Role of genetic testing in the evaluation of patients with chronic lung diseases- Interstitial Lung Disease and Bronchiectasis

DM Seminar

09/04/2021

Dr Kritarth

Brief Outline

- Genetic Testing -Overview
- Challenges to Genetic Testing
- Specific diseases
  - ILDs esp IPF, Familial IPF/NSIP, Sarcoidosis and SSc-ILD
  - Bronchiectasis esp CF, PCD, Immunodef
- Summary

- DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine

- Different types of sequencing techniques include
  - Sanger sequencing: based on chain termination; modified di-deoxynucleotide triphosphates (ddNTPs), the latter of which terminate DNA strand elongation
  - High-throughput sequencing, which includes exome sequencing, genome sequencing

Comparison between genetic testing methods

	Single gene sequencing	Targeted Gene Panel
Advantages	1. High Accuracy 2. Lowest Cost 3. Rapid Result 4. Less likely to report incidental findings	1. Seqeunces multiple genes 2. Has lower cost than WES/WGS 3. More accurate than WES/WGS
Limitations	1. Only sequences 1 gene 2. Poor coverage for G-C rich DNA segments	1. Only sequences regions included in panel 2. Panels become dated 3. Low potential for discovery of novel genes 4. Low intrionic coverage

Chinen et al, J Inflamm Res Ann Allergy Asthma Immunol 2019 123 (5) 433-9

	Whole Exome Sequencing	Whole Genome Sequencing
Advantages	1. Seqeunces >90cent coding regions 2. Can evaluate for mosacism 3. Lower cost than WGS 4. Can discover new genes	1. Reads all coding and noncoding regions 2. Can detect intronic/regulatory mutations 3. Detects CNV 4. More consistent coverage of exonic sequences 5. Can discover new genes 6. Lower false-positive rate as compared with WES
Limitations	1. Low coverage for psuedogenes, G-C rich DNA segments 2. Poor detection of CNV 3. Pathogenic intronic variants can be missed 4. Likely to identify numerous VUS 5. Costs more than single-gene or TGS 6. Less accurate than single-gene or TGS	1. Identifies the most VUS 2. Has the highest cost 3. Has the highest error rate 4. Requires longest data analysis time

Chinen et al, J Inflamm Res Ann Allergy Asthma Immunol 2019 123 (5) 433-9

Role of Genetic Testing

- Understanding Pathophysiology; Disease predisposition
- Diagnosis
- Drug SelectionTreatment
- Prognosis
- Genetic Counselling

Chinen et al, J Inflamm Res Ann Allergy Asthma Immunol 2019 123 (5) 433-9

Challenges to using genetic testing

- 3 major challenges
  - Cost
  - Accessibility

Heimall et al, J Clin Immunol .2018;38(3):320-329

Challenges to using genetic testing

- Interpretation
  - Interpretation of genetic test results requires correlation between a positive finding and the clinical disease phenotype
  - Finding a change in a specific gene or chromosome does not always translate into a disease or a specific diagnosis and not all changes will have the same effect.
  - Changes in DNA sequence are referred to as “variants”, which highlights the large number of variants in each individual, the vast fraction of which do not cause disease or harm to the individual
  - With novel variants, it remains challenging to decipher which variants are disease-causing and proving their association with specific phenotypic patterns

Heimall et al, J Clin Immunol .2018;38(3):320-329

Classification of ILDs

- Diffuse Lung Disease
  - IIPs
  - DPLD of known cause
    - Drug Induced
    - Collagen vascular diseases
    - Granulamatous disorders, Sarcoidosis
    - Exposure
      - Pneumoconiosis, Radiation Exposure, Hypersensitivity Pneumonitis, Toxic Inhalation
    - Misc
      - Cystic Diseases, Airway diseases, Infiltrative diseases

ATS/ERS Statement. Am J Respir Crit Care Med 2002; 165;277–304

- Major idiopathic interstitial pneumonias
  - Idiopathic pulmonary fibrosis
  - Idiopathic nonspecific interstitial pneumonia
  - Respiratory bronchiolitis–interstitial lung disease
  - Desquamative interstitial pneumonia
  - Cryptogenic organizing pneumonia
  - Acute interstitial pneumonia
- Rare idiopathic interstitial pneumonias
  - Idiopathic lymphoid interstitial pneumonia
  - Idiopathic pleuroparenchymal fibroelastosis
- Unclassifiable idiopathic interstitial pneumonias

Travis et al, Am J Respir Crit Care Med 2013 188(6) 733–74

Genetic Variants Associated with IPF

Common variants associated with IPF	Gene Function	Gene with Risk allele
	Airway mucin production	MUC5B, MUC2
	Cell-cell adhesion	DSP, DPP9
	Toll-like receptor signaling	TOLLIP, TLR3, ATP11A
	Cytokine/growth factor signaling	IL1RN, IL8, IL4 TGFB1
	Telomere maintenance	TERT, OBFC1
	Cell cycle regulation	KIF15, MAD1L1, CDKN1A, TP53

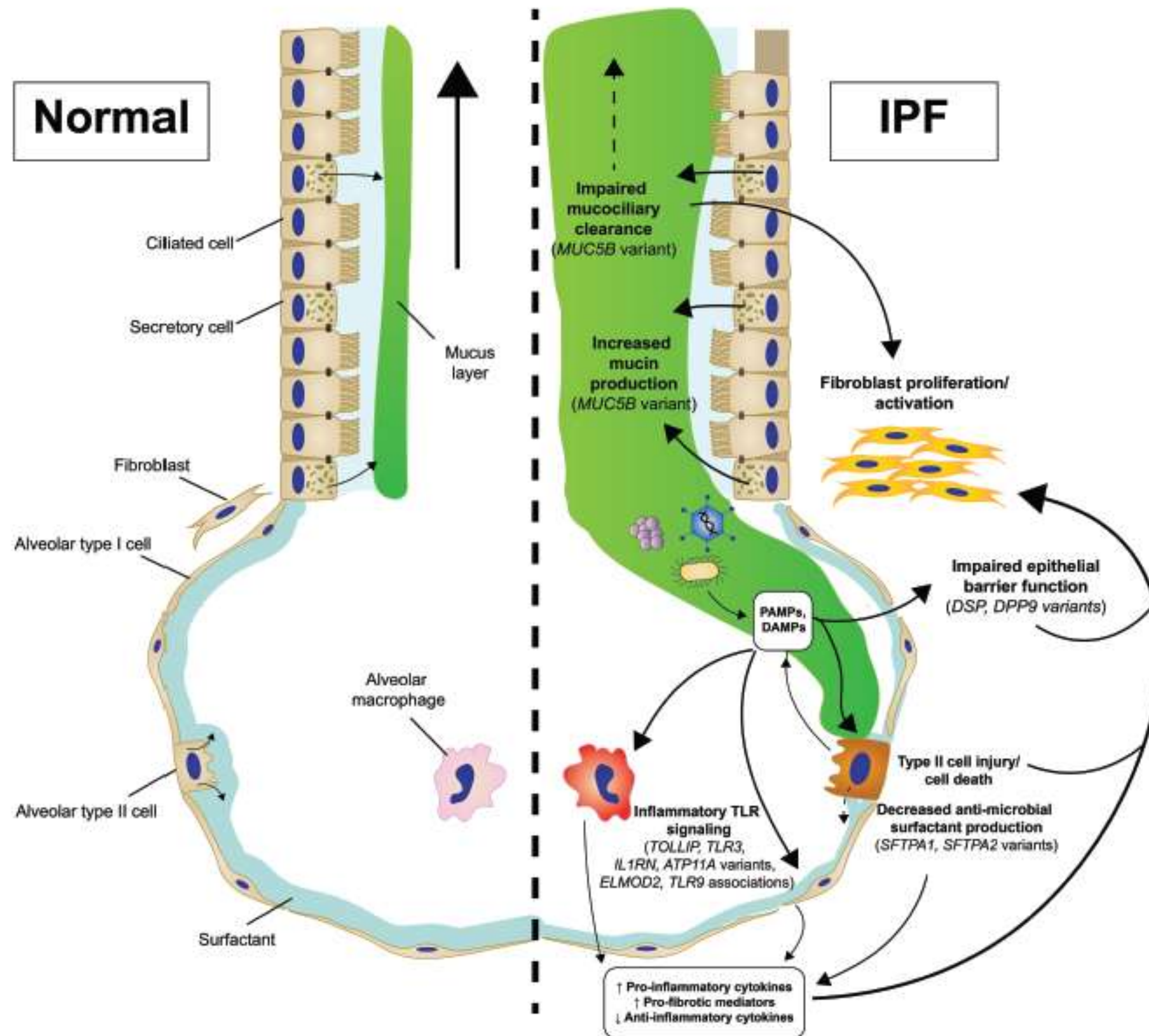
Michalski et al, J Inflamm Res. 2021 ;13:1305-18

