Genetics and Genomics for the Pulmonary Physician

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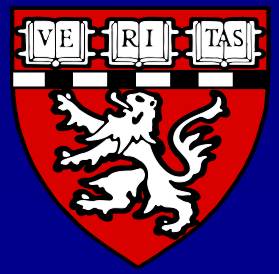


Statement of conflict of interest

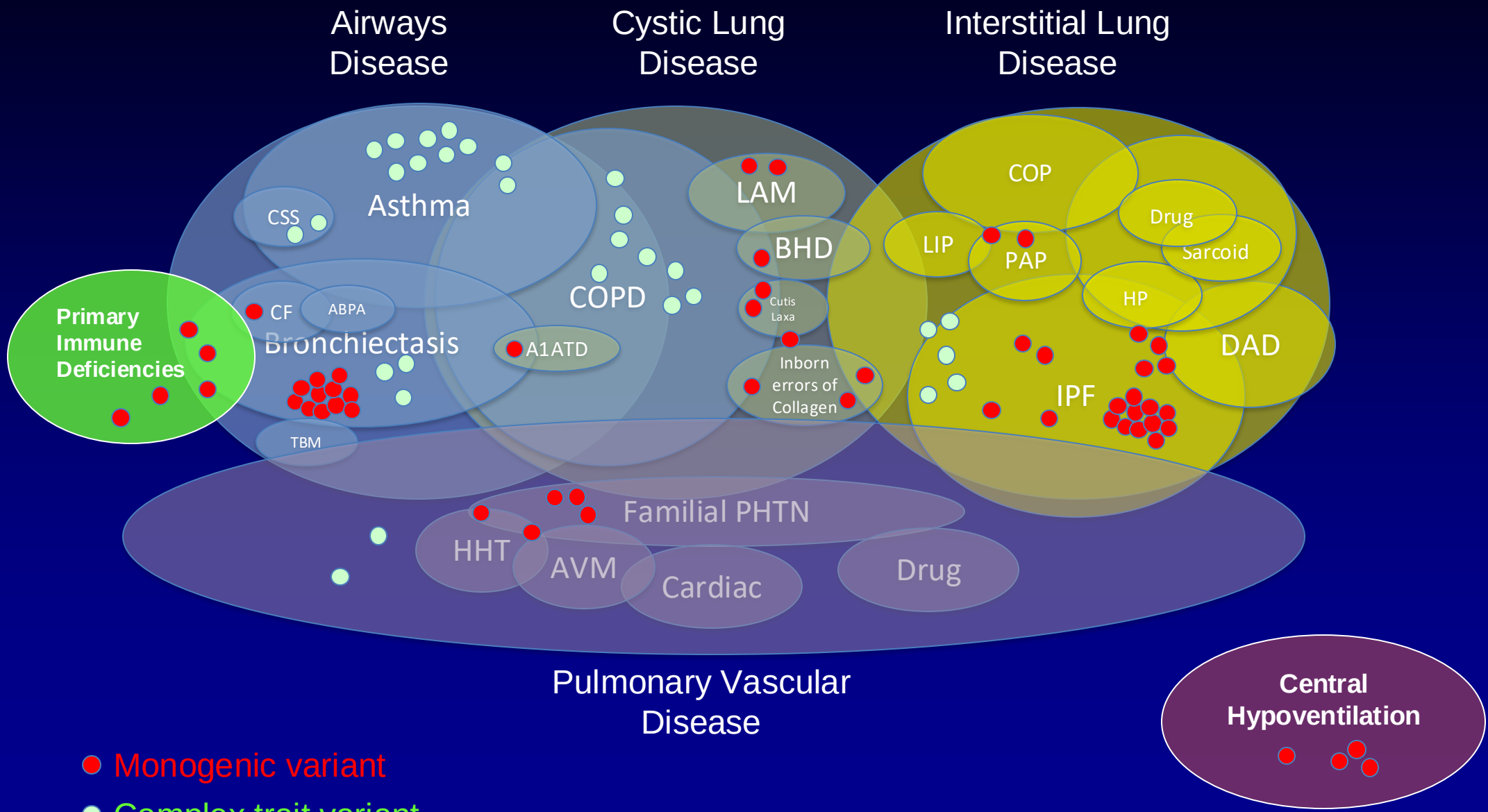
- Dr. Raby is Director of the Pulmonary Genetics Center at Brigham and Women's Hospital, and has assisted the Laboratory of Molecular Medicine (LMM) in the development of the PulmoGene™ Sequencing Panels. He has no financial relationship with the LMM and does not receive royalties related to the Pulmogene Sequencing Panel or any other genetic test.

- Dr. Raby is the Genetics Section Editor for UpToDate, Inc. and receives editorial royalties for these efforts.

Outline

- The genetic landscape of pulmonary disease
- The value of genetics in pulmonary medicine
- Rare vs. complex genetic traits
- Selected Mendelian disorders in pulmonary medicine
  - Diseases of the airway: CF and PCD
  - Cystic parenchymal lung diseases: A1ATD and BHD
  - Interstitial lung disease: TBDs
- Recognizing genetic disease
- Genetic counseling
- Online Clinical Genetic Databases
  - OMIM: Online Mendelian Inheritance of Man
  - GeneTests

The genetic landscape of pulmonary medicine: a conceptual framework



Pulmonary Genetic Disorders (more than 100 genes!)

Bronchiectasis (>40)

Cystic Fibrosis (1)
Primary Ciliary Dyskinesia (~35)
Alpha-1-Antitrypsin Deficiency (1)

Fibrotic Lung Disease (>35)

Surfactant Deficiencies (3)
Short Telomere Syndrome (15)
Hermansky Pudlak Syndrome (>7)
Primary Alveolar Proteinosis (2)
Fibrosis and hypothyroidism (1)
Common IPF (>16)

Miscellaneous (9)

Central Hypoventilation (5)
Ichthiosis Vulgaris / Asthma (1)
Hyperimmunoglobulin E (2)
Hypereosinophilic syndrome (1)

Cystic Lung Disease (21)

Alpha-1-antitrypsin deficiency (1)
Lymphangioleiomyomatosis (2)
Birt-Hogg-Dubé Syndrome (1)
Cutis Laxa (4)
Marfans (1)
Loey-Dietz (3)
Ehlers-Danlos (9)

Pulmonary Vascular Disease (13)

Pulmonary Hypertension (8)
Hereditary Hemorrhagic Telangiectasia (4)
Pulmonary Veno-Occlusive Disease (1)

Primary immune deficiencies

More than 100 genes implicated that can present with recurrent pulmonary infection, bronchiectasis, interstitial lung disease…

Why should we test? Why make the diagnosis?
Role of genetics in clinical pulmonary medicine

- Diagnostics
  - Non-invasive testing
  - “Why did this happen to me?”
- Therapeutics
  - Disease-specific therapies
  - Gene-specific therapies
  - Mutation-specific therapies
- Preclinical screening
  - Early intervention
  - Risk factor modification
- Genetic counseling
  - Assessment in at-risk relatives
  - Reproductive counseling

Rare vs. Common Genetic Diseases

Rare

- Single gene disorders
- Mendelian inheritance
- Mutations are usually coding
- Diagnostic testing available
- Interventions and counseling more specific

- Testing recommended

Common

- Polygenic (100's of genes)
- Environment +++
- Non-Mendelian inheritance
- Variants usually non-coding
- Diagnostic testing NOT available

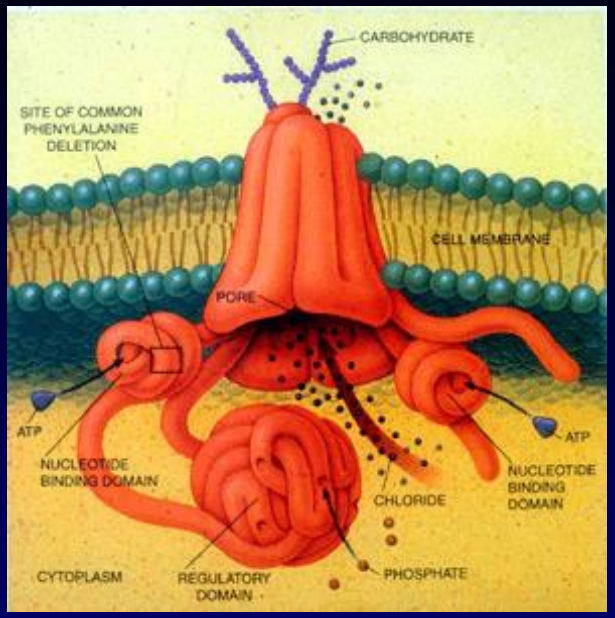
- Phenotypic screening helpful (i.e. IPF)

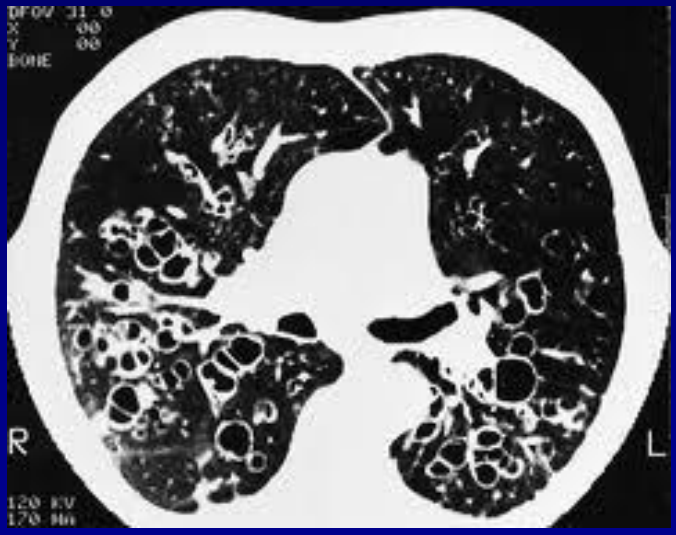
Monogenic Airways disease

Cystic fibrosis
Primary Ciliary Dyskinesia

Cystic Fibrosis

- Autosomal recessive – need two copies of defective gene
- Caused by mutations in CFTR: a chloride channel expressed in airway epithelium, epithelium of ducts of the pancreas, biliary tract, vas deferens, sweat ducts
- Manifestations:
  - Airways disease
    - Diffuse, purulent bronchiectasis
    - Sinus disease
    - Accelerated decline in pulmonary function
  - Pancreatic Insufficiency
    - Malabsorption
  - Obstructive Biliary Disease
  - Male infertility

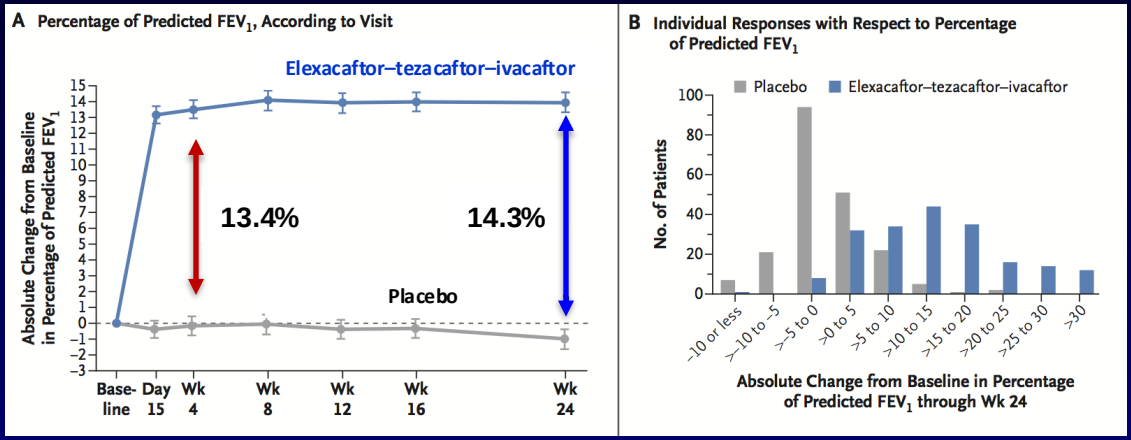




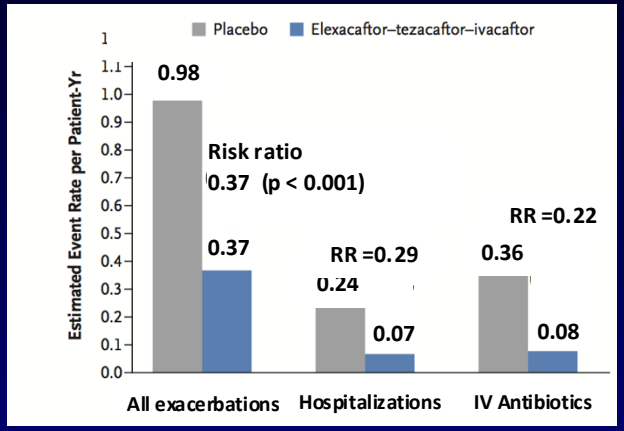
Triple therapy for Cystic Fibrosis

Middleton PG et al. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. N Engl J Med. 2019;381:1809-1819.

