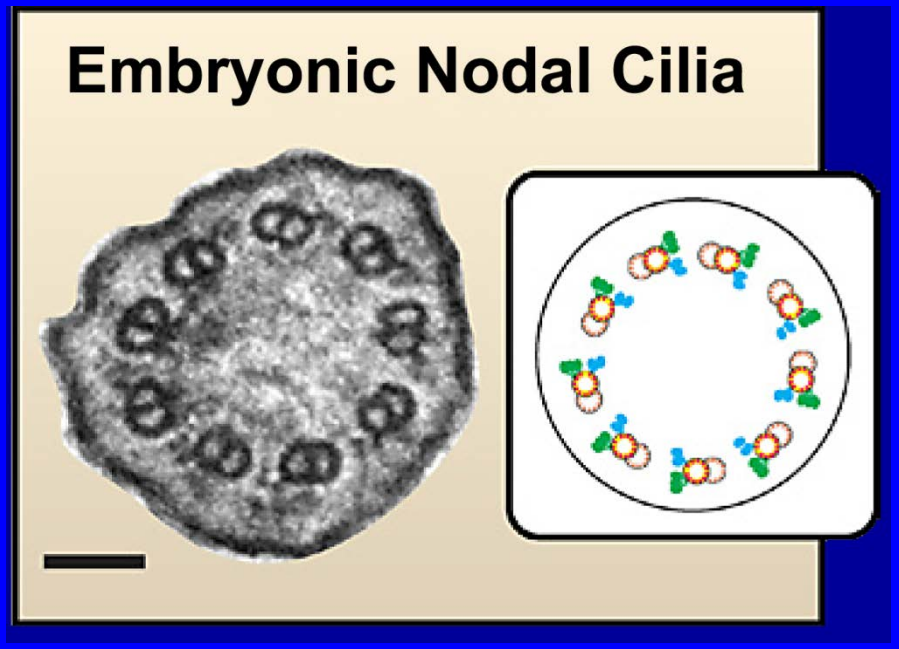
1. Unexplained neonatal respiratory distress (lobar collapse and/or need for CPAP and/or $O_2 \geq 24$ hrs
2. Daily, year-round wet cough starting in 1st year of life
3. Daily, chronic nasal congestion starting in 1st year of life
4. Organ laterality defect

# Clinical Criteria met (max=4)	Sensitivity	Specificity	Probability of PCD
4	16.8%	99.2%	0.96
3	47.2%	97.3%	0.88
2	73.6%	77.6%	0.66
1	93.6%	53.2%	0.34

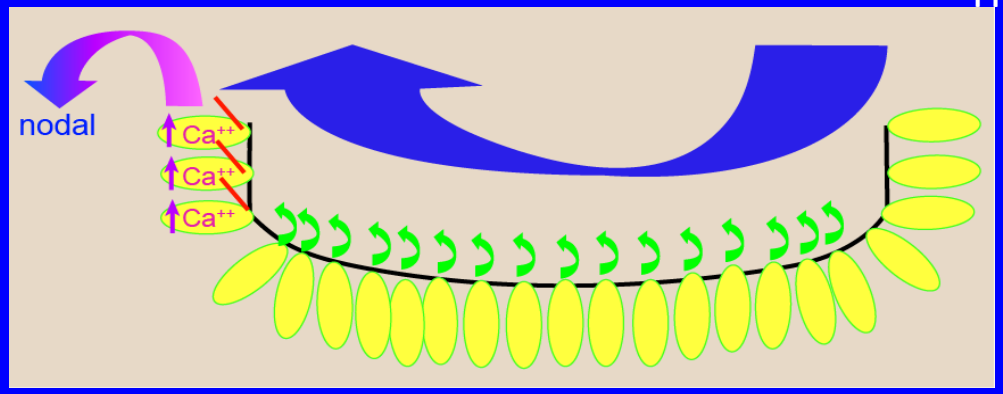
Leigh, MW, et al, 2016, Annals ATS

Thoraco-Abdominal Asymmetry

- Situs Solitus – Normal (99.99%)
- Situs Inversus Totalis
- Heterotaxy (Situs Ambiguus)



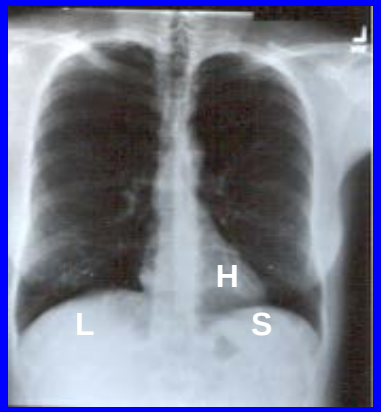
Hirokawa et al., 2006, *Cell*



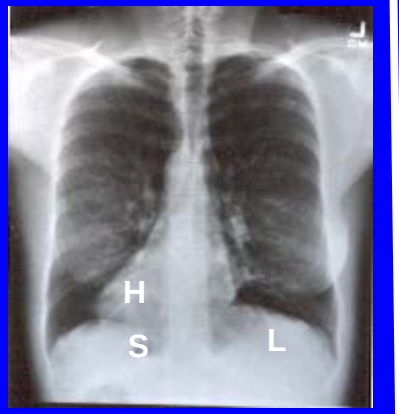
McGrath and Brueckner, 2003, *Curr. Opin. Genet. Dev.*