Genetic Variants Associated with IPF

Rare variants associated with IPF	Gene Function	Gene (Mutations)
	Surfactant production/ secretion	SFTPA1 (T622C, W211R) SFTPA2 (G231V, F198S) SFTPC (I73T, M71V) ABCA3 (S1261G, R288K)
	Telomere maintenance	TERT (L55Q, R901W, T1110M) TERC (98G>A, 37A>G) TINF2 (K280E, R282H, R282S) DKC1 (T405A) RTEL1 (R213W, T49M, F964L) PARN (A383V)

Michalski et al, J Inflamm Res. 2021 ;13:1305-18

Relationship Between Genetic Variants and IPF Immunopathogenesis



Michalski et al, J Inflamm Res. 2021 ;13:1305-1318

Role of MUC5B Gene

- MUC5B promoter variant (rs35705950) is the strongest and most validated risk factor (genetic and otherwise) for IPF and preclinical pulmonary fibrosis (PrePF)
- A novel entity, PrePF has been defined as:
  - abnormalities on chest HRCT consistent with probable or definite fibrosis (e.g., bilateral subpleural reticular changes, honeycombing, or traction bronchiectasis—radiographic findings are concordant with IPF)
  - asymptomatic subjects of 40 years of age or older
  - recruited from at-risk populations (first-degree relatives of patients with IPF)
- PrePF is an early diagnostic sign of IPF and a harbinger of progressive fibrosis, occurring in 1.8% of the general population and 15–20% of high risk populations of those aged 40 years or greater

Schwartz et al, Annals Am Thorac Soc.2018;15(S3);S192–197

- During a 5- to 6-year period of observation, approximately 75% of subjects with PrePF progressed radiographically, and that radiographic progression of PrePF is associated with a greater decline in FVC and an increased risk of death
- Emerging clinical phenotype of PrePF creates a window of opportunity to identify at-risk individuals with preclinical stages of pulmonary fibrosis before the injury/repair/regenerative process has permanently damaged substantial lung parenchyma
- That would also allow for the treatment of disease before significant, irreversible loss of functional lung parenchyma has occurred

Schwartz et al, Annals Am Thorac Soc.2018;15(S3);S192–197

- Excessive production of MUC5B by stem cells that attempt to regenerate injured bronchiolar and alveolar epithelium disrupt normal developmental pathways and hijack the normal reparative mechanisms in the distal lung, resulting in chronic fibroproliferation and honeycomb cyst formation

- Too much MUC5B may impair mucociliary function, cause excess retention of inhaled substances (air pollutants, cigarette smoke, microorganisms, etc.), and, over time, the foci of lung injury may lead to scar tissue and persistent fibroproliferation that expands and displaces normal lung tissue

Schwartz et al, Annals Am Thorac Soc.2018;15(S3);S192–197

Familial Interstitial Pneumonia

- FIP is defined by the diagnosis of an idiopathic interstitial pneumonia (IIP), predominantly IPF, in two or more relatives who share common ancestry
- Often Implicated Genes:

Telomere related	TERT, hTR, DKC1, TINF2, RTEL1, PARN
Surfactant related	SFTPC, SFTPA2 and ABCA3
	Common variants at 10 loci (3q26, 4q22, 5p15, 6p24, 7q22, 10q24, 11p15, 13q34, 15q14–15, and 19q13)

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- ABCA3 has a recessive mode and DKC1 is X-linked, but the other disease-associated rare genetic variants are inherited in an autosomal dominant manner
- Disease penetrance in carriers of these rare genetic variants is incomplete
- Mutations in currently identified FIP causing genes are found in approximately 20% of affected families; thus rare genetic variants are yet to be identified in 80% of FIP families
- In addition to FIP, pulmonary fibrosis is also a feature of some multisystem genetic disorders, including Hermansky–Pudlak syndrome and dyskeratosis congenita

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- In all subjects with IPF, a thorough family history should be performed regardless of patient age