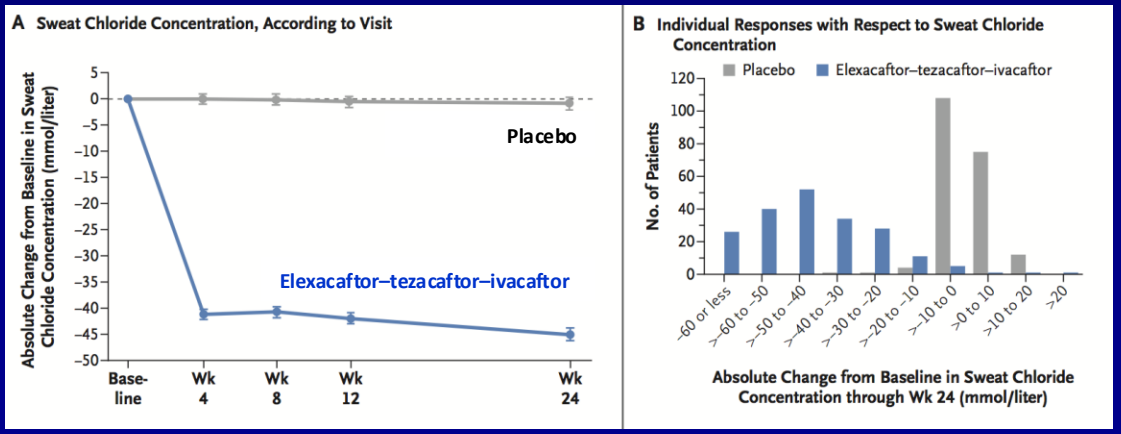
Improved Lung Function



Fewer Exacerbations



Improved Sweat Chloride Test



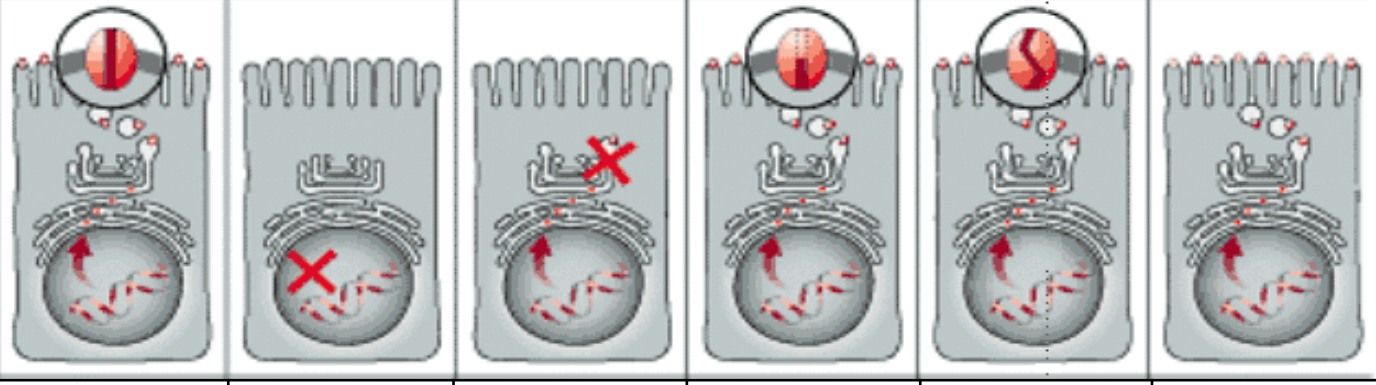
Triple therapy improves chloride channel function in up to 90% of patients. However, patients with null mutations do not benefit.

Mutation characterization now critical in CF patient care.

ClinicalTrials.gov number, NCT03525444

Mutational Spectrum of Cystic Fibrosis

There are >1,800 CFTR variants reported, > 100 are pathogenic

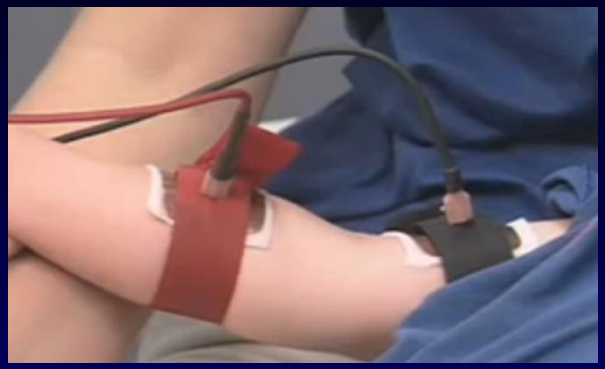
Class	Normal	I	II	III	IV	V
						
Channel Defect		No synthesis	Abnormal protein trafficking	Altered conductance	Blocked conductance	Reduced protein synthesis
Typical mutations		Nonsense Frameshift	Missense In frame deletions	Missense	Missense	Missense Non-coding Alternative splicing
Examples		G542X	ΔF508	G551D	R117H R347P	A445E
Modulator responsiveness		NO	Yes	Yes	Yes	Yes

Severity

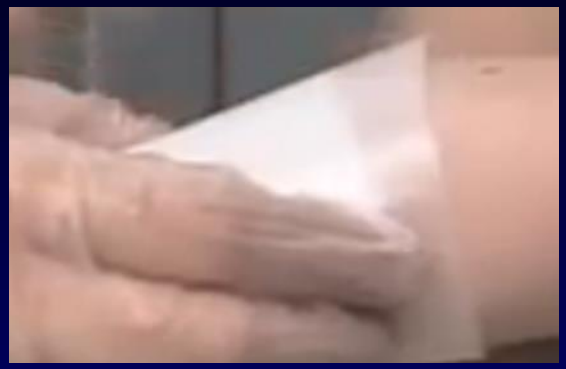
What is the first-line diagnostic test for CF?

Sweat chloride test: the initial diagnostic test

Pilocarpine iontophoresis

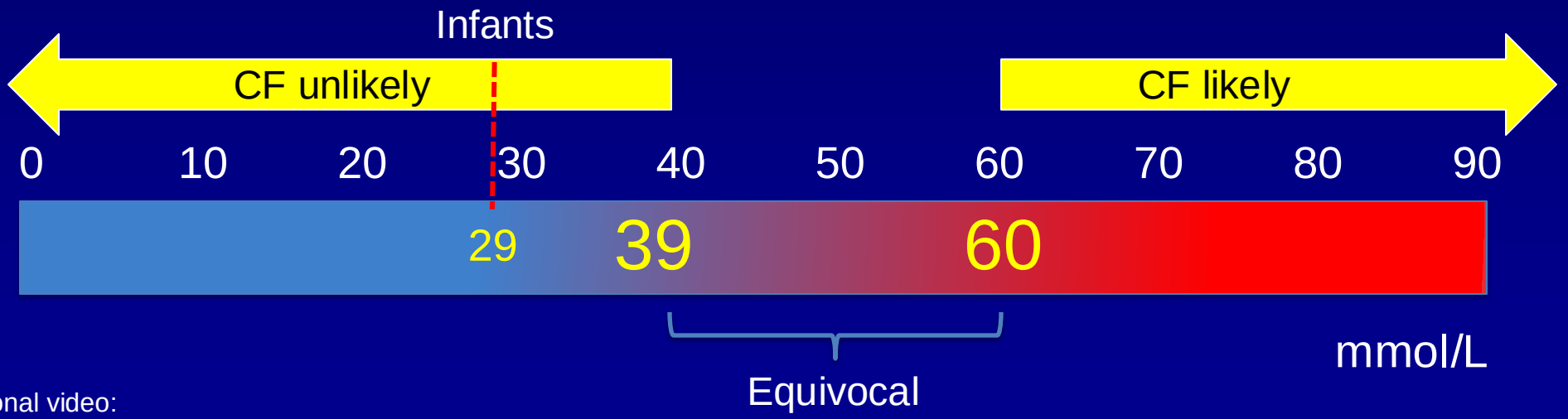


Electric stimulation
(5 min)



Sweat collection
(30 min)
Wescor Macroduct coil: 15 µl
Gibson-Cooke procedure: 75 mg

Chloride
concentration



Instructional video:
http://www.cff.org/aboutcf/testing/sweattest/

Sweat chloride and genetic testing

- Use sweat chloride test to make diagnosis
  - A normal test should make you think of something else
    (false negative rate <2%)

- Use genetic testing if:
  - Sweat test is inconclusive:
    - identification of 2 pathogenic would establish diagnosis
  - Sweat chloride is positive:
    - Determine eligibility for modulator therapy

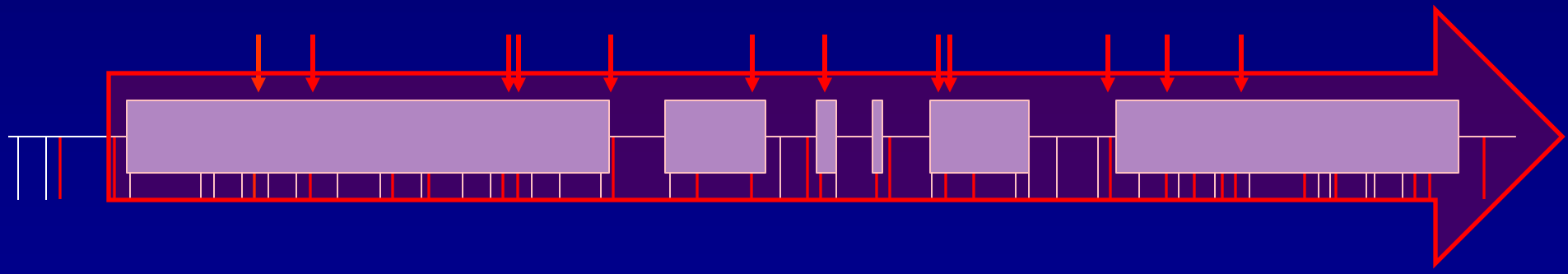
Genetic testing for CF

Genotyping

- Predetermined panel of most common variants, including 23 ACMG - recommended
- Cost-effective in majority of cases as stand alone test
- False negatives more common in non-European populations, consanguineous parents

Sequencing

- Comprehensive characterization of all bases in CFTR gene.
- Identifies common, rare and novel variation in most cases.
- Robust to ethnicity but expensive – remains second line test.