Thoraco-Abdominal Asymmetry

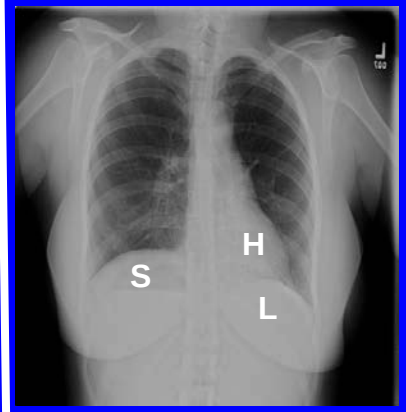
Situs Solitus



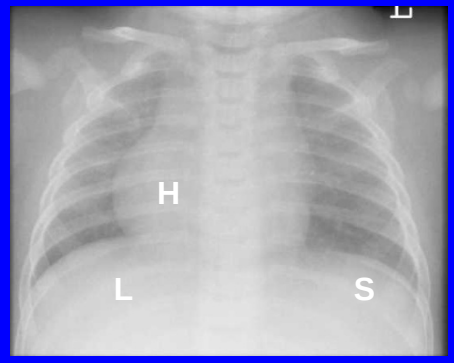
Situs Inversus Totalis



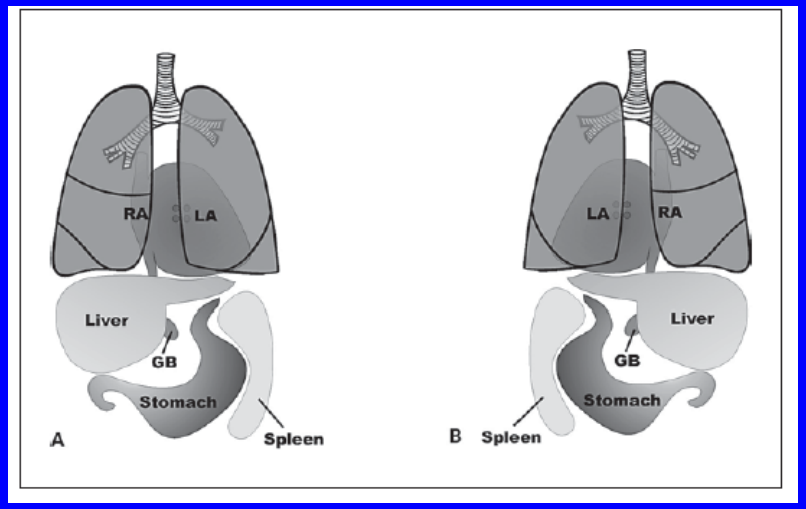
Abd Situs Inversus



Isolated Dextrocardia

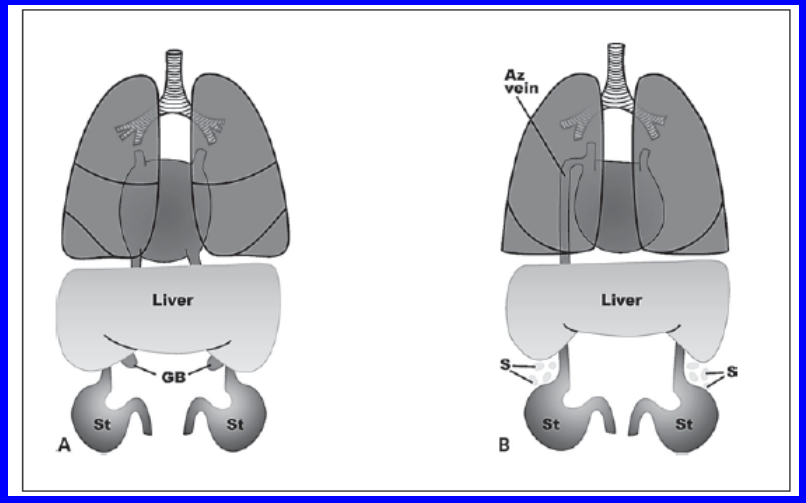


Heterotaxy (Situs Ambiguus)