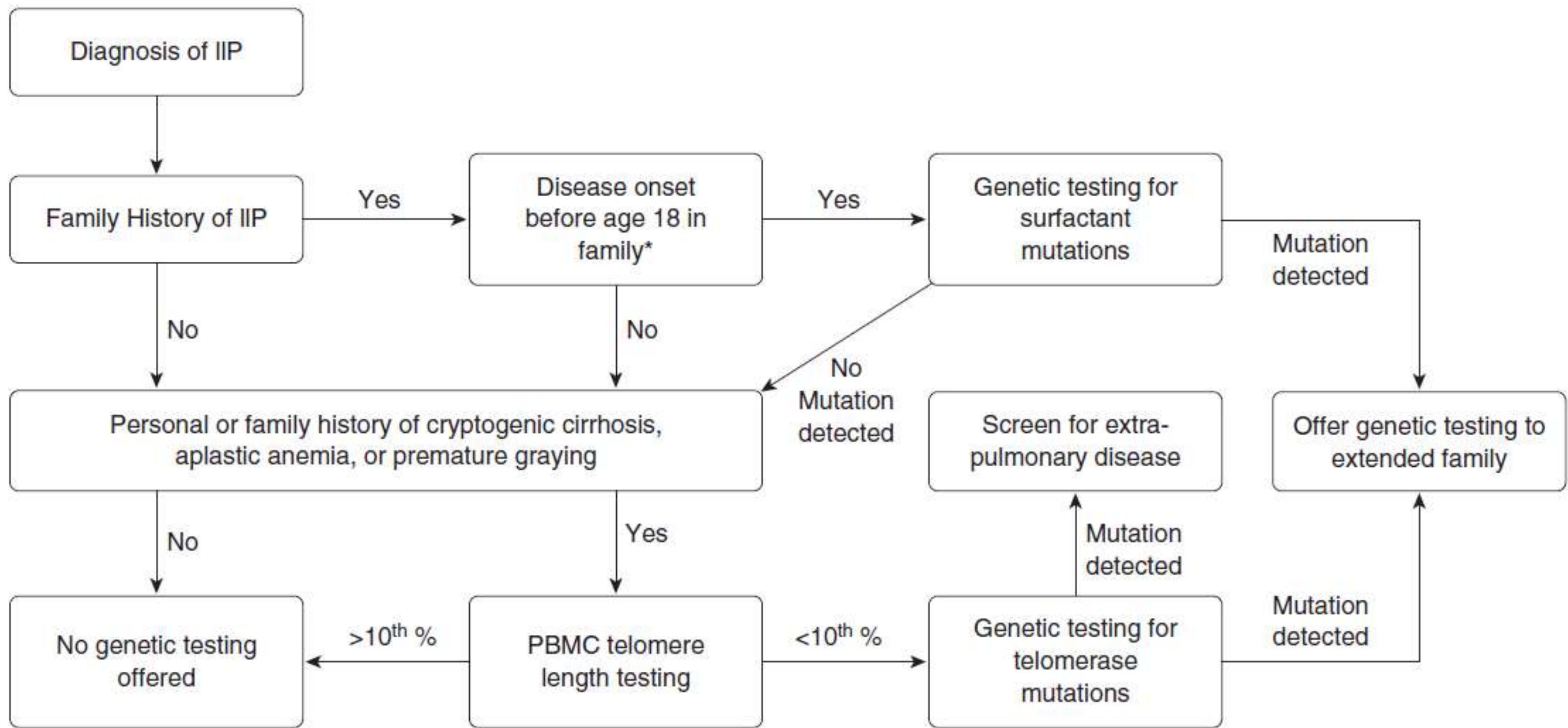
- Key elements should include

	Short Telomere Syndromes
Adult ILD	++
Premature graying	++
Cryptogenic cirrhosis	++
Aplastic anemia	++
Myelodysplasia/leukemia	+

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- A family history of neonatal respiratory distress or childhood interstitial lung disease is relevant for suggesting the possibility of a surfactant-related disorder, which can present across a wide age spectrum

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28



Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- In cases with history suggestive of short telomere syndrome, peripheral blood mononuclear cell (PBMC) telomere length testing through flow cytometry is recommended
- If the PBMC telomere length is short, (10% for age), the likelihood of identifying a pathogenic mutation in a known telomerase-related gene is high
- It is possible to inherit short telomeres (and disease risk) without inheriting a mutation; this has been termed “occult genetic disease”

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- Identification of a disease-associated rare genetic variant in a patient with FIP may suggest the need for additional screening and/or testing for extrapulmonary disease. For example, subjects with telomerase pathway mutations may undergo periodic monitoring of blood counts and liver tests

- In addition, the presence of an FIP-associated mutation in an affected family member should prompt consideration of genetic testing for at-risk asymptomatic family members in concert with genetic counseling

- In particular, in families with telomerase mutations, earlier-onset and more severe extrapulmonary disease have been observed in successively younger generations, suggesting that closer monitoring of asymptomatic mutation carriers may be beneficial

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

Sporadic IPF

- Patients with IPF who do not have a known family history of IIP are classified as having sporadic IPF
- Genetic testing in individuals with sporadic IPF or other IIPs unless their family history suggests a short telomere syndrome as described previously
- If the personal and/or family history does suggest a short telomere syndrome, clinical telomere length testing should be done as the next step, similar to patients with FIP

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

Clinical Implications

- Regardless of etiology, it appears that PBMC telomere length provides prognostic information in IPF
- In the United States, it was reported that PBMC telomere length was independently associated with transplantation-free survival
- It is also suggested that PBMC telomere length testing should be considered as a component of the pretransplantation evaluation of those patients with IPF in whom this treatment is being considered
- Future studies are needed to more comprehensively define the lung transplantation outcomes of patients with short telomeres and to determine optimal approaches to minimize posttransplantation complications related to short telomeres.

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- Common genetic variants, including a polymorphism in the mucin 5B (MUC5B) promoter, have been linked to risk of developing familial and sporadic IPF;

- However, because the positive and negative predictive values of risk allele carrier status are modest (i.e., disease penetrance in carriers of this polymorphism is low), testing for these common polymorphisms as a screening approach for IPF is currently not suggested

- In addition to correlations with disease risk, common polymorphisms in the MUC5B promoter and in toll interacting protein (TOLLIP) have been associated with outcomes in patients with IPF and offer promise as prognostic indicators

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- Carriers of the allele that confers risk for IPF have better outcomes than patients with IPF who do not have these alleles, suggesting that these variants identify a prognostic (and possibly biologically distinct) subtype of IPF

- Carriers of at least one T allele of the MUC5B polymorphism appear to have at least 50% improved survival compared with GG carriers. Interestingly, T allele carriers have modestly better lung function than do G allele carriers at similar ages, consistent with a slower disease course.

- Carriers of the TOLLIP risk polymorphism rs5743890 A allele have approximately 35% reduced risk of mortality compared with G allele homozygotes

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

- Another area of possible usefulness for testing of common genetic variants in IPF is stratification of patient populations in clinical intervention trials.
- It has been suggested that carriers of a TOLLIP polymorphism may benefit from treatment with N-acetylcysteine, whereas other single-nucleotide polymorphisms may predict harm from this intervention.
- Further studies are required to understand if and how presently known genetic risk factors for disease progression can be used to personalize IPF treatment

Kropski et al, Am J Respir Crit Care Med.2017; 195(11):1423-28

Genetic testing registry; ILD panel

- 24 Genes and variants

  ABCA3 (16p13.3)
  CFTR (7q31.2)
  CSF2RB (22q12.3)
  DKC1 (Xq28)
  FLCN (17p11.2)
  HPS1 (10q24.2)
  HPS4 (22q12.1)
  ITGA3 (17q21.33)
  NF1 (17q11.2)
  NKX2-1 (14q13.3)
  PARN (16p13.12)
  RTEL1 (20q13.33)
  SFTPA2 (10q22.3)
  SFTPB (2p11.2)
  SFTPC (8p21.3)
  SLC34A2 (4p15.2)
  SLC7A7 (14q11.2)
  SMPD1 (11p15.4)
  STAT3 (17q21.2)
  TERC (3q26.2)
  TERT (5p15.33)
  TINF2 (14q12)
  TSC1 (9q34.13)
  TSC2 (16p13.3)