CFTR mutations and ethnicity

Prevalence varies by ethnicity

Ethnicity	Prevalence
Caucasian	1 in 3,300
Ashkenazi Jewish	1 in 3,300
Hispanic	1 in 8,464
African American	1 in 16,900
Asian American	1 in 32,400

Spectrum of mutation varies by ethnicity

	△F508	Other common pathogenic variants
Caucasian	70%	G542X, G551D, 621+1G>T, W1282X, N1303K
Southern Europe		G542X, R1162X, N1303K
Ashkenazi Jewish	31%	**W1282X (26%-35%),** G542X (7.5%), 3842+10kbC>T (4.8%), N1303K
Hispanic	54%	G542X (5%), R553X (2.3%), R334W (1.8%), N1303K (1.7%), 3842+10kbC>T (1.6%)
African American	44%	3120+1G>A (9.6%), R553X (2.3%), △I507 (1.9%), G542X (1.5%), G551D (1.2%), 621+1G>T (1.1%)
Asian	Rare	

Primary Ciliary Dyskinesia

Primary Ciliary Dyskinesia

- Recessive disorder
- Mucociliary clearance disorder caused by defects of the ciliary apparatus
- Manifestations:
  - Bronchiectasis,
  - Chronic sinus disease,
  - Infertility
  - Dextrocardia

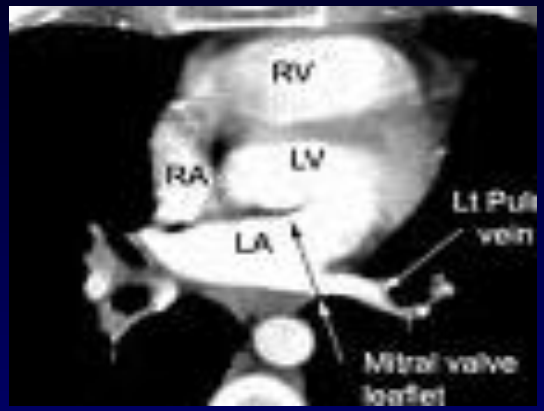
- Genetics suggest 17,500-35,000 cases in US, but only a fraction have been diagnosed!
    - Lack of awareness of disease prevalence
    - Symptoms in childhood are rather non-specific
    - Diagnostic testing challenging and often difficult to access
    - “Gold-standard” test of EM studies of nasal epithelium does not address functional deficits.

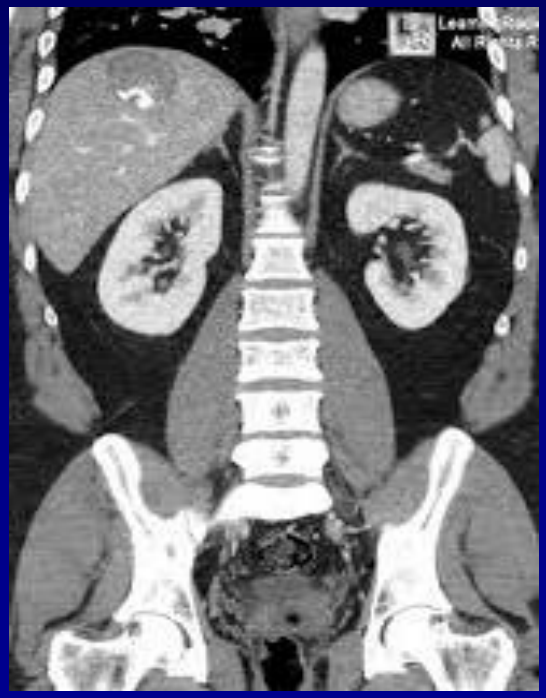
Clinical manifestations

- Diagnostic clues:
  - Neonatal respiratory distress (80%) (“transient tachypnea”)
  - Infertility is almost universal in men with PCD; females less so, but ectopic pregnancy reported
  - Laterality defects (in 50% of patients):
    - Heterotaxia syndromes: including polysplenia and asplenia
    - ~6% situs ambiguous → a 200x increase in structural congenital heart disease
- Associated disorders:
  - Pectus Excavatum (10%, 33x population average);
  - Scoliosis in 5-10%;
  - Cardiac malformations, retinitis pigmentosa, hydrocephalus
  - Bardet-Biedl or Alstrom syndromes

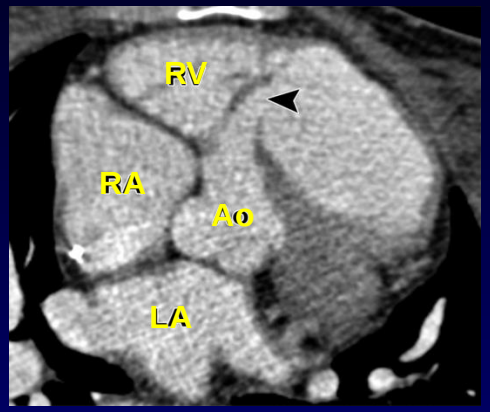
Heterotaxia

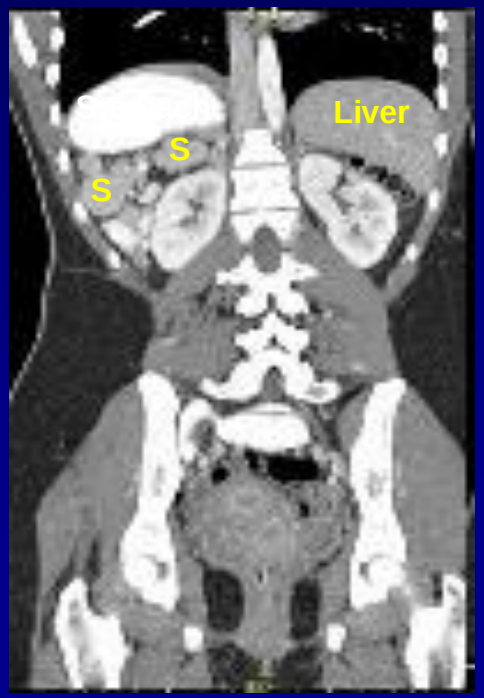
Situs solitus



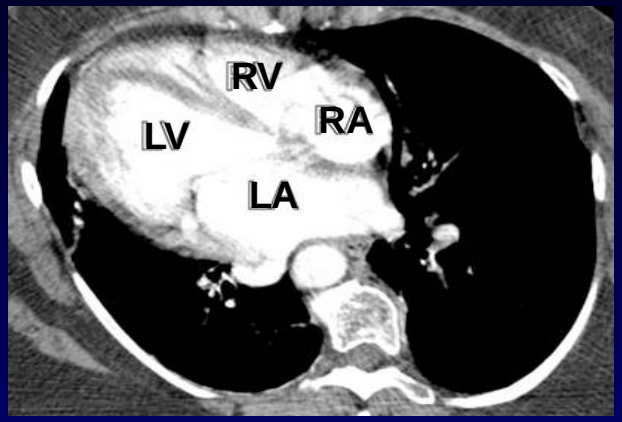


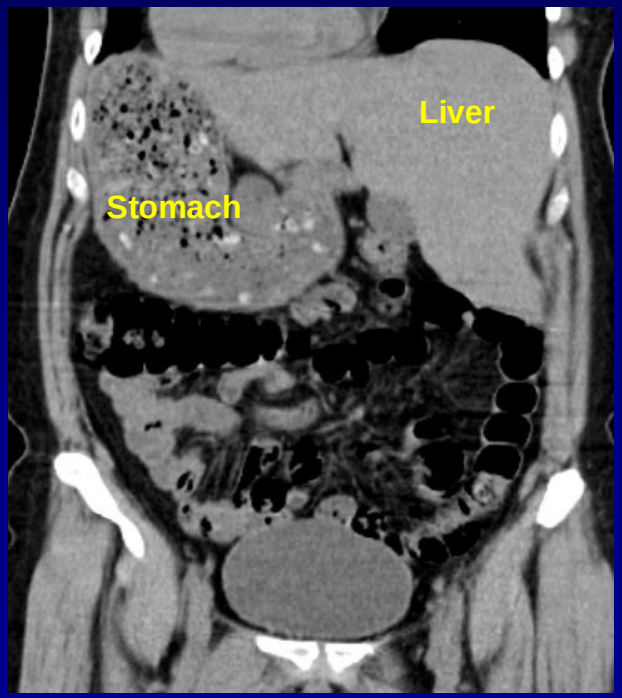
Situs ambiguus





Situs inversus





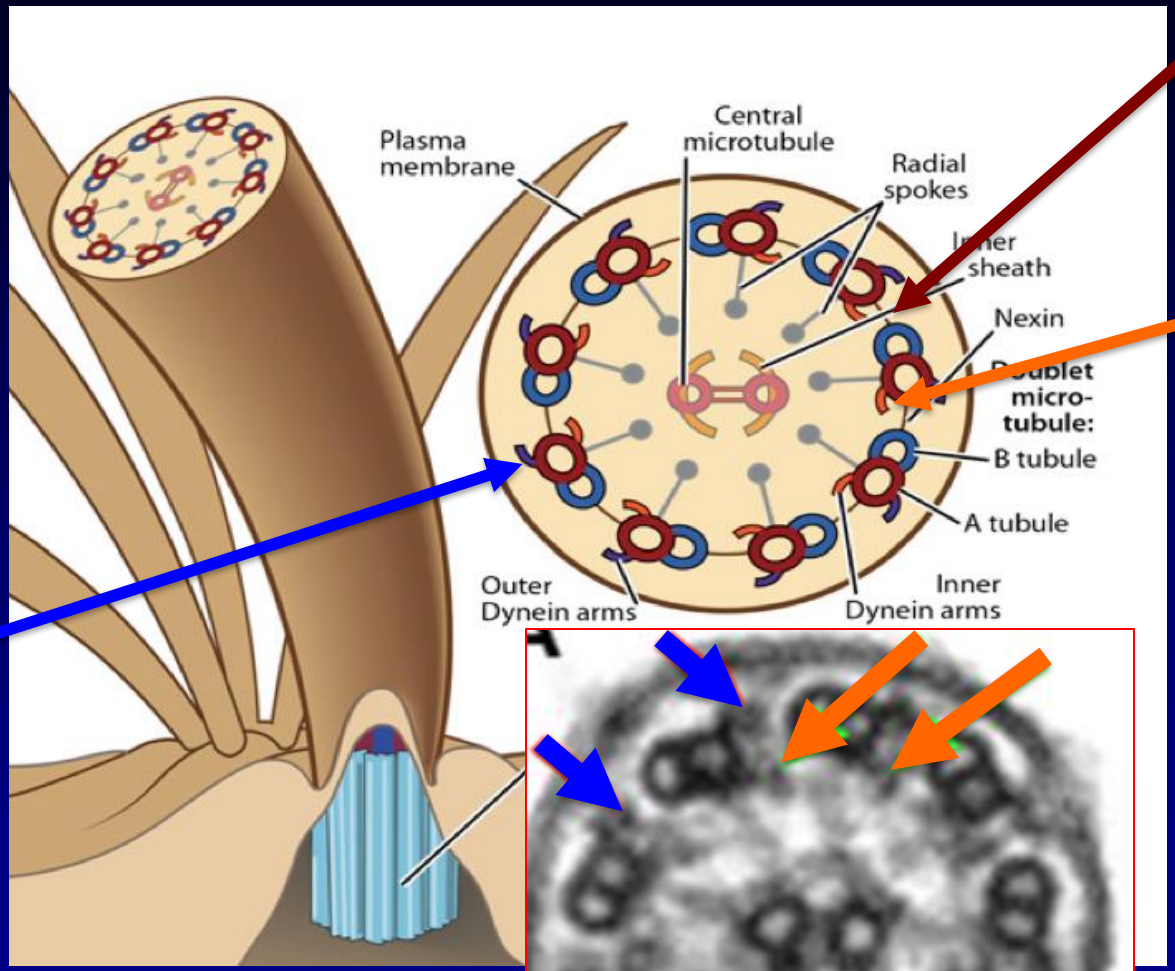
Genetic Basis of PCD

Normal Ultrastructure:

DNAH11
RSPH4A
RSPH9
HYDIN
OFD1

Outer Dynein Arm:

DNAH5
DNAI1
DNAI2
DNAL1
CCDC114
TXNDC3



Nexin link:

CCDC164

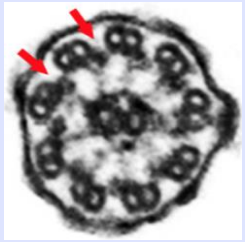
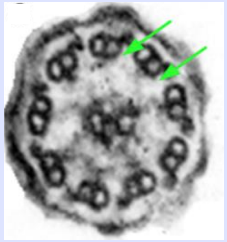
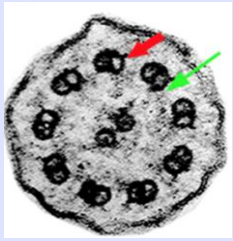
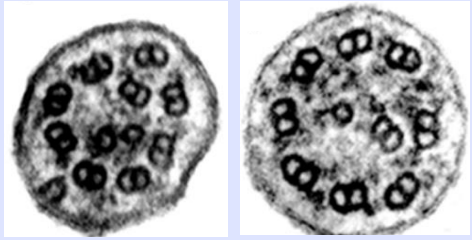
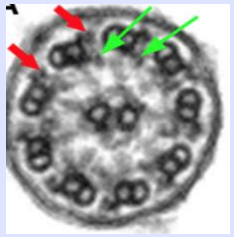
Isolated Inner Dynein Arm:

CCDC39
CCDC40

Mixed (ODA+ IDA):

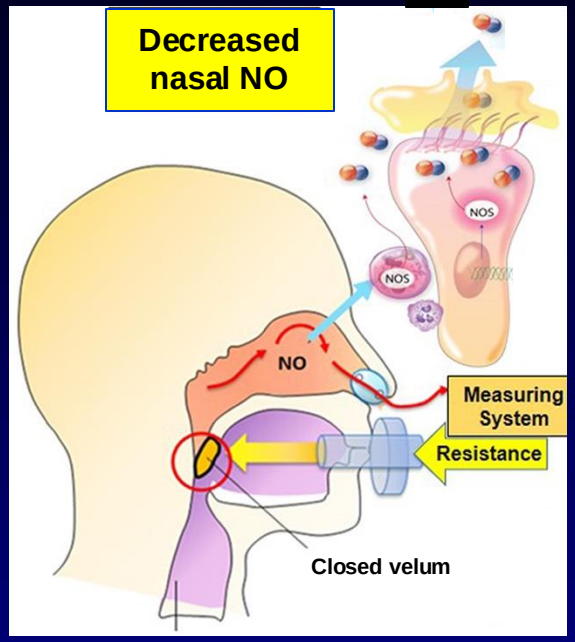
DNAAF1 (LRRC50)
DNAAF2 (KTU)
DNAAF3 (C19ORF51)
CCDC103
HEATR2
LRRC6
RPGR

Diagnostic “gold standard”: Nasal ciliary electron microscopy

Outer arm (40%)	Inner arm (5-10%)	Mixed (15%)	Central Apparatus (10%)	Normal Structure (30%)
				
DNAH5 DNAI1 DNAI2 CCDC114 DNAL1 & TXNDC3	CCDC39 CCDC40	DNAAF1 DNAAF2 LRRC6 DNAAF3 CCDC103 HEATR2	CCDC164	DNAH11 RSPH4A RSPH9 HYDIN OFD1

Availability of sequencing and identification of more genes has revealed a substantially higher false negative rate of ciliary EM (~40%)

nasal Nitric Oxide levels (nNO) in PCD



nNO levels are markedly reduced in majority of PCD

Cut-off of 300 ppb or 77 nl/min provides highest combined sensitivity (89.5%) and specificity (87.3%)

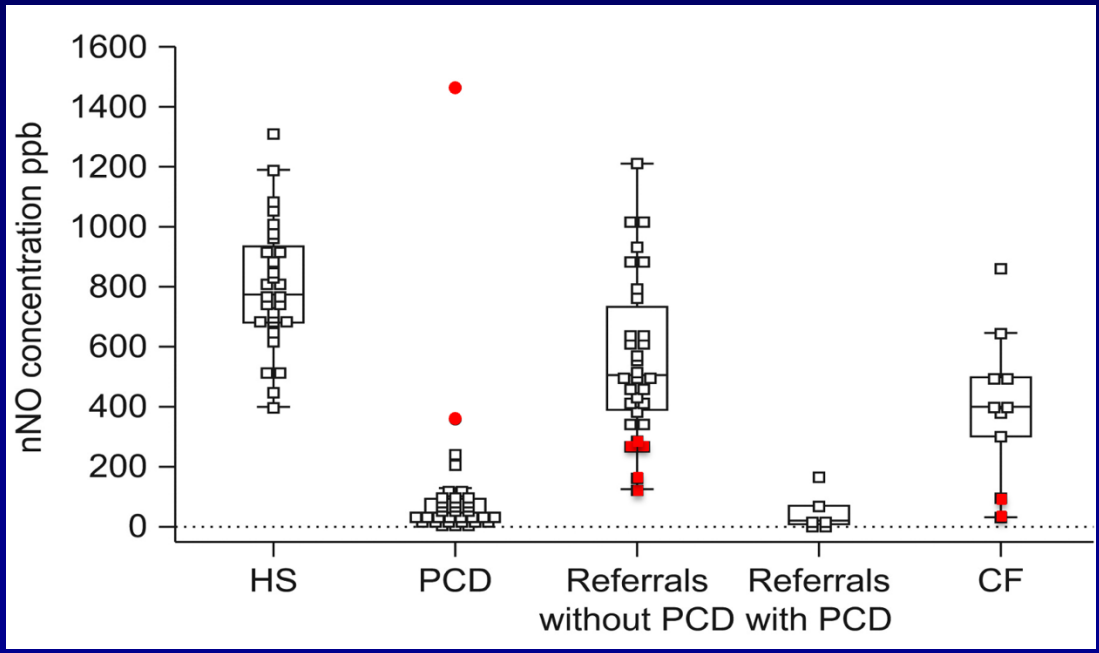
Unfortunately, not FDA approved

False positive (low levels):

- Nose bleeds
- Sinusitis
- Viral infection
- CF

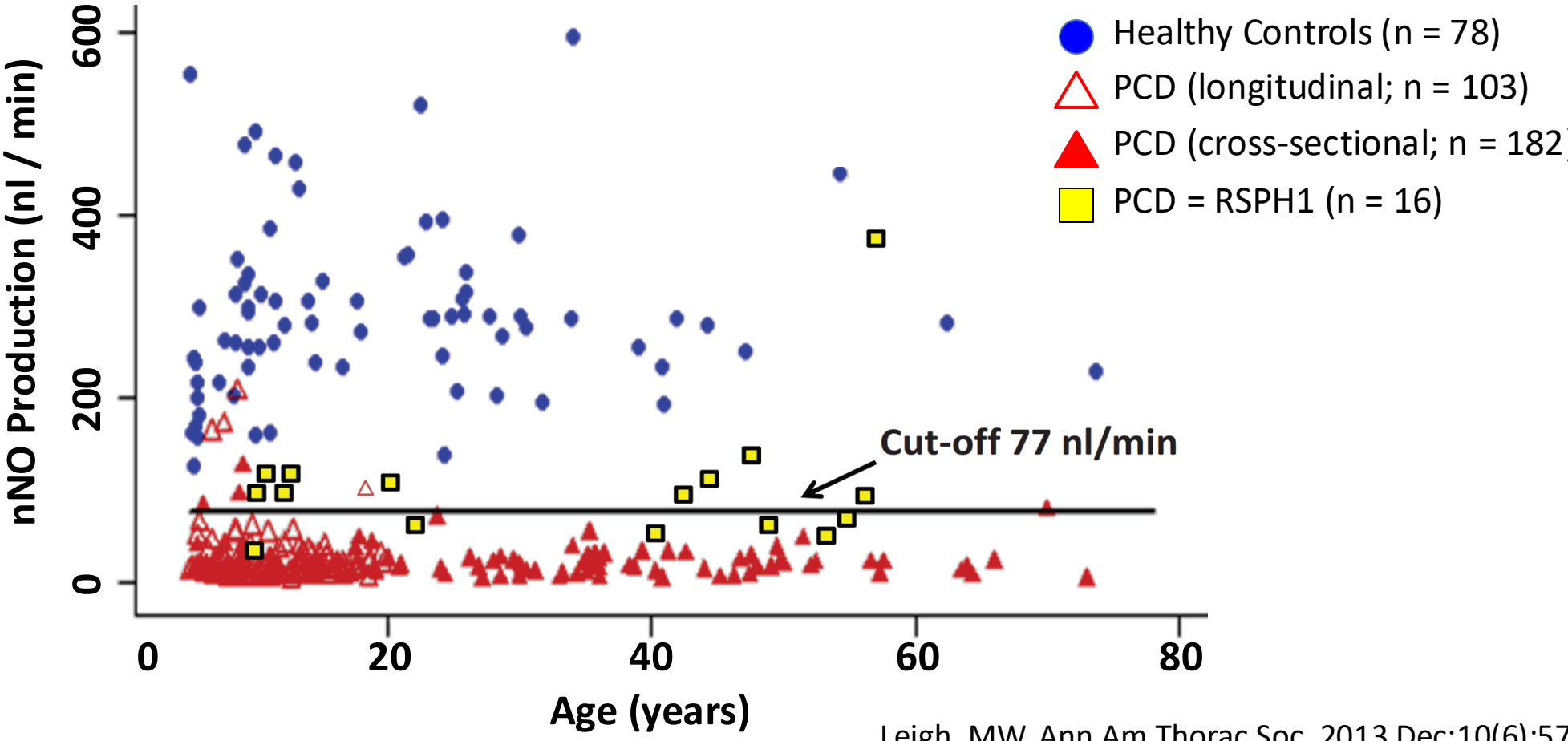
False negatives (normal levels):

- Radial spoke head genes



J.K. Marthin & K.G. Nielsen; ERJ 2011; 37: 559-565

nNO in PCD vs. Healthy Controls

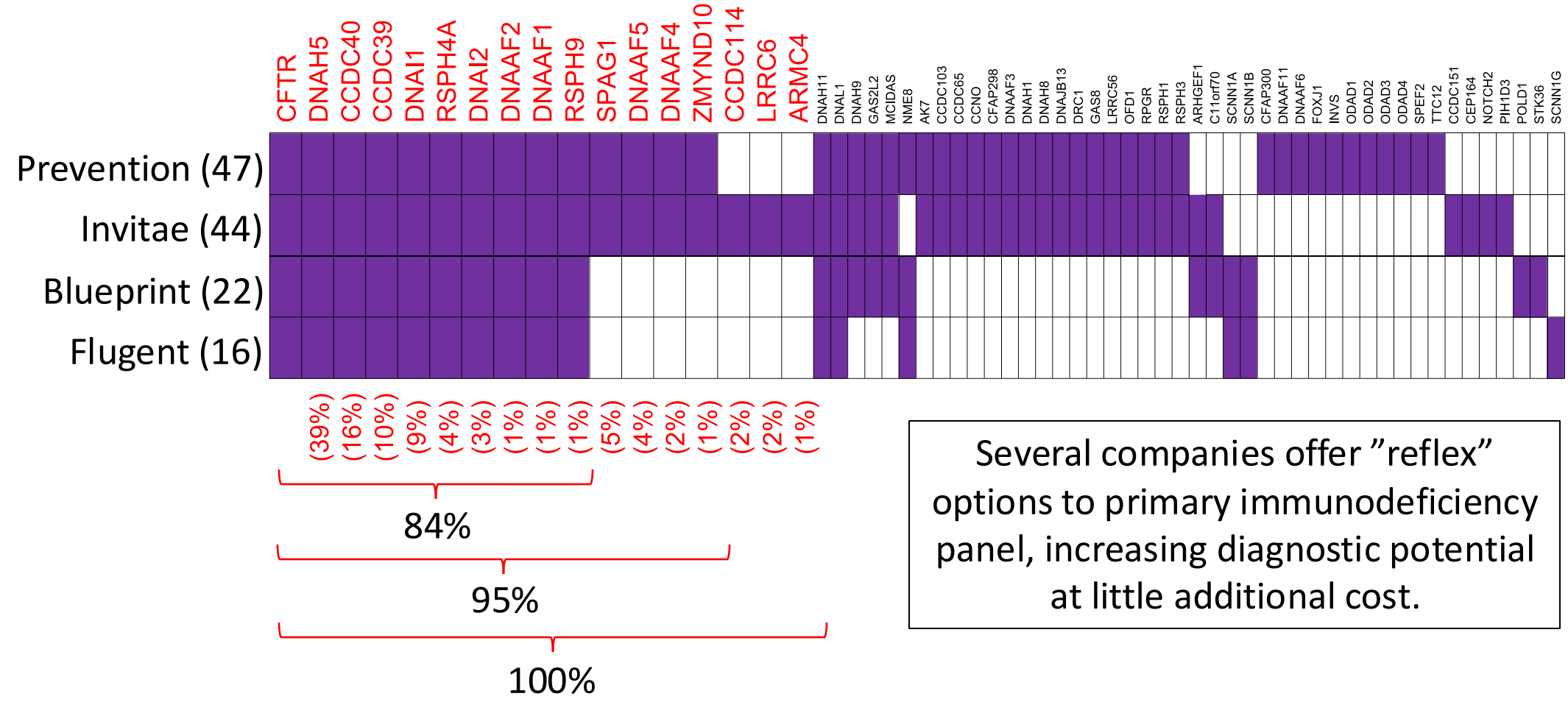


Leigh, MW, Ann Am Thorac Soc. 2013 Dec:10(6):574-81.