Situs Solitus

Situs Inversus Totalis

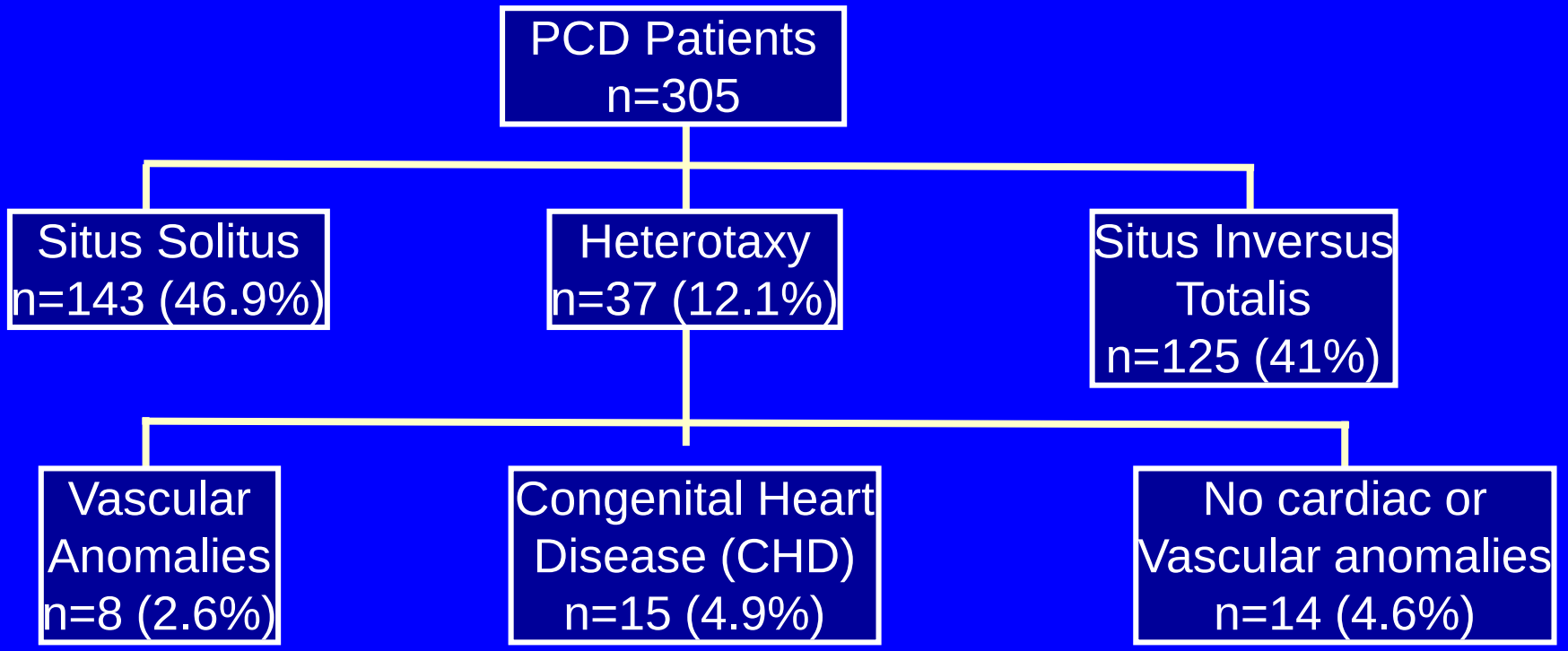


Right Isomerism* (Asplenia Syndrome)

Left Isomerism* (Polysplenia Syndrome)

Bartram et al., 2005, *Biol. Neonate*

Situs Status and Congenital Heart Disease in PCD Patients*



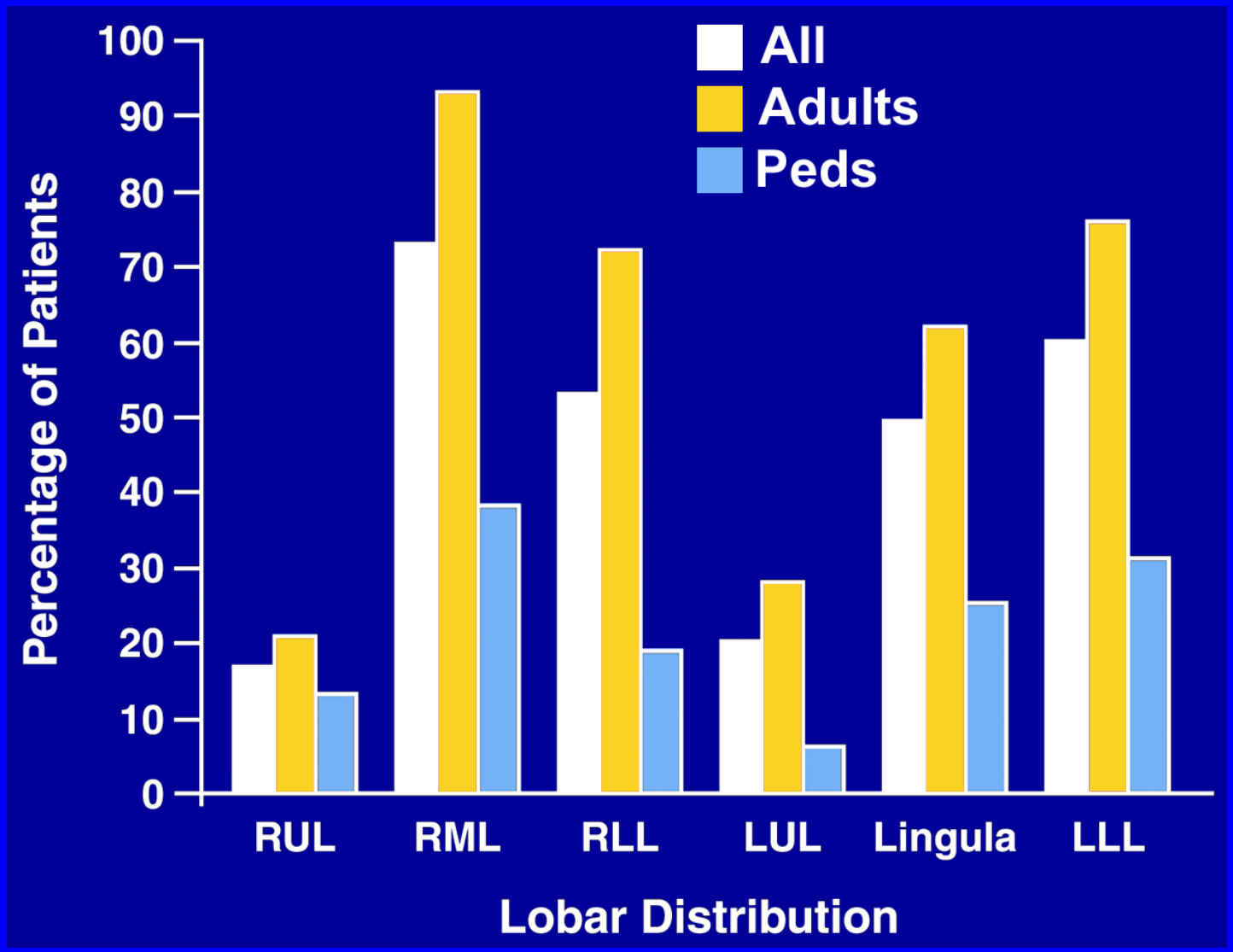
Patients with congenital heart disease and heterotaxy have worse outcome after cardiac surgery; possibly due to PCD and lung complications?

New Report: Some patients with CHD and heterotaxy have ciliary dysfunction[ł].