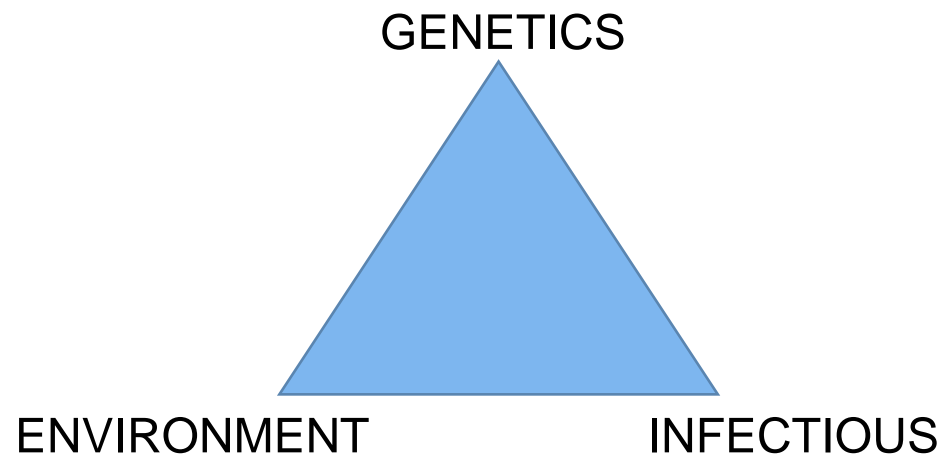
Retrieved on 09/04/21 from https://www.ncbi.nlm.nih.gov/gtr/tests/556382/

- Genetic pathways in IPF ("TSUNAMI")
  - Telomere related
  - Surfactant related
  - Unclassified
  - No-Not known yet
  - Adhesion cell mol
  - Mucin and Matrix metalloprotienases
  - Immune mediated

Courtesy Dr Sahajal Dhooria

Sarcoidosis

- Characterized by the formation and accumulation of non-necrotizing epithelioid cell granulomas in the lungs, although the skin, eyes, bones, liver, spleen, heart, upper respiratory tract, and nervous system can also be a affected
- Inflammatory, multifactorial disease with complex interplay; etiopathogenesis still not clear



Moller et al. Annals Am Thorac Soc. 2017;14(S6):S429-36.

- Epidemiology of sarcoidosis suggests that genetic background in different ethnicities may favor the occurrence of the disease.
- Higher incidence rates have been reported in Northern European countries(Sweden and Iceland), African-Americans in the United States
- ACCESS (A Case-Control Etiologic Study of Sarcoidosis) confirmed the possibility of familial clustering of sarcoidosis, supporting an inherited susceptibility to sarcoidosis. Siblings of patients with sarcoidosis were shown to have around a fivefold increased risk

Moller et al. Annals Am Thorac Soc. 2017;14(S6):S429-36.

Gene	Effect	Population
HLA-DRB1*11:01	Risk	Afrcan Americans(2.04) and whites (2.05)
HLA-DRB1 *1201	Risk	African Americans (2.67)
HLA-DRB1 *1503	Protective	African Americans (0.56)
HLA-DRB1 *1501	Risk	European Americans (2.08)
HLA-DRB1 *0401	Protective	European Americans (0.44)
BTNL2	Risk	Germans (1.60- 2.75), European Americans (1.7- 2.63)
ANXA11	Protective	Germans (0.62), Czechs (0.77), African Americans (0.84)
ANXA11	Risk	African Americans (1.31)
NOTCH4	Risk	African Americans (1.30-1.52)
XAF1	Protective	African Americans (0.74)

Moller et al. Annals Am Thorac Soc. 2017;14(S6):S429-36.

Gene	Exposures
HLA DRB1*11:01	Insecticide exposure; exposure to mold and musty odors
DRB1*15:01	Insecticide exposure
Fucosyltransferase 9 gene on chromosome 6q16.1	Insecticide exposure

Moller et al. Annals Am Thorac Soc. 2017;14(S6):S429-36.

Gene	Organ-specific involvement
HLA-DRB1*04/*15	Extrapulmonary involvement
HLA-DRB1*03:01	Lofgren syndrome
HLA-DQB1*06:01	Cardiac sarcoidosis
HLA-DRB1*04	Uveitis
TAB1, TAB2	Skin and bone/joint involvement
MAPK13	Eye, skin and bone/joint sarcoidosis
a SNP in a zinc finger gene, ZNF592	Neurosarcoidosis in African-Americans and European Americans

Moller et al. Annals Am Thorac Soc. 2017;14(S6):S429-36.

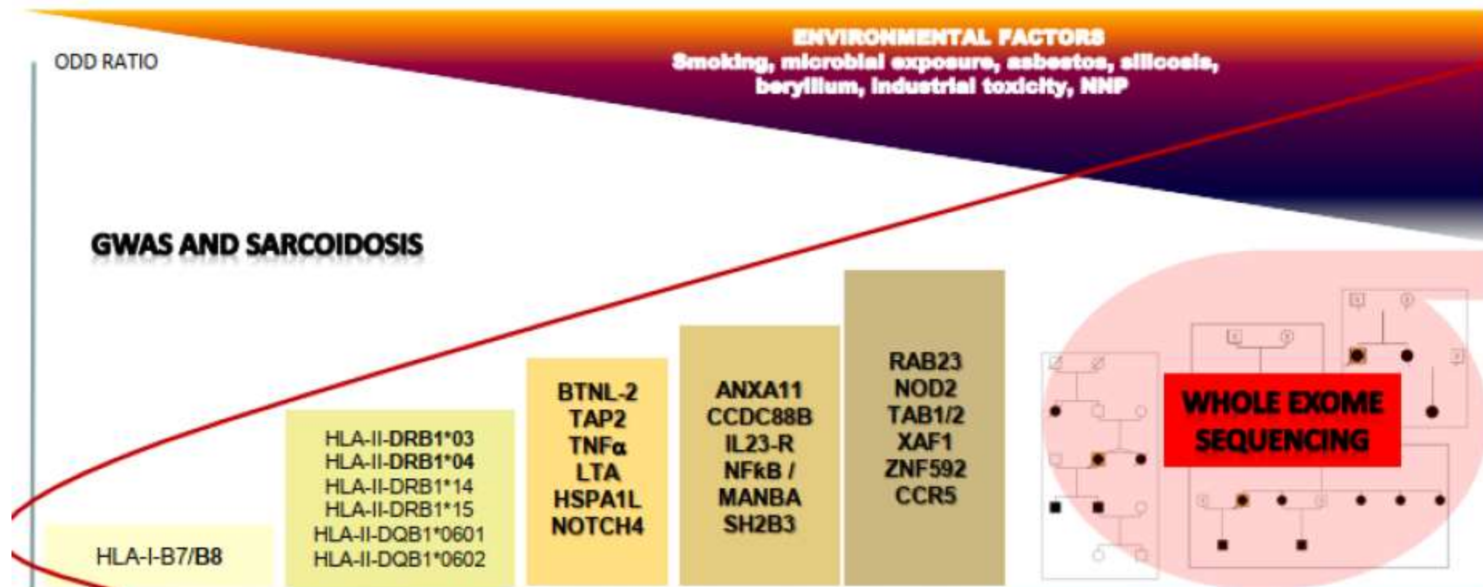


Figure 1. A representation of genes identified by genome wide association (GWAS) studies in sarcoidosis and the cumulative risk induced by the accumulation of variants closely associated with the negative impact of environmental factors. All the gene names are detailed in the text. The red line represents only a theoretical representation of the progression of the odds ratio to sarcoidosis when a patient accumulates polymorphisms in the different groups of genes. The knowledge provided by GWAS is complementary to the data from whole exome sequencing in families predisposed to sarcoidosis. NNP, nanoparticle.