Genetic testing: Sequencing panels

- Simultaneous direct resequencing of large numbers of PCD-causing genes
- Identifies known and novel mutation
- Multiple commercial products available, some with > 40 genes
  - None fully comprehensive
  - Technical performance generally excellent (CLIA-standards)
  - Diagnostic performance has not been determined

Gene panels for bronchiectasis



Several companies offer ”reflex” options to primary immunodeficiency panel, increasing diagnostic potential at little additional cost.

Diagnostics in PCD

- High clinical suspicion in patients with non-CF bronchiectasis
  - “Wet phenotype”
  - Respiratory distress at birth
  - Situs / dextrocardia (patient or family)
- Nasal NO testing
  - Sensitivity good, specificity excellent (FP rate low)
  - Not readily available
- EM testing not reliable
  - False negative rate increasing (50%)
  - False positive rates non-trivial
- Panel sequencing
  - Convenient and accessible
  - 65%-70% cases explained

Cystic parenchymal lung diseases

Alpha-1-antitrypsin deficiency
Birt-Hogg-Dubé Syndrome

Alpha-1 anti-trypsin deficiency

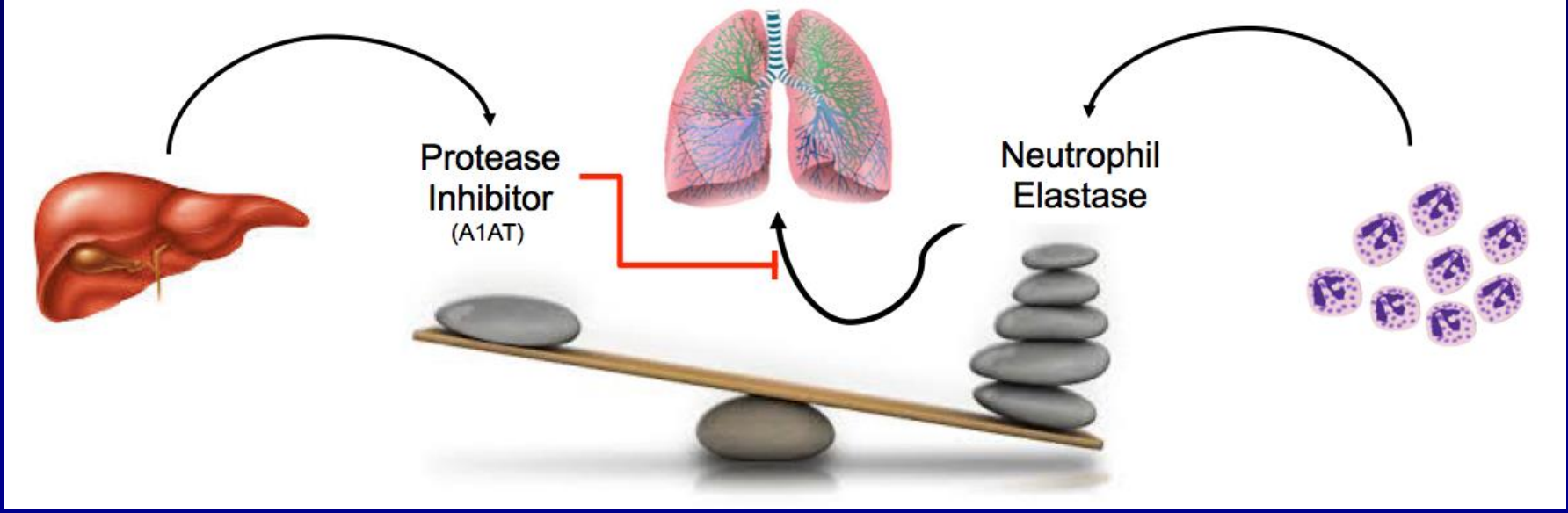
- The first proven genetic risk factor for COPD
  - Explains ~1% of COPD cases
  - A1ATD prevalence of 1/3000-1/5000

- A1ATD not easily identifiable by clinical suspicion alone!
  - Associated with earlier onset, more severe disease
  - Often with less impressive smoking history
  - Autosomal recessive condition
    - » No parental history of disease
    - » Possible history of disease among siblings
    - » Screen for history of consanguinity

Molecular genetics of A1ATD

Caused by loss-of-function mutations in SERPINA1, which codes for protease inhibitor (PI) for neutrophil elastase.

PI deficiency results in elastase – inhibitor imbalance



SERPINA1 Alleles and Mutations

Allele	Serum PI levels	Electrophoresis phenotype	Comment
M	Normal levels (> 20 umol/L)	M	Normal allele
S	Normal levels	S	COPD risk unclear
Z	Low levels (7-10 umol/L)	Z	Most common risk allele Accumulates in liver
Null	Undetectable	M	Protein absent
Others	Normal, low	M, unusual	Includes variants of abnormal function

Diagnostic testing:
PI levels, PI phenotyping, genetics

Genotype	Serum PI levels	Protein electropharesis phenotype	COPD risk	Risk of liver disease
MM	> 20 umol/L	M	Low	None
MS	~ 20 umol/L	MS	Low	None
MZ	10-20 umol/L	MZ	Possibly increased	Possibly increased
M/Null	10-20 umol/L	M	Unknown	None
SZ	8-15 umol/L	SZ	Increased	Possibly increased
Z/Null	2.5-7 umol/L	Z	High	Unknown
ZZ	2.5-7 umol/L	Z	High	High
Null / Null	0 umol/L	None	High	None

Serum levels

- Widely available
- Detects null variants
- Falsely elevated in inflammatory states

Genotyping

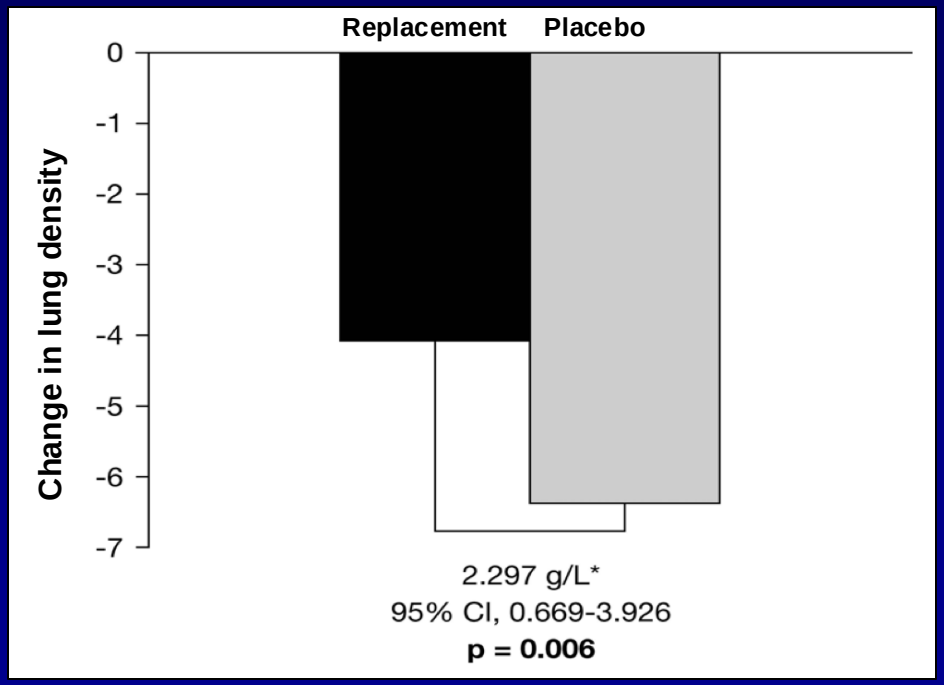
- Common tests assay S and Z alleles only
- Sequencing for null and rare alleles available

PI Phenotype

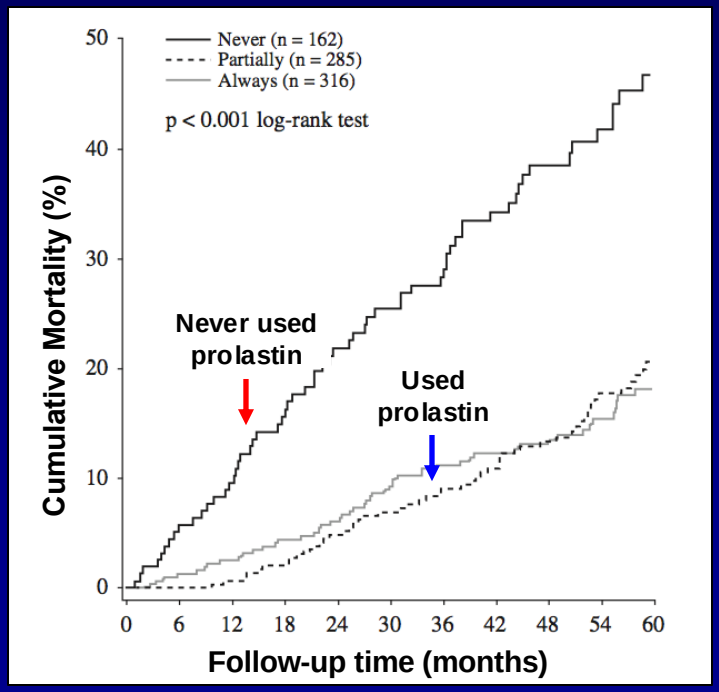
- Specialized laboratories
- Can’t detect null variants
- Robust to inflammation

Augmentation Therapy

- FDA approved for patients with:
  - » evidence of COPD and
  - » A1AT protein levels < 11umol/L
- Most efficacy data based on observational studies, not clinical trials
  - » Reductions in emphysema progression
  - » CT-based metrics