*Shapiro, A, et al 2014, *Chest*
[ł]Nakhleh, N, 2012, *Circulation*

Radiographic (CT) Imaging* in PCD

Bronchiectasis: Adults (35; 100%); Pediatrics (56; 70%)



*Jain et al., 2007, *Clin. Radiol.;* Kennedy et al., 2007, *AJR*; Santamaria et al., 2008, *Chest*

Consensus-based PCD Diagnostic Criteria*

Newborns (0 to 1 month of age)
Situs inversus totalis and unexplained NRD at term birth, **plus** at least one of the following:
Diagnostic ciliary ultrastructure electon micrographs or two mutations in PCD-associated gene
Children (1 month to 5 years)
Two or more major PCD clinical criteria (NRD; wet cough; nasal congestion; laterality defect), **plus** at least one of the following (nasal nitric oxide included in this age group, since it is not yet sufficiently tested):
Diagnostic ciliary ultrastructure on electron micrographs
Two mutations in one PCD-associated gene
Persistent and diagnostic ciliary waveform abnormalities on high-speed videomicroscopy, on multiple occasions
Children and adults 5-18 years of age
Two or more PCD clinical criteria (NRD; wet cough; nasal congestion; laterality defect), **plus** at least one of the following:
Nasal nitric oxide during plateau <77nL/min on 2 occasions, >2 months apart (with cystic fibrosis excluded)
Diagnostic ciliary ultrastructure on electron micrographs
Two mutations in one PCD-associated gene
Persistent and diagnostic ciliary waveform abnormalities on high-speed videomicroscopy, on multiple occasions

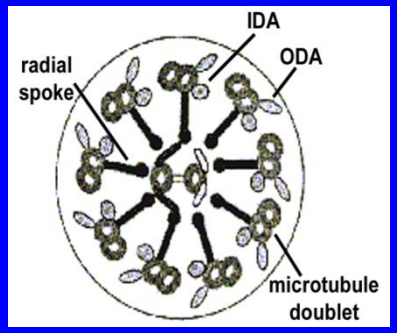
* Pediatric Pulmonology, Shapiro et al, Sept 2015

Ciliary Ultrastructure: Electron Microscopy

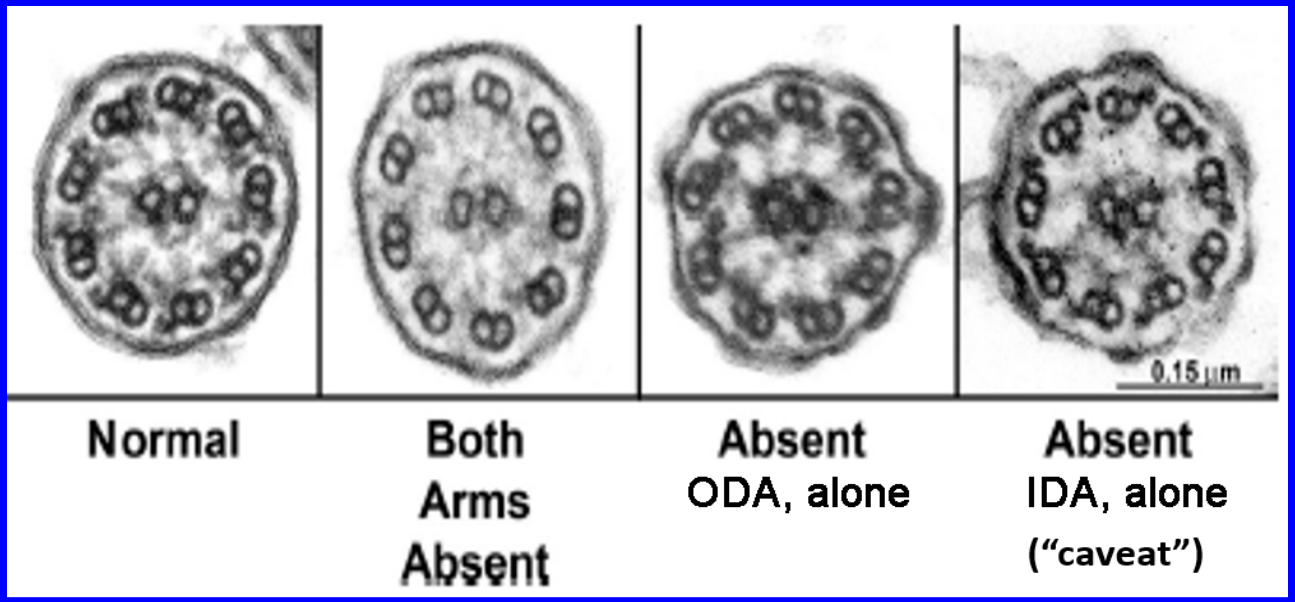
Ciliary Ultrastructural Defects (previously “gold std” for dx)

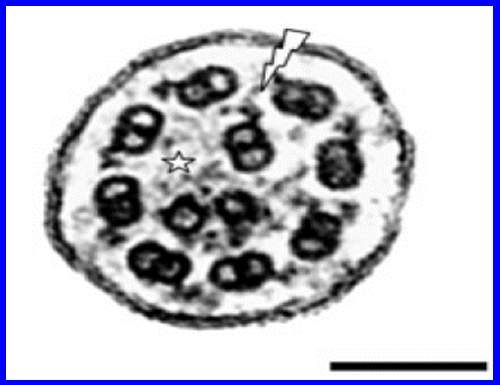
Samples obtained without anesthesia from the inferior nasal turbinate using brush or curette

- **Dynein Arms Defects**
  - **Absence/shortening ODA, alone**
  - **Absence /shortening IDA, alone (“caveat”)**
  - **Absence/shortening ODA+IDA**
  - **Absence of IDA + Microtubular Disorganization**
- **Central Complex Defects (up to 90% may appear normal)**



9+2 configuration





Absent IDA + MTD

~ 30% of PCD patients have normal (or non-dx) ciliary ultrastructure.