Calender et al, J. Clin. Med. 2020; 9(8): 2633

- Two polymorphisms of TNF-alphaare associated with susceptibility to sarcoidosis in European population. Both are located in regulatory regions of the gene and, strikingly, one of them, 308 A > G, was shown to decrease the response to TNF inhibitors sarcoidosis

Calender et al, J. Clin. Med. 2020; 9(8): 2633

Systemic Sclerosis

- Systemic sclerosis (SSc) is a chronic multisystem autoimmune disease that is characterized by autoimmunity, tissue fibrosis, and vasculopathy
- Interstitial lung disease (ILD) is a life-threatening complication and is one of the frequent causes of mortality in SSc, along with pulmonary arterial hypertension
- Key pathogenesis of SSc-ILD is thought to be initiated by a persistent injury to lung cells that induces profibrotic stimuli, which leads to fibroblast activation and myofibroblast transition
- Lung injury results in the release of profibrotic mediators such as transforming growth factor β1 (TGF-β1) and connective tissue growth factor (CTGF)

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Genetics and SSc

- Incidence of SSc is 1.5%–1.7% in people with a family history of SSc, while it is 0.026% in the general population
- SSc is not inherited in a Mendelian fashion; however, having family members who have SSc or another autoimmune disease increases the relative risk
- Having a sibling with SSc results in a 15- to 19-fold increased risk, and first-degree relatives have a 13- to 15-fold increased risk of having SSc compared with the general population

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Genetics and SSc

- Observation studies among Caucasian and African-American patients have suggested that African-Americans have a genetic tendency to develop SSc and also have a higher morbidity and mortality.
- Early genetic studies indicated that SSc was associated with the human leukocyte antigen (HLA) region.
- Moreover, many candidate gene studies have identified multiple novel polymorphisms that increase susceptibility to SSc.

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Genetic Association in SSc-ILD
HLA Dependent Genes

HLA	Alleles	Population
HLA-C	Cw*0602	Caucasian
HLA-DR	DRB1*1102	South African
	DRB5*01:05	Japanese

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Genetic Association in SSc-ILD non-HLA Genes

Gene	Risk Alleles	Population	Odd Ratio
CTGF	GG Genotype	UK Caucasian	3.1
	G allele	Japanese	2.0
CD226	T allele	European Caucasian	1.27
NLRP1	CC Genotype	European Caucasian	1.43
MMP12	AA Genotype	Italian Caucasian	2.94
IRF5	T allele	French Caucasian	1.44
HGF	TT genotype	Japanese	8.1

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Gene Polymorphisms of CTGF Associated with SSc-ILD

- CTGF was found to have increased expression in the circulation, skin, and fibroblasts of patients with SSc
- CTGF regulates proliferation of fibroblasts and production of extracellular matrix
- Studies in the UK and Japan reported that an SNP in the promoter region of CTGF rs6918698 was significantly associated with SSc
- CTGF is constitutively overexpressed by fibroblasts in fibrotic skin lesions as well as in lung fibroblasts of SSc patients
- Furthermore, higher levels of CTGF are seen in bronchoalveolar lavage fluid from active SSc-ILD patients

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Gene Polymorphisms of CTGF Associated with SSc-ILD

- However, the study of the CTGF gene in North American patients (white Americans, African-Americans, and Hispanic Americans) did not validate susceptibility to any form of SSc

- Similarly, a multicenter study in seven independent case–control sets, including Spanish, Dutch, German, French, British, Swedish, and North American groups, also failed to confirm an association between SNP and SSc.

- In contrast, in Asians, CC and CG genotypes of CTGF rs6918698 were significantly associated with a reduced SSc risk

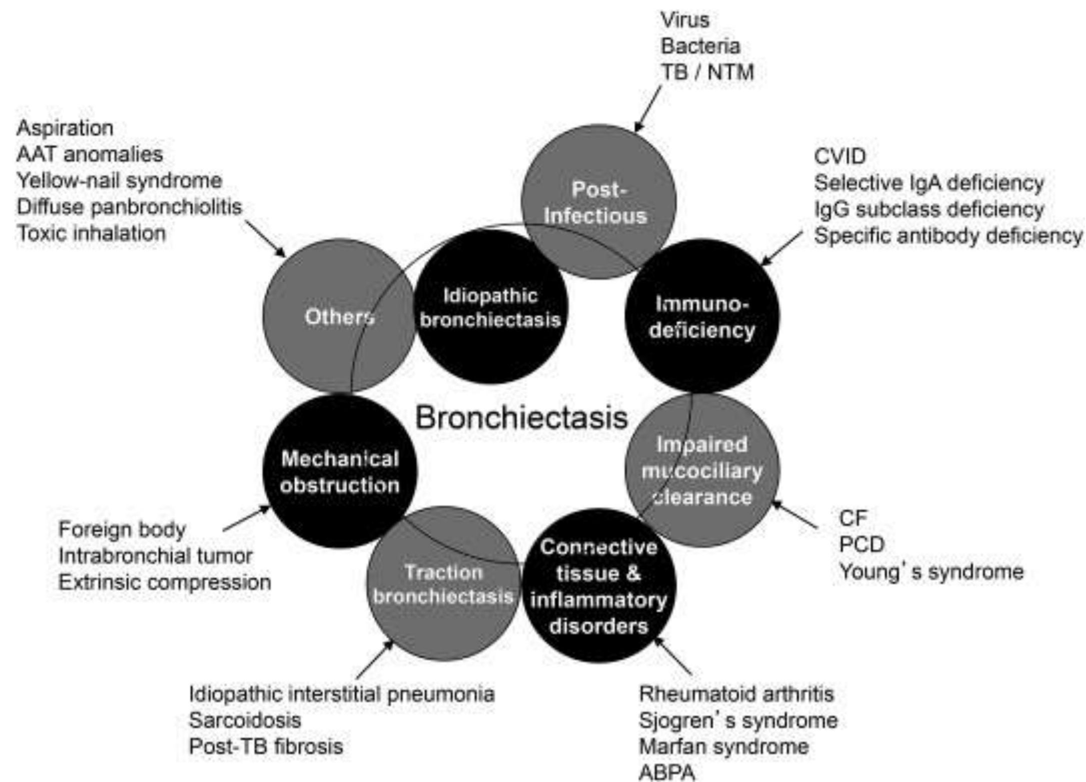
Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Gene Polymorphisms of CTGF Associated with SSc-ILD

- Discrepancies in the genetic association studies of different races have been reported
- One of the possible explanations for this discrepancy between Asians and Caucasians could be a result of different gene–environment interactions among populations

Tochimoto et al, Clin Med Insights Circ Respir Pulm Med 2015:9(S1) 135–140

Causes of Bronchiectasis



Chan et al. Respiratory Medicine: X. 2019;1:100006.