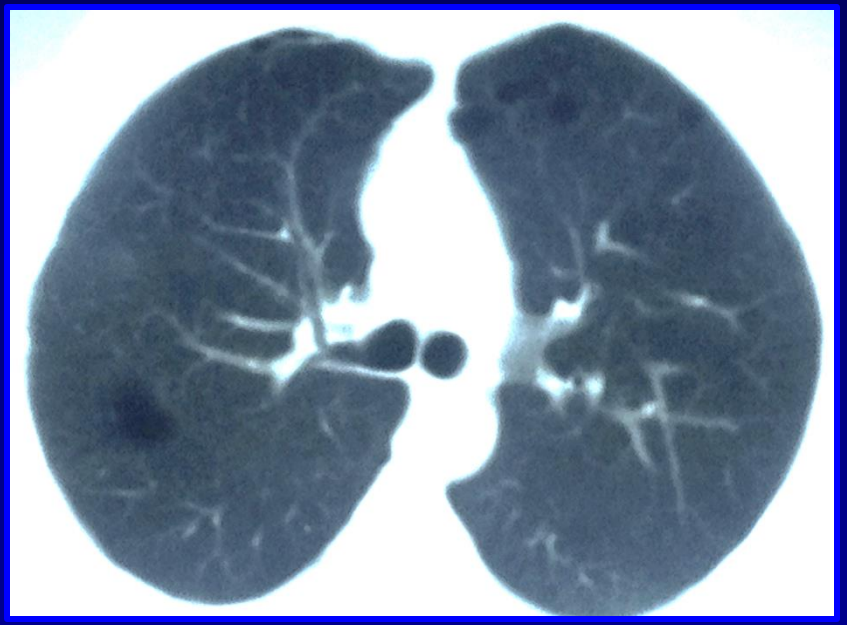
Stockley et al. Respiratory Research 2010, 11:136

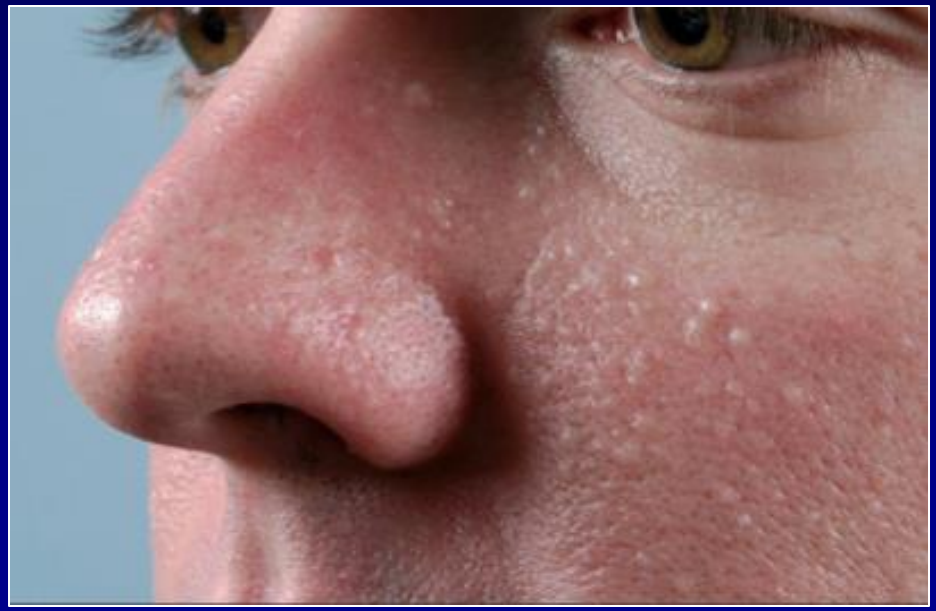


AJRCCM (1998): 158:49-59.

Birt-Hogg-Dubé Syndrome (BHD)

- Cystic lung disease caused by autosomal dominant mutations in FLCN gene.
  - Spontaneous pneumothoraces (25-40%)
  - Thin-walled lung cysts (90%)
  - Fibrofolliculomas & trichodiscomas (68%)
  - Renal tumors (40%)

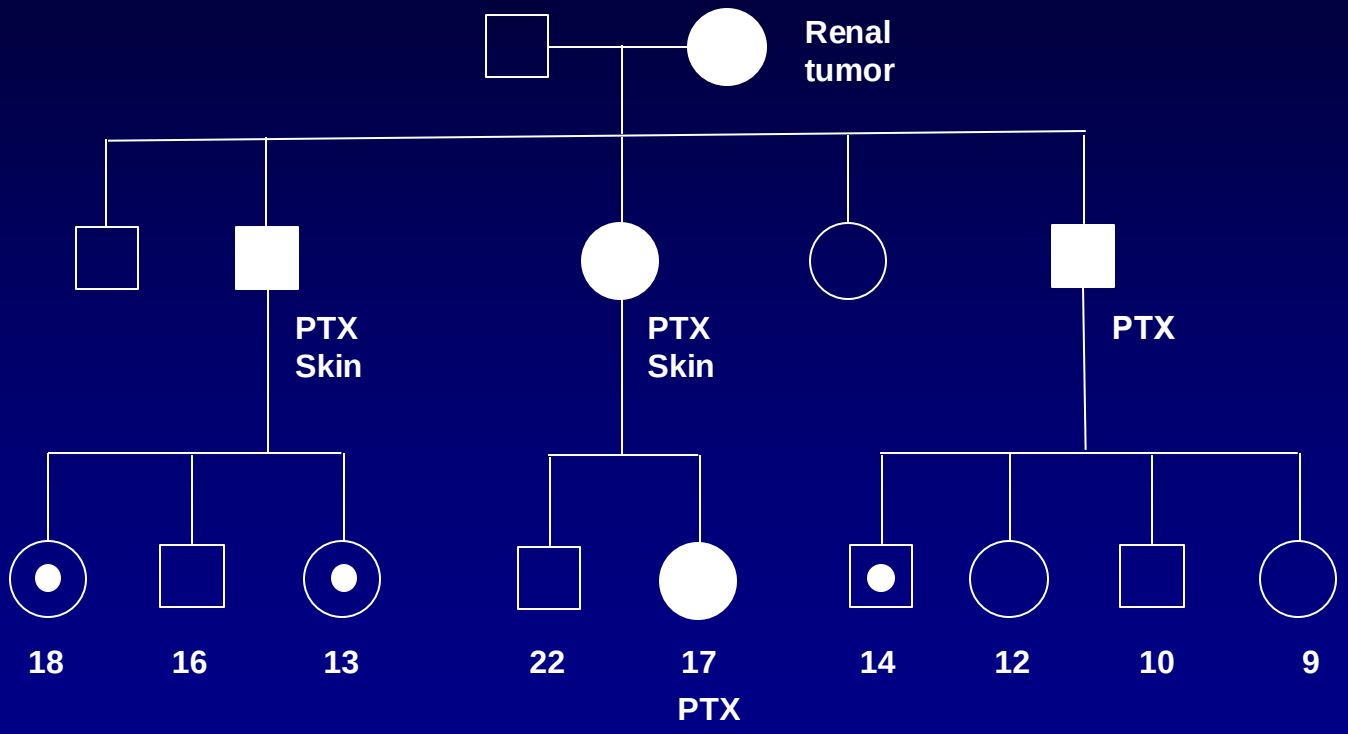




Menko et al.
Lancet Oncology (2009); 10: 1199–1206.

Birt-Hogg-Dubé Syndrome: Diagnosis

- Gene sequencing: identification of truncating frameshift mutations



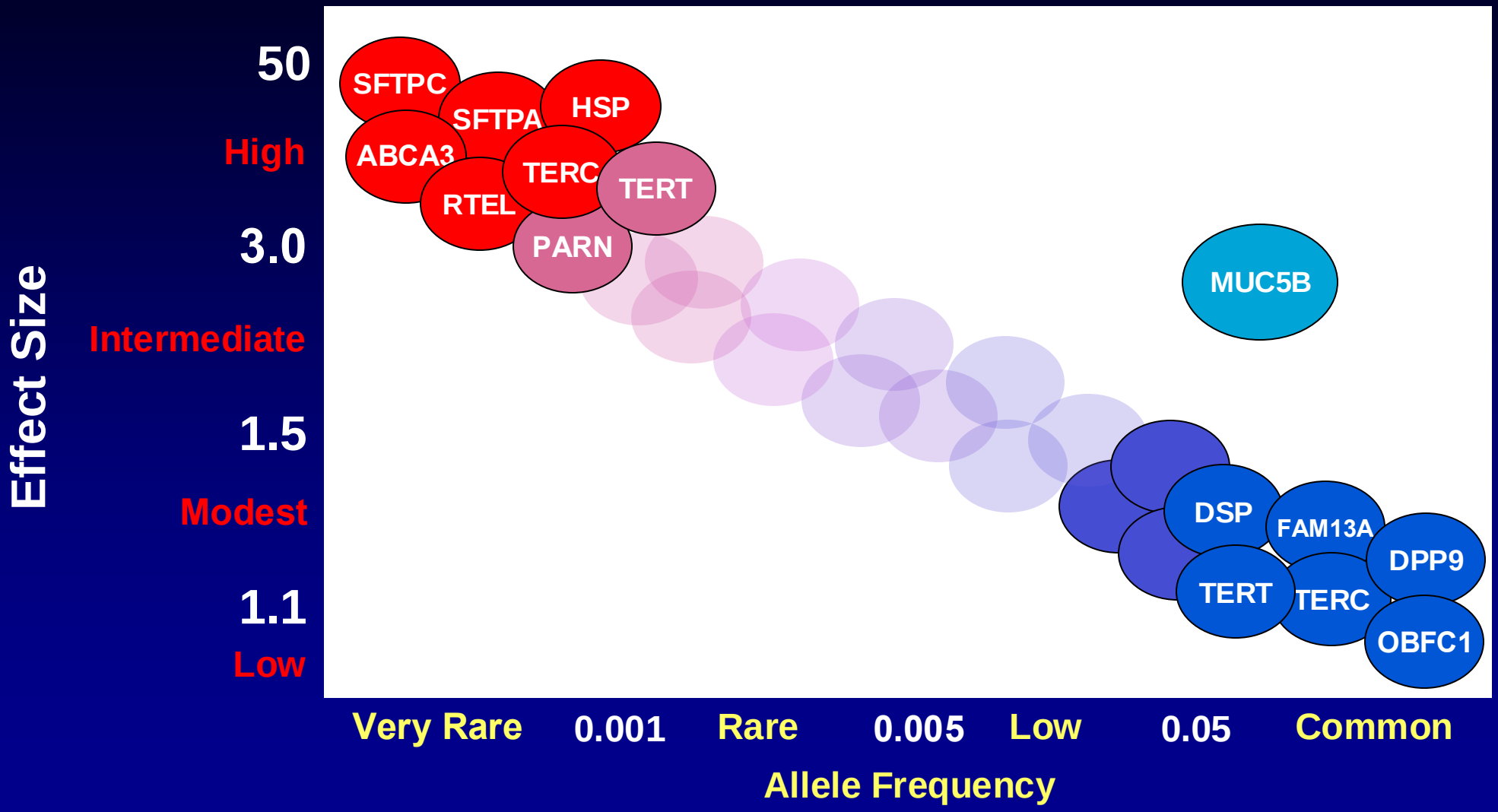
Probability of observing a similar pedigree with only one of nine offspring of carriers inheriting mutation is very low:

$$(½)^8 = 0.004$$

Interstitial lung diseases

Short Telomere Syndrome
Surfactant Disorders
Hermansky Pudlak Syndrome
Common polymorphisms (MUC5B)