Inner Dynein Arm (IDA) Defect, alone: Transient*

- IDAs have low contrast on EMS
- 5 of 21 patients with IDA defect (IDAs missing) were retested, and found to have normal IDAs and normal ciliary function.*
- IDA may appear (falsely) to be missing, perhaps due to non-specific biological or technical artifacts.
- Patients with an IDA defect should undergo repeat study to confirm.

O'Callaghan, C., *et. al.,* Eur. Respir. J., 2011; 38(3):603-7.

NOT All PCD has EM Defects

Well recognized there are many patients (~30% of PCD) with a strong clinical phenotype for PCD (NRD; respiratory and ear symptoms; situs inversus), and low NO (+/- abnormal HSVM), with normal (or non-diagnostic) EMs.

Many of these patients have genetic confirmation of PCD with loss-of-function mutations in DNAH11 or RSPH genes or nexin link genes.

PCD cannot be confirmed in such patients by ciliary ultrastructural studies.

Confounding Factors for Using Ciliary EMs for Confirmatory Diagnosis of PCD

1. **Need ciliated cell biopsies, and inflammation/infection can cause *"secondary" changes* to ciliary ultrastructure.**

2. **Adequate *fixation* is critical.**

3. **These small samples are processed by *multiple technical steps*, including getting adequate ultrathin cross-sections for EM, and technician must select correct images (multiple cells).**

4. ***Evaluation requires an adequate (>20) number of interpretable images from different cells, including good contrast.***

5. ***Interpretation requires recognition of normal variability*, and an understanding of EM changes that may occur as non-specific ("secondary") effects, such as absent central microtubules, "extra" central and peripheral microtubules, and compound cilia. Further complicated by recent genetic etiologies, where as many as 90% of cilia may appear normal (RSPH; nexin links).**

Primary Ciliary Dyskinesia Genetics

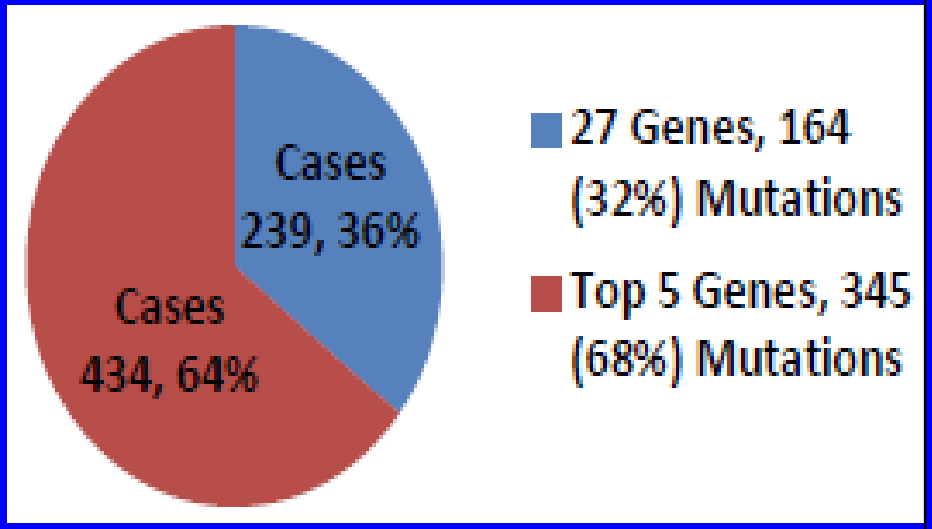
Discovery of PCD-Associated Genes: 37 Genes Sorted by Year of Discovery

Years	Genes
1999	*DNAI1*
2002	*DNAH5*
2004-07	*OFD1, RPGR, NME8*
2008	*DNAAF2, DNAI2, DNAH11*
2009	*DNAAF1, RSPH4A, RSPH9*
2011	*DNAL1, CCDC39, CCDC40*
2012	*CCDC103, DNAAF3, HEATR2, HYDIN, LRRC6*
2013	*ARMC4, C21orf59, CCDC114, CCDC164, CCDC65, DNAH8, DYX1C1, RSPH1, SPAG1, ZMYND10*
2014	*CCNO, MCIDAS, CCDC151*
2015	*DNAH1, RSPH3, GAS8*
2016	*TTC25, DNAJB13*

Mutation Statistics: Published & UNC unpublished to Mid-2014

- Total 509 independent Mutant Alleles from 32 genes
- Total 673 unrelated cases with biallelic mutations in PCD gene

Total 673 Unrelated Cases *Top 5* Vs 27 Remaining Genes



Top 5 genes contributes ~ 2/3rd of all solved cases (42% of all cases)

~4/5th alleles are LOF in both

Top 5 genes (based on published literature):

DNAH5 (15-29%),

DNAH11 (6-9%),

CCDC39 (4-9%),

DNAI1 (2-10%),

CCDC40 (3-4%).

Ciliary Ultrastructural Association: Currently 37 Genes Associated with PCD

37 Genes: accounts for ~ 2/3rd of all PCD
33 Genes: for Ciliary Structure & Function
2 Genes: for Cilia Biogenesis
2 Genes: associated with other Syndromes as well

ODA defects
*DNAH5**
*DNAI1**
DNAI2
DNAL1
NME8 (TXNDC3)
CCDC114
CCDC151
ARMC4

ODA+IDA defects
DNAAF1 (LRRC50)
DNAAF2 (KTU)
DNAAF3
LRRC6
C21orf59
HEATR2
ZMYND10
DYX1C1
SPAG1
CCDC103

IDA+MTD defects
*CCDC39**
*CCDC40**

RS/CP defects
RSPH1
RSPH3
RSPH4A
RSPH9
HYDIN

Nexin-link defects
CCDC164 (DRC1)
CCDC65 (DRC2)

Normal EM
*DNAH11**

Oligocilia
CCNO
MCIDAS

EM not available
DNAH8
DNAH1

PCD+other syndrome (n=2)
RPGR (X-linked RP+PCD)
OFD1 (X-linked MR+PCD)

*** Top 5 mutated genes**
Mut in 65% cases
(42% of all)

Clinical Manifestations in *RSPH1* Subjects in Consortium

Seem to resemble PCD with dynein arm defects: all had bronchiectasis by mid-late teenage years, and most had recurrent otitis media and sinusitis in childhood.