Cystic Fibrosis

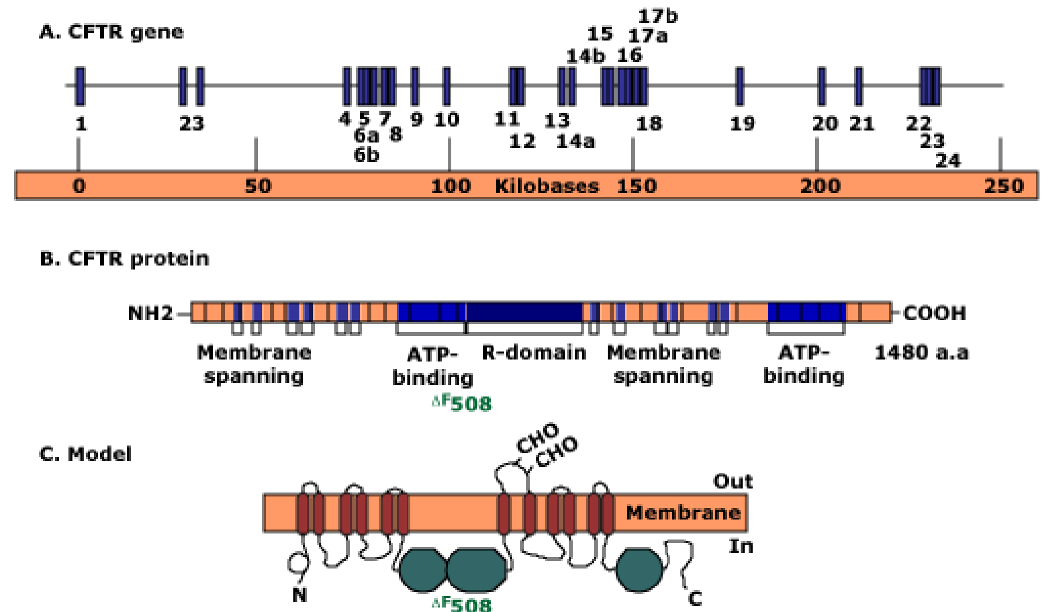
- Multisystem, autosomal recessive disease
- Caused by mutations in the CF transmembrane conductance regulator (CFTR) gene located on chromosome 7

Rommens et al, Science 1989;245(4922):1059-65

Normal CFTR gene

- CFTR functions as a regulated Cl^- channel, which, in turn, may regulate the activity of other Cl^- and Na^+ at the cell surface

- The CFTR gene spans 250 kb on chr 7, encoding 1480 AA in the mature protein



Schematic representation of the *CFTR* gene and its encoded polypeptide. ΔF508 refers to the site of the most common variant causing cystic fibrosis, also known as F508del.

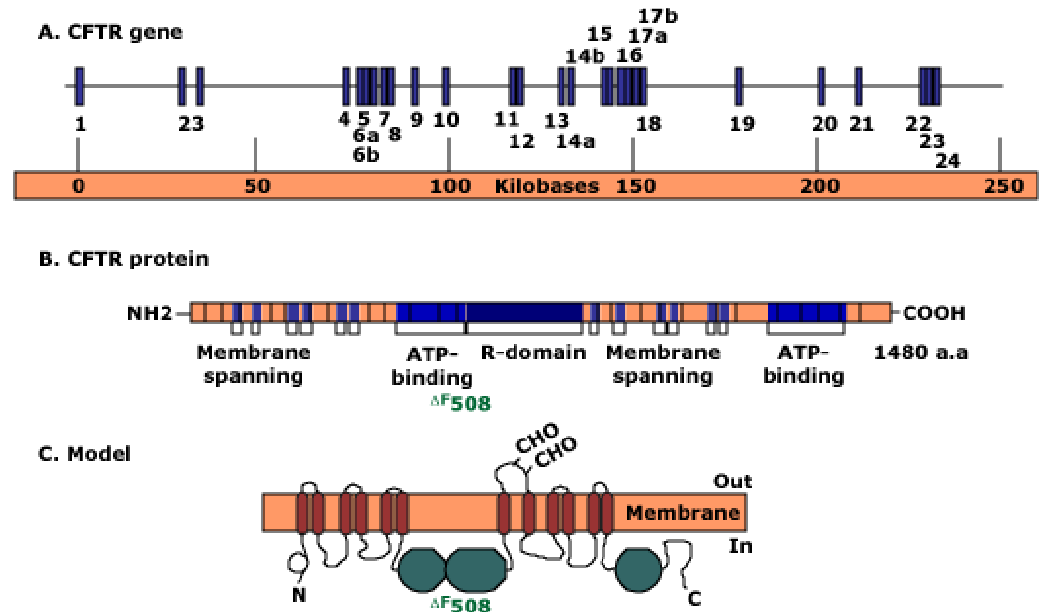
Katkin et al, Uptodate 2021

Normal CFTR gene

- Protein has 2 groups of 6 membrane-spanning regions, 2 intracellular nucleotide-binding folds (NBFs), and a highly charged "R domain" containing multiple phosphorylation sites

- Activation of the Cl^- channel requires phosphokinase A-mediated phosphorylation of the R domain and the continuous presence of ATP in the NBFs

CFTR gene (cystic fibrosis transmembrane conductance regulator)



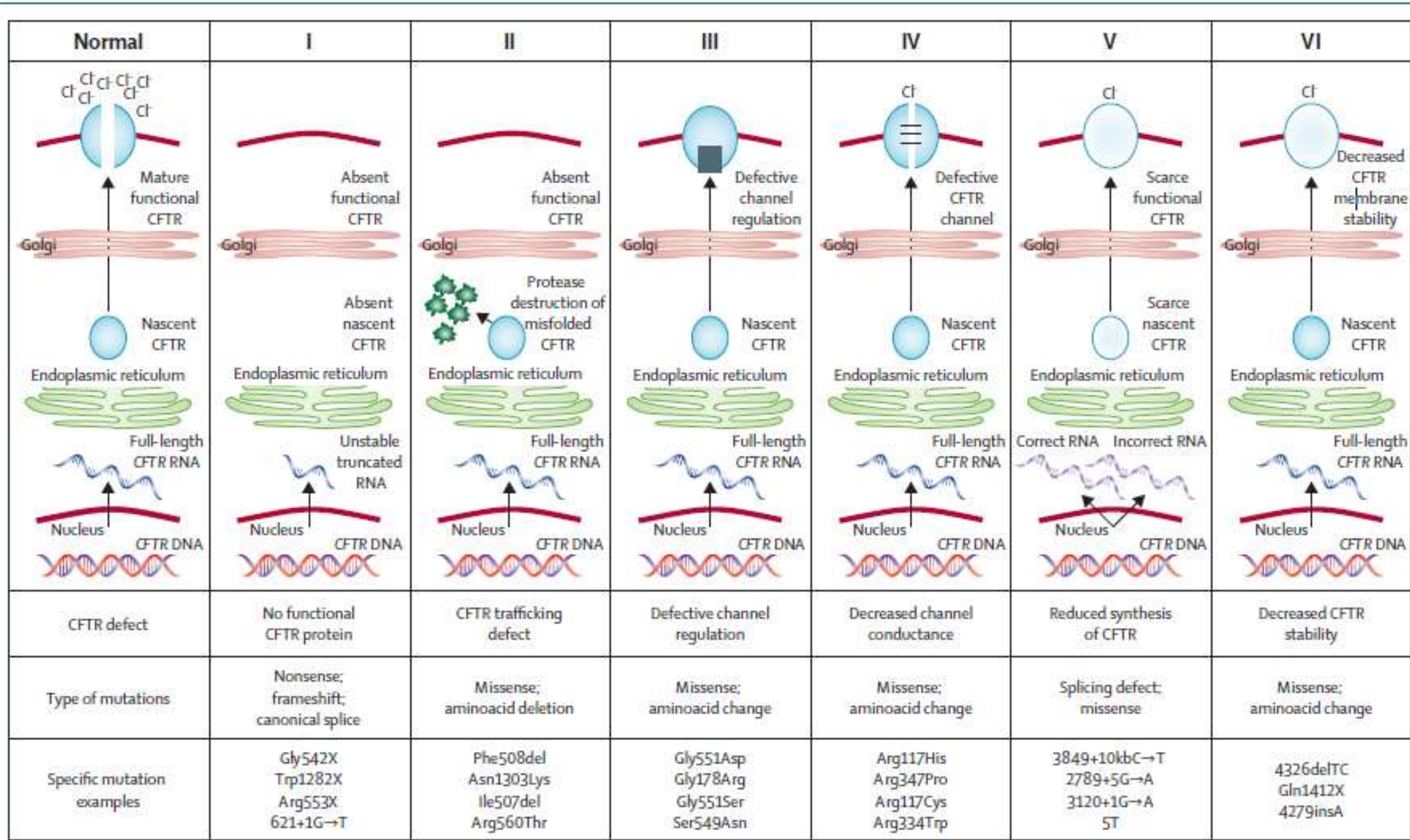
Schematic representation of the *CFTR* gene and its encoded polypeptide. $^{\Delta F}$508 refers to the site of the most common variant causing cystic fibrosis, also known as F508del.

Katkin et al, Uptodate 2021

Genetic changes in CFTR

- Phenotypic expression of disease varies widely, primarily as a function of the specific mutation (or mutations) present
- More than 2000 gene variants have been identified, many of which have been associated with disease causation.
- The most common pathogenic mutation is F508del.

J Stuart Elborn, Lancet 2016; 388(10059);2519-31



J Stuart Elborn, Lancet 2016; 388(10059);2519-31

- Diagnosis of CF requires clinical symptoms consistent with CF in at least one organ system and evidence of CFTR dysfunction (elevated sweat chloride, presence of two disease-causing mutations in the CFTR gene, or abnormal nasal potential difference [NPD]

Farrell et al,J Pediatr.2017 ;181S:S4-S15

Incomplete Phenotype

CFTR-related disorder — defined as clinical disease limited to only one organ system associated with some evidence of CFTR dysfunction that does not meet full genetic for a CF diagnosis

Clinical manifestations may include isolated obstructive azoospermia, chronic rhinosinusitis, chronic pancreatitis, or pulmonary disease in adulthood

Pathogenetic mechanism responsible for clinical disease without two CFTR gene mutations is unclear, but other genetic or environmental factors likely contribute to the risk in some cases

Farrell et al,J Pediatr.2017 ;181S:S4-S15

Diagnosis of CRMS or CFSPID

CRMS/CFSPID is a provisional diagnosis given to an asymptomatic infant with a positive newborn screening test for CF and either:

•An intermediate sweat chloride value (30 to 59 mmol/L) and fewer than 2 CF-causing gene mutations OR

•Normal sweat chloride results (<30 mmol/L) and 2 CFTR gene variants, at least 1 of which is not clearly categorized as CF-causing

Farrell et al,J Pediatr.2017 ;181S:S4-S15

- To date, more than 2000 different variants have been reported in the CFTR coding and flanking sequences, either pathogenic, neutral or of uncertain significance

- A number of studies on CF populations have demonstrated a high allelic heterogeneity worldwide.

- Although only five CF-causing variants account for about two-thirds of all CF alleles, e.g., p.Phe508del (66%), p.Gly542* (2.4%), p.Gly551Asp (1.6%), p.Asn1303Lys (1.3%), and p.Trp1282* (1.2%), the remaining third of alleles is substantially heterogeneous, with 10 to 20 less common CF-causing variants whose reported frequency reaches 0.1% worldwide, and a large fraction of rare or private variations.

- Moreover, the spectrum and frequency of individual CFTR variants vary relative to specific population groups and geographic locations

Bergougnoux et al, Expert Rev Respir Med 2018; 12(5):415-426

Classification of CFTR variants with regard to their clinical consequences

Variants may be grouped in four categories:

(A) loss-of-function variants that cause CF disease when paired together;

(B) variants that retain residual CFTR function and are compatible with milder phenotypes such as CFTR-RD, when paired with a CF-causing variant or paired together;

(C) variants with no clinical consequences;

(D) variants of unproven or uncertain clinical significance (VUS or VUCS).

Some variants that may cause CF disease or CFTR-RD depending on individuals (A/B) are now called “of varying clinical consequence”.

Castellani et al, J Cyst Fibros 2008;7(3):179-96

Case 1

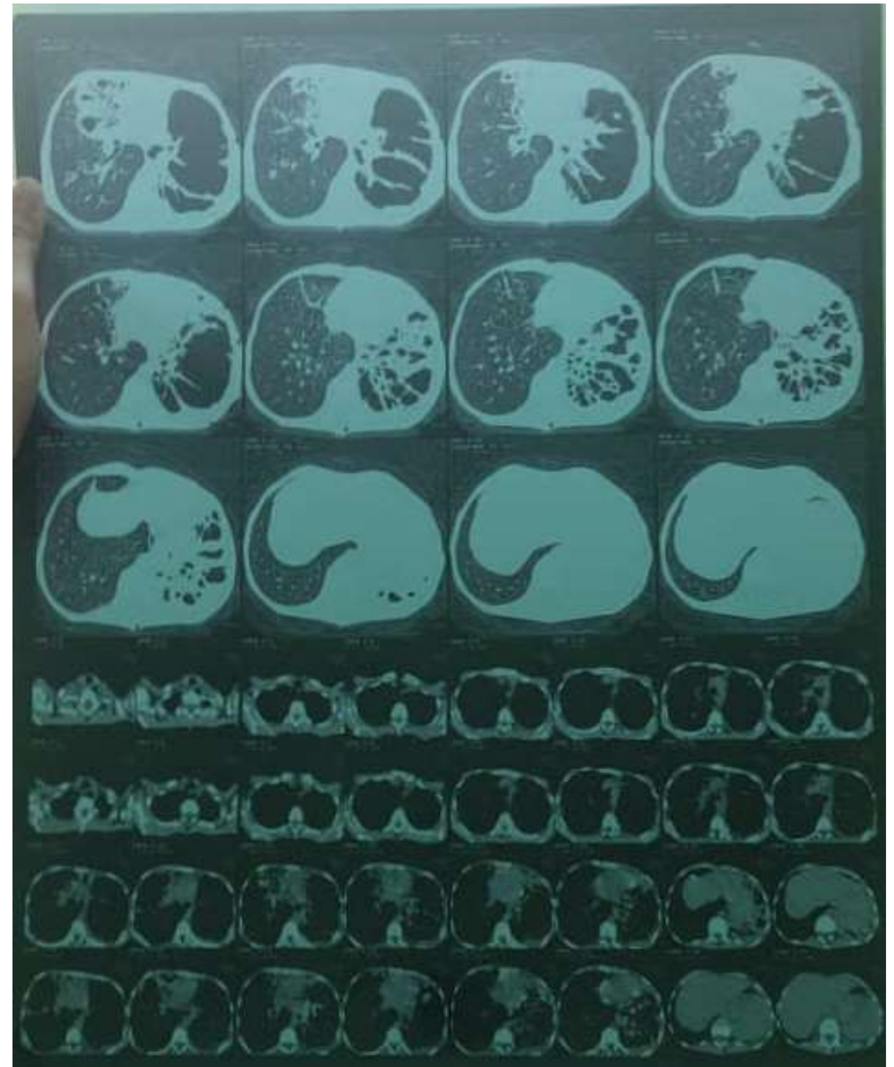
SM, 24 years, male, non smoker, no previous h.o PTB presented with complaints of

- •Cough with expectoration since 20 years;
- •Breathlessness since 1 years;
- •Loss of weight since 1 years;
- •Fever since 3 months

Courtesy Dr Inderpaul S Sehgal

Hb gm/dl	9.6
TLC cells/mm^3 (N/L/M/E)	17,400 (70/09/08/01)
PLT (cells/mm3)	404000

Courtesy Dr Inderpaul S Sehgal



Courtesy Dr Inderpaul S Sehgal

- Af IgE (kUA/L): 0.04
- A. f IgG (kUA/L): 62
- S. IgG/ IgA/ IgM: 2160/ 467/273

Courtesy Dr Inderpaul S Sehgal

- Sweat chloride levels: 39

Courtesy Dr Inderpaul S Sehgal

LIKELY COMPOUND HETEROZYGOUS PATHOGENIC VARIANTS CAUSATIVE OF THE REPORTED PHENOTYPE WERE DETECTED

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
CFTR **(+)** (ENST00000003084.11)	Intron 10	c.1393-1G>A **(3' splice site)**	Heterozygous	Cystic fibrosis	Autosomal recessive	Pathogenic
	Exon 4	c.349C>T **(p.Arg117Cys)**	Heterozygous			Pathogenic

Courtesy Dr Inderpaul S Sehgal

Case 2

- PS, 15 years male, presented with clinical indications of recurrent upper respiratory tract infections, fever, cough with sputum production, asthma and wheezing since childhood

- On examination, he was found to have bilateral bronchiectasis and crackles

Courtesy Dr Inderpaul S Sehgal

- Sweat chloride Levels: 19 and 22

Courtesy Dr Inderpaul S Sehgal

PATHOGENIC VARIANT CAUSATIVE OF THE REPORTED PHENOTYPE WAS DETECTED

Gene (Transcript) [#]	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
CFTR **(+)** (ENST00000003084.6)	Exon 8	c.1055G>A **(p.Arg352Gln)**	Homozygous	Cystic fibrosis	Autosomal recessive	Pathogenic

Courtesy Dr Inderpaul S Sehgal

Case 3

- LS, 25 years male, born of a non-consanguineous marriage, presented with clinical indications of recurrent mycobacterial infections, chronic pulmonary aspergillosis, pulmonary tuberculosis reactivation and AFB negative. He is suspected to be affected with immunodeficiency and has been evaluated for pathogenic variations

Courtesy Dr Inderpaul S Sehgal

- Sweat chloride Levels: 6

Courtesy Dr Inderpaul S Sehgal

Gene (Transcript) #	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
CFTR **(+)** (ENST00000003084.11)	Exon 22	c.3689T>C **(p.Ile1230Thr)**	Heterozygous	Cystic fibrosis	Autosomal recessive	Uncertain Significance

Courtesy Dr Inderpaul S Sehgal

Targeted therapy in CF

IVACAFTOR

•Oral drug; G551D mutation in at least one of their CFTR genes

•Indications — monotherapy for patients ≥4 months of age with eligible mutations if the patient is not otherwise eligible for dual or triple therapy

•Dosing for ivacaftor is as follows :

- 4 to <6 months and >5 kg body weight (and no hepatic impairment) – 25 mg packet taken orally every 12 hours
- six months to five years:
  - 5 kg to <7 kg body weight – 25 mg packet taken orally every 12 hours
  - 7 kg to <14 kg body weight – 50 mg packet taken orally every 12 hours
  - ≥14 kg body weight – 75 mg packet taken orally every 12 hours
- ≥6 years – 150 mg tablet taken orally every 12 hours

Uptodate 2021

- Ivacaftor should be taken with fat-containing foods.
- If packets are used, the dose should be mixed with a small amount (1 teaspoon) of soft food or liquid.
- Dose reductions are needed for patients with hepatic impairment or those who are taking drugs that are inhibitors of cytochrome P450 3A4 (CYP3A4) such as itraconazole, clarithromycin, or fluconazole
- Liver function tests are recommended prior to ivacaftor treatment, every three months for the first year, and then annually thereafter.
- Dosing should be interrupted if the alanine aminotransferase (ALT) or aspartate aminotransferase (AST) concentrations are more than five times the upper limit of normal.

Uptodate 2021

- Adverse effects: Elevation in liver enzymes, Non congenital lens opacities

Uptodate 2021

Population	• **Double blind RCT** • **12 years of age or older with cystic fibrosis and at least one G551D-CFTR mutation**
Intervention	150 mg of ivacaftor every 12 hours (n=84) placebo (n=83) for 48 weeks
Outcome	Primary end point: Estimated mean change from baseline through week 24 in the percent of predicted forced expiratory volume in 1 second (FEV(1)
Results	• Change from baseline through wk 24 in the percent of predicted FEV(1) was greater by 10.6 percentage points in the ivacaftor group than in the placebo group (P<0.001). • Effects on pulmonary function were noted by 2 wks, and a significant treatment effect maintained through wk 48 • Substantial improvements were also observed in the risk of pulmonary exacerbations, patient-reported respiratory symptoms, weight, and concentration of sweat chloride.

Ramsey et al, N Engl J Med 2011 ;365(18):1663-72

Other drugs approved for use:

- ELEXACAFTOR-TEZACAFTOR-IVACAFTOR
  - ELEXACAFTOR and TEZACAFTOR: Corrector
  - IVACAFTOR: Potentiator
- TEZACAFTOR-IVACAFTOR
- LUMACAFTOR-IVACAFTOR
  - Lumacaftor:Corrector