Genetic Landscape of Pulmonary Fibrosis

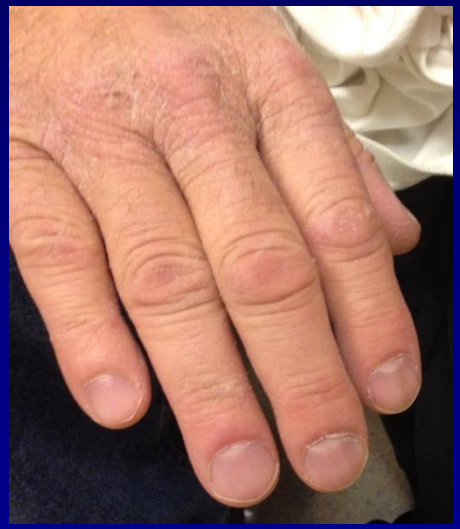


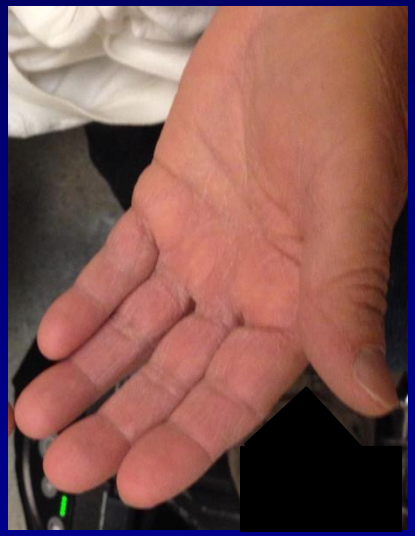
Telomere biology disorders: Short Telomere Syndrome

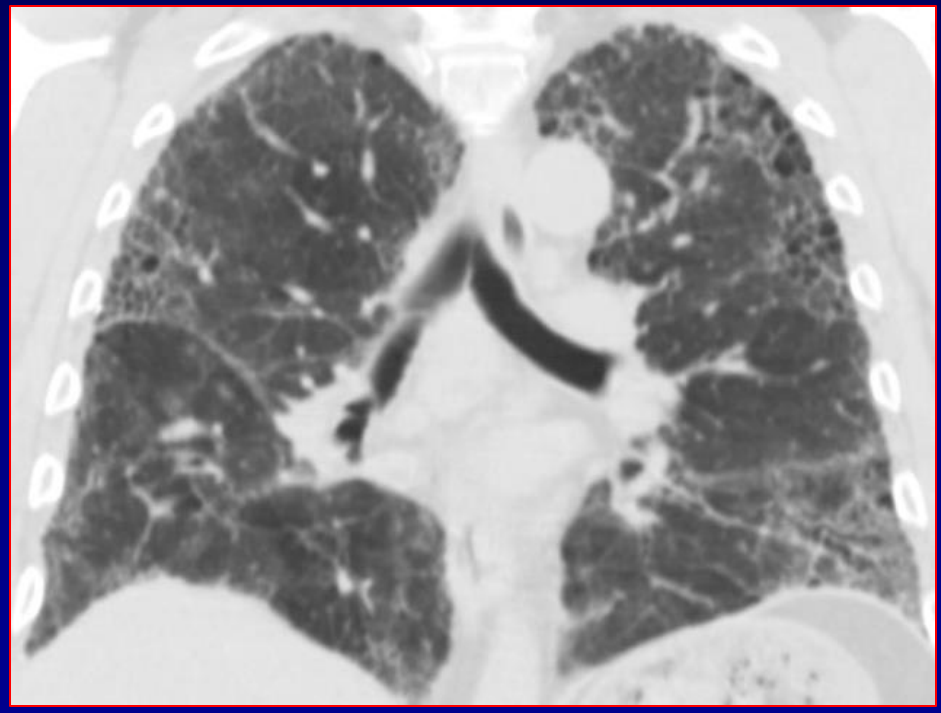
- Dysfunctional telomerase activity results in premature shortening of chromosomal telomeres
- Autosomal dominant in adults (TERT, PARN, RTEL1, TERC)
- Spectrum of disease:
  - Dyskeratosis congenita
  - Premature greying of hair
  - Myelodysplasia
  - Cirrhosis
  - Pulmonary fibrosis
- Lung transplant patients at risk of bone marrow and liver failure in post-operative period

Short Telomere Syndrome: Diagnosis

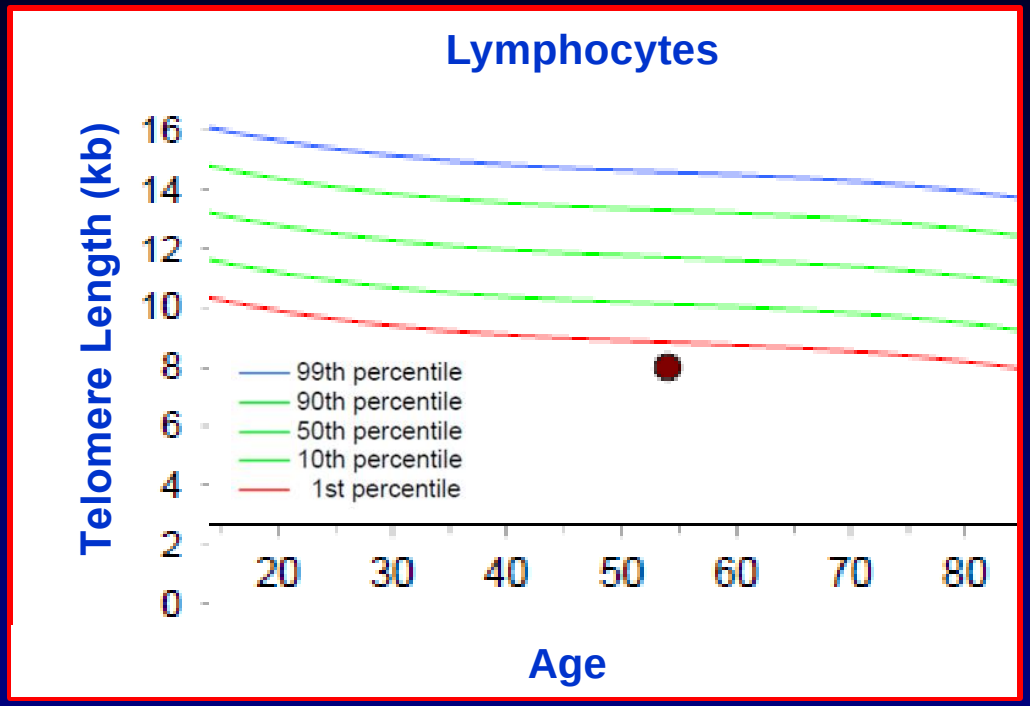
- High suspicion for diagnosis:
  - Family history of fibrosis, cirrhosis, hematological disorders, premature greying
  - Elevated MCV, low platelets or evidence of liver dysfunction
  - Dyskeratosis
- Measure telomere lengths in granulocytes and lymphoctyes

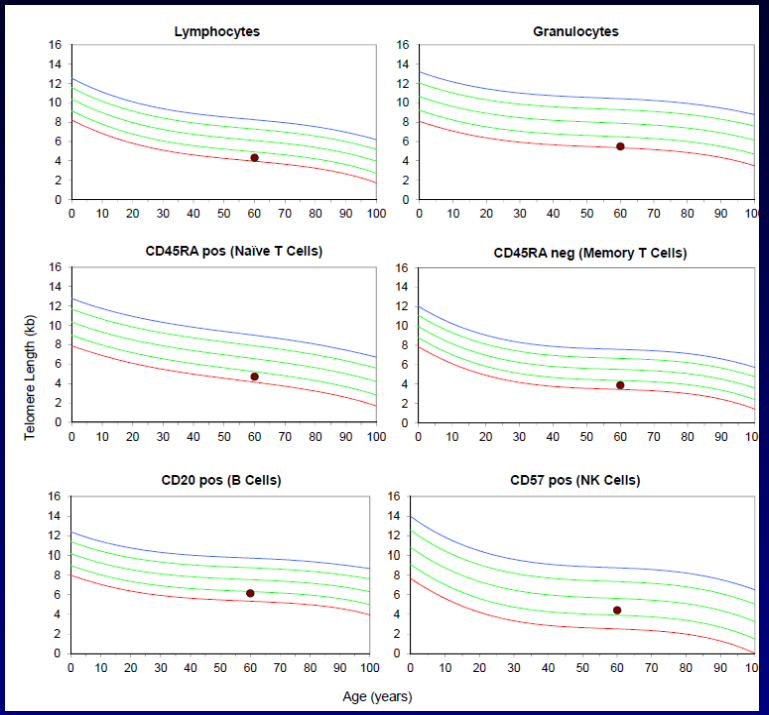






Telomere length assays





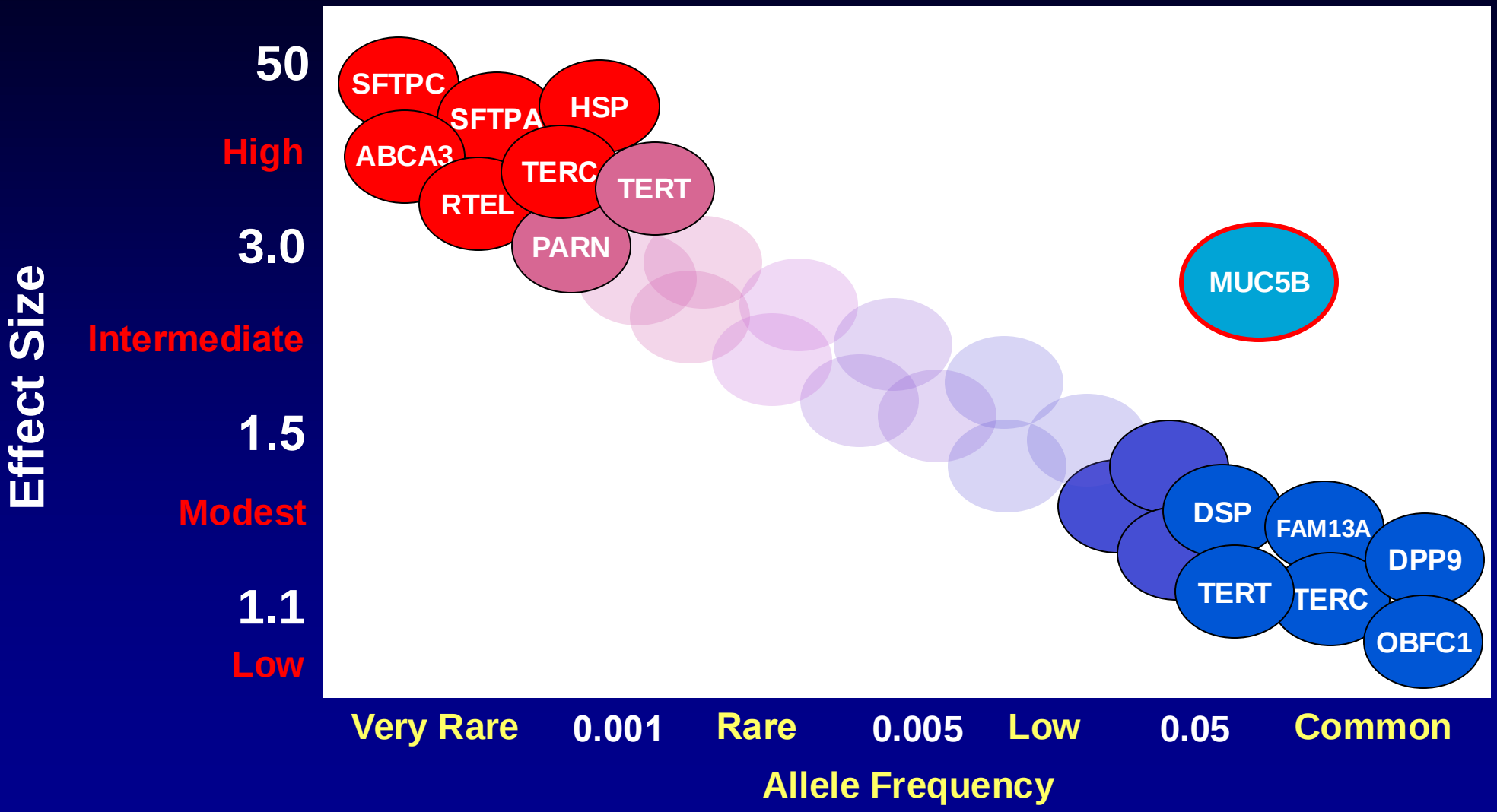
Telomere lengths below 10th percentile for age are considered low and trigger sequencing-based search for pathogenic variants.