HOWEVER

- All had situs solitus.
- Neonatal Respiratory Distress: only 50% vs expected >80%.
- Daily, year round wet cough in 1st year of life: only 13% vs >90%.
- Lung function better vs age and gender matched PCD.

Subjects	N	Mean age (years)	Mean FEV_1 (% Pred.)
RSPH1	15	35.3 ± 18.6	73.0 ± 23.9
All PCD	75	34.2 ± 17.6	61.8 ± 22.8*
ODA/ODA+IDA	45	35.7 ± 18.8	61.6 ± 23.2†

Published in final edited form as:
Thorax. 2012 May ; 67(5): 433–441. doi:10.1136/thoraxjnl-2011-200301.

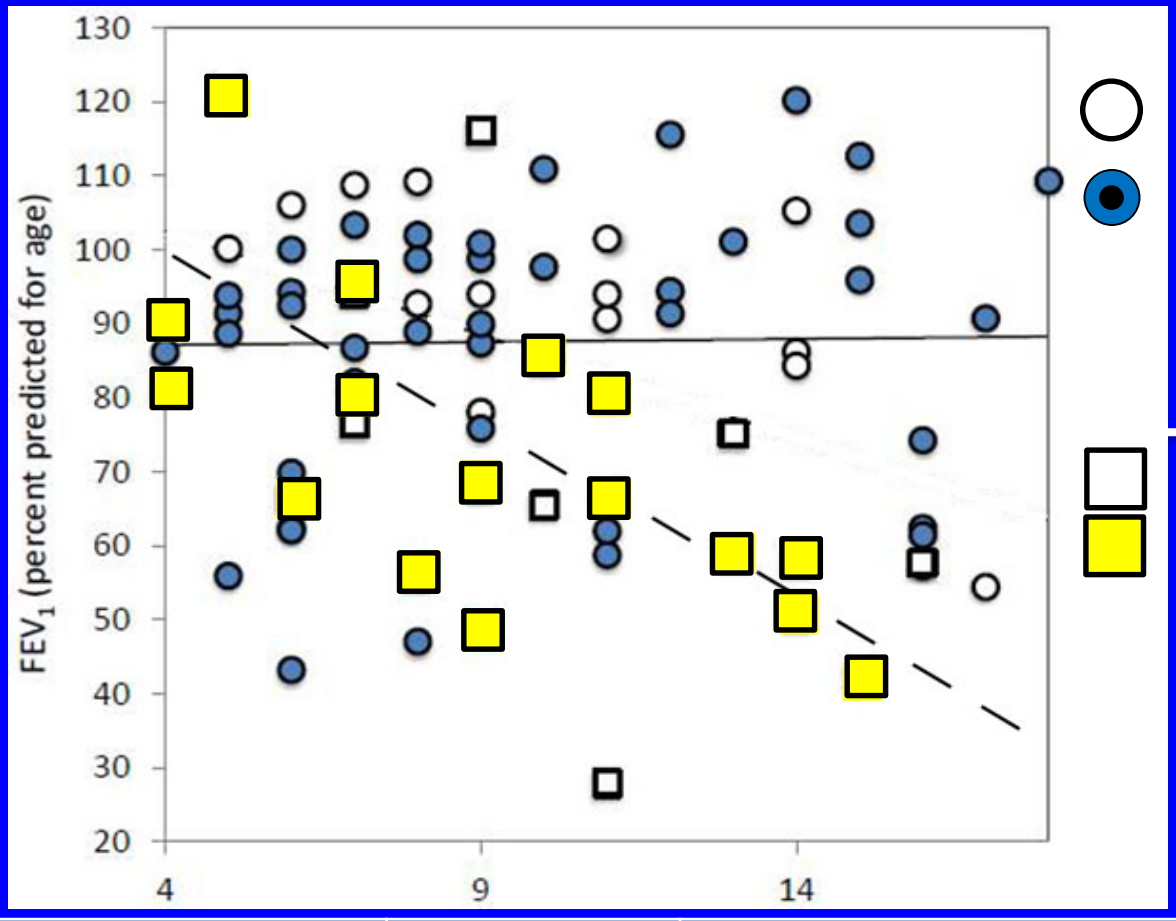
Mutations of *DNAH11* in Primary Ciliary Dyskinesia Patients with Normal Ciliary Ultrastructure

Michael R Knowles[1], **Margaret W Leigh**[2], **Johnny L Carson**[2], **Stephanie D Davis**[2], **Sharon D Dell**[3], **Thomas W Ferkol**[4], **Kenneth N Olivier**[5], **Scott D Sagel**[6], **Margaret Rosenfeld**[7], **Kimberlie A. Burns**[1], **Susan L Minnix**[1], **Michael C Armstrong**[1], **Adriana Lori**[1], **Milan J Hazucha**[1], **Niki T Loges**[8,10,11], **Heike Olbrich**[10], **Anita Becker-Heck**[8,10,11], **Miriam Schmidts**[8], **Claudius Werner**[10], **Heymut Omran**[8,10], **Maimoona A Zariwala**[9], and **The Genetic Disorders of Mucociliary Clearance Consortium**

[1]Department of Medicine, UNC School of Medicine, Chapel Hill, NC, USA

[2]Department of Pediatrics, UNC School of Medicine, Chapel Hill, NC, USA

CCDC39/CCDC40 (FEV_1 % Pred.)



Dynein arm defect

Dynein arm defect with defined genetic defect: *DNAH5, DNAI1, DNAI2, CDC114, ARMC4, LRRC6, HEATR2, SPAG1, DNAAF1, DNAAF2*

Microtubular disorganization

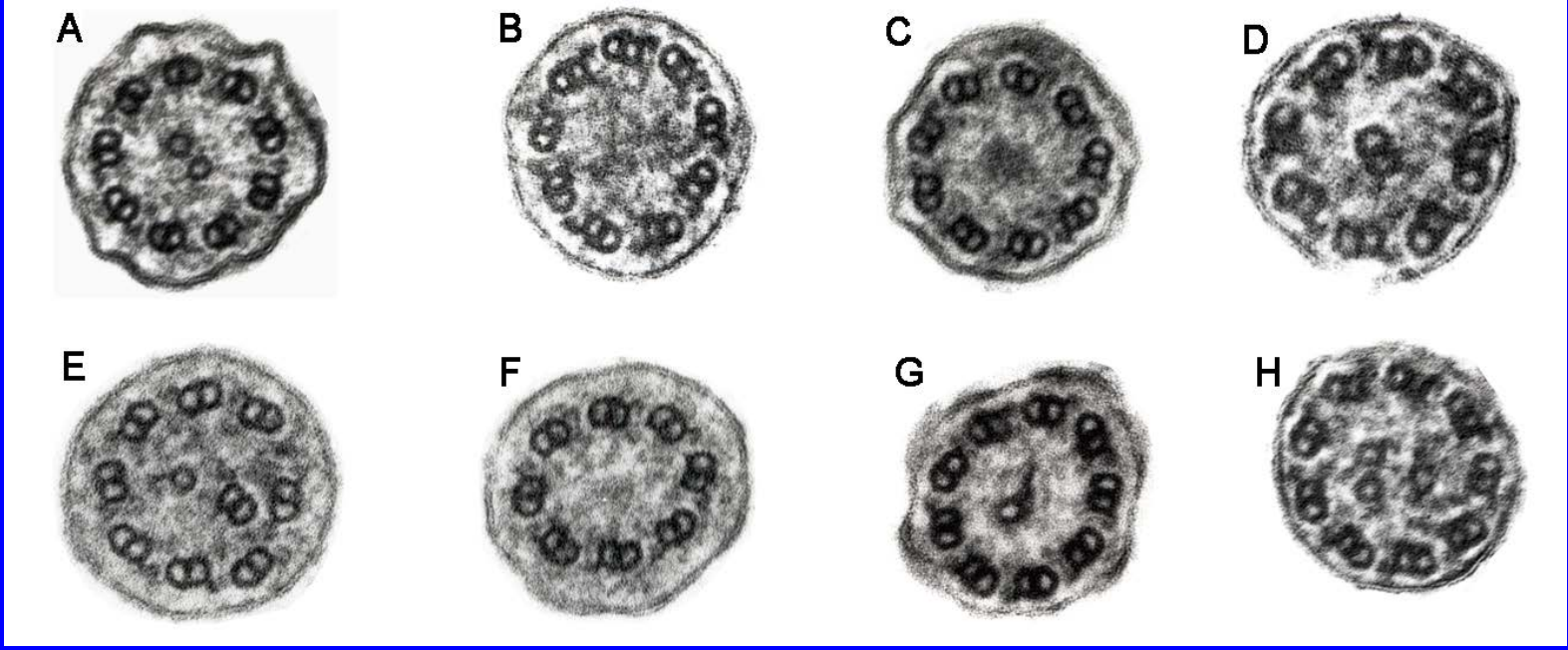
Microtubular disorganization with defined genetic defect: *CCDC39, CCDC40*

Adult Subjects	N	Mean age (years)	Mean FEV_1 (% Pred.)
CCDC39; CCDC40	24	39.9	52.1*
ODA/ODA+IDA	96	38.8	62.8

*p=0.012, worse than ODA/ODA+IDA

Davis, S, et al, *2015, AJRCCM.*

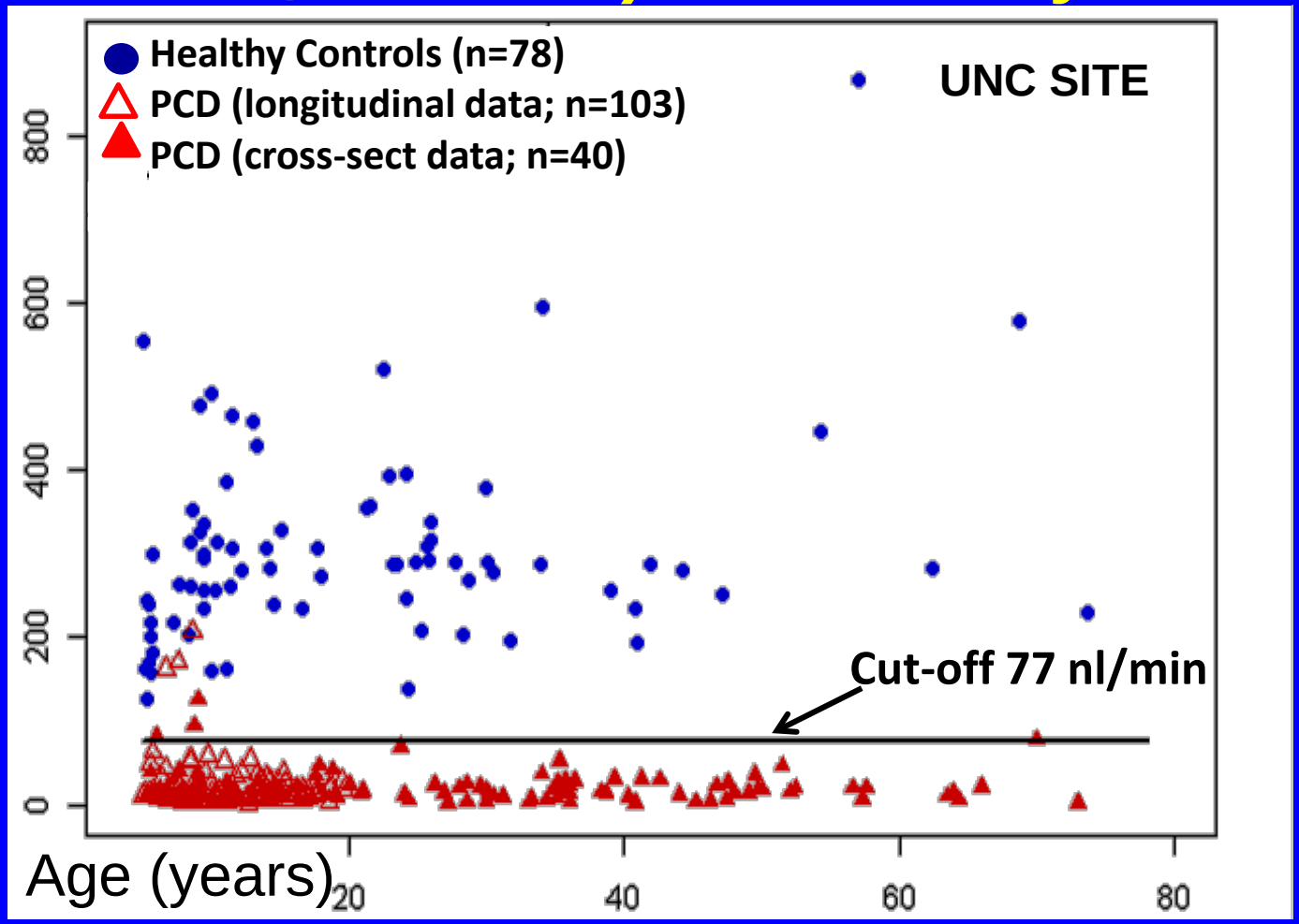
Ciliary EMs for RSPH1 Mutations



	(A) Normal Axonemal Structure	Absence of Central Pair **(B)** or Electron Dense Material **(C)**	Translocation of Outer Doublet to Cilia **(D)** Center or Other Site **(E)** or Other Changes (e.g., 8+0) **(F)**	**(G)** Single Microtubule in Center	**(H)** Off-Center Central Pair
Mean	80.1%	12.1%	5.1%	1.4%	1.2%
Range	63.4 – 93.1%	0 - 24.7%	3.0 – 14.1%	0 – 5.9 %	0 – 6.9%

Primary Ciliary Dyskinesia: nasal Nitric Oxide

Nasal Nitric Oxide (nNO) in PCD (age ≥5 years) (EM defect; DNAH11) and Healthy Controls*



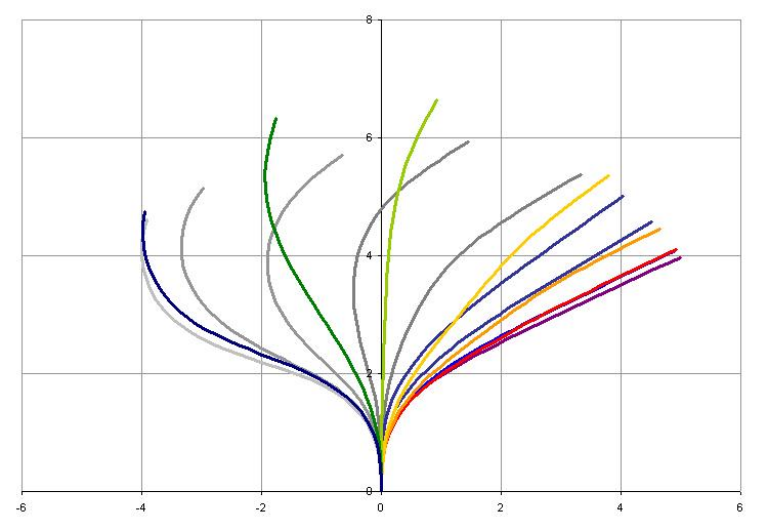
Also studied >150 Disease Controls, and Replicated at other Consortium Sites. Specificity >0.99; Positive and Negative Predictive Value >0.95 & >0.99, ***if rule out CF.***

*Leigh MW, Ann Am Thorac Soc. 2013 Dec:10(6):574-81.

Primary Ciliary Dyskinesia:

- Diagnostic ciliary waveform abnormalities on high-speed videomicroscopy

- Offered at specialized PCD centers of excellence



Conclusions

1. PCD is a recessive genetic disease caused by genetic variation in the proteins that are involved in the structure, function and biogenesis of cilia.

2. The clinical presentation of PCD varies considerably but includes a) neonatal respiratory distress syndrome, b) chronic sino-pulmonary disease c) recurrent otitis media, d) thoraco-abdominal assymetry including situs inversus and heterotaxy.

3. A PCD diagnosis requires an integrated approach that uses clinical parameters, ciliary electron microscopy, genetic testing, nasal nitric oxide assessments and potentially the use of high speed videomicroscopy.

4. Electron microscopy is normal in 30% of patients and is technically challenging to perform and interpret

5. Genetic screening is commercially available and in some cases informs prognosis but does not currently capture all cases

6. Nasal nitric oxide and videomicroscopy are specialized techniques available at centers of excellence.

4 Consortium Sites(2004); soon, 24 PCD sites