Correlation between telomere length and lung function demonstrated in IPF populations. Role as prognostic marker in STS unclear

Short telomere syndrome: Management

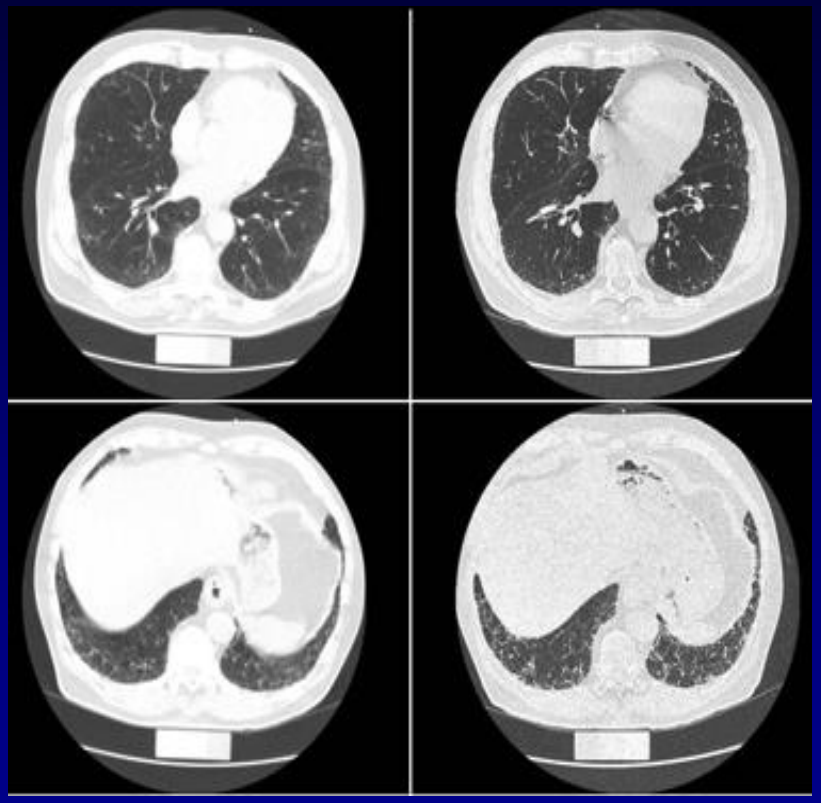
- Baseline evaluation of bone marrow and liver function:
  - Bone marrow biopsy to assess cellularity
  - Liver biopsy only if clinical evidence of hepatic dysfunction
- Tobacco, alcohol and hepatotoxin avoidance
- Annual surveillance for pulmonary, hepatic, and myeloid dysfunction, secondary malignancy
- Diagnosis is not a contra-indication to lung transplantation
  - Decision based on physiological evaluation
  - Tailoring of immunosuppression regimen?
- Genetic counseling and case identification
  - Early lifestyle modifications in at-risk individuals

Genetic Landscape of Pulmonary Fibrosis



MUC5B is associated with early-stage disease

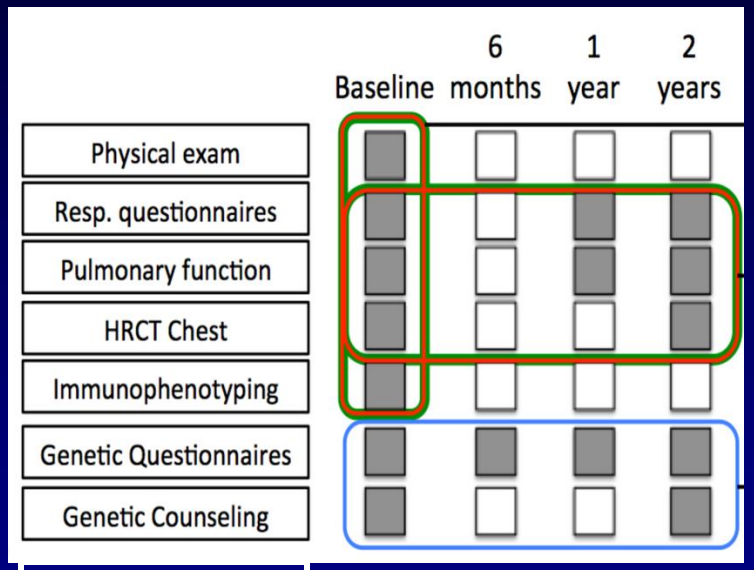
Baseline **Year 5**



Hunninghake GM, et al. (2013) NEJM

Status of Interstitial Lung Abnormalities	Adjusted Odds Ratio with Covariates (95% CI)‡	P Value
Absence of interstitial lung abnormalities	1.0	
Presence of interstitial lung abnormalities	2.8 (2.0–3.9)	<0.001
Definite fibrosis§	6.3 (3.1–12.7)	<0.001

A simple question: Do we find evidence of ILA or early IPF among relatives of patients with IPF?



Clinical Genetics and Screening for Pulmonary Fibrosis
(1R01HL130974; Raby, Rosas, Hunninghake)

Screening first-degree relatives for IPF

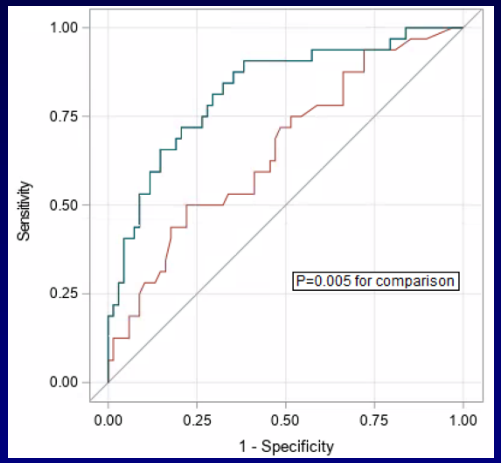
History of Familial Pulmonary Fibrosis
105 individuals from 53 families

Prevalence of Interstitial Lung Abnormalities

31%
(from 27 of 53 families)

Prevalence of Pulmonary Fibrosis

Definite IPF: 18%



Clinical covariates
(AUC 0.66)

Clinical & physiology
& genetics
(AUC 0.82)

3 started on anti-fibrotic therapy and one referred for transplant evaluation

Hunninghake et al. AJRCCM 2020

Recognizing genetic disease

- Personal history:

  Atypical / rare symptoms

  Early onset of adult disease

  Severe disease

- The family history is the key:

  Autosomal dominant disease = Multigenerational incidence of disease

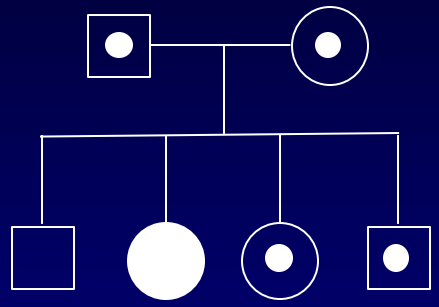
  Autosomal recessive disease = Affected siblings only (not parents)
  Consanguinity

  Familial manifestations of related disease features:

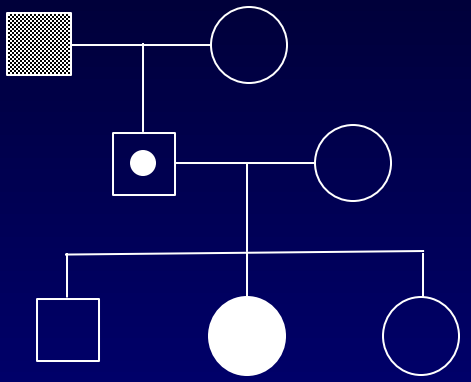
  - situs inversus in primary ciliary dyskinesia
  - premature greying in short telomere syndrome
  - “heart failure” in pulmonary hypertension

A negative family history: very common

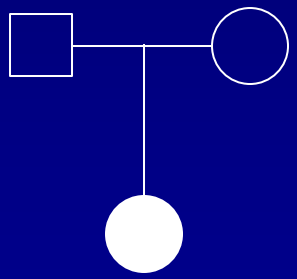
Autosomal recessive diseases
(Cystic Fibrosis, Alpha-1 antitrypsin deficiency, primary ciliary dyskinesia)



Incomplete penetrance
(including Gene x Environment Interaction)

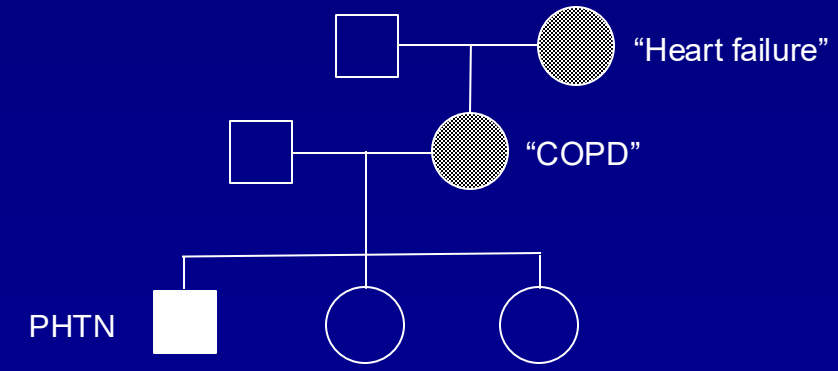


Spontaneous mutation



FIP1L1-PDGFA
Hypereosinophilic syndrome

Inaccurate or unavailable history
(in diagnosis, non-paternity, adoption)



Recognizing genetic disease is challenging

- Family history often lacking
- Suspecting rare disease requires vigilance
- Lack of pathognomonic manifestations:
  - Forme Fruste disease - late onset, “mild” cystic fibrosis
  - Limited expressivity - lack of extra-pulmonary manifestations
- Available exposures for attribution
  - Smoking history
  - Occupational exposures

VUS: Variants of Unknown Significance are to be expected

Table 2. SNPs Identified through Whole-Genome Sequencing of DNA from the Proband.*

SNP Type	No. of SNPs
Nongene	2,255,102
Gene	1,165,204
Intron	1,064,655
Promoter	60,075
3′ UTR	16,350
5′ UTR	3,517
Splice regulatory site	2,089
Splice site	112
Synonymous	9,337
Stop→stop	17
Nonsynonymous	9,069
Stop→gain	121
Stop→loss	27
Total	3,420,306

In this representative genome, more than 9,000 variants were identified that alter protein coding sequence.

On average, that would be one in every three genes!

If we sequence 10 genes, we might find 3 coding variants of unknown significance.

Interpreting these variants is challenging, requires expertise in annotation, clinical correlation

The importance of genetic counseling

Lupski JR et al N Engl J Med. (April 2010) PMID: 20220177.

The importance of genetic counseling

- Pre-test:
  - Understanding role of testing in clinical evaluation
  - Preparation for VUS and “incidentalisms”
  - Determine the patient-specific appropriateness of test
  - Consenting process

- Post-test:
  - Result reporting and interpretation
  - Review of result implications
  - Role of genetic determinism
  - Family counseling
  - Reproductive counseling

Online resources

- GeneTests:
  - Clinical resource for genetic testing, including:
    - » a laboratory directory of over 600 labs offering testing;
    - » a Clinic Directory of over 1000 international genetics clinics
    - » GeneReviews – summaries of diseases and genes
    - » www.genetests.org
- OMIM: Online Mendelian Inheritance of Man
    - » Annotated catalog of disease-associated genes and genetic traits
    - » omim.org
- Disease Foundations:

Summary

- Monogenic subgroups of virtually all forms of lung disease have a described, and making specific diagnoses can impact management and have familial implications.
- Genetic assays are rapidly gaining traction as first-line testing, but only in specific situations.
- CFTR modulator therapies are now FDA approved as first-line therapy for patients 12 years of age or older. Indicated for 90% of patients.
- When considering genetic testing using panels, exomes, or genomes, the involvement of a certified genetic counselor prior to testing is recommended.

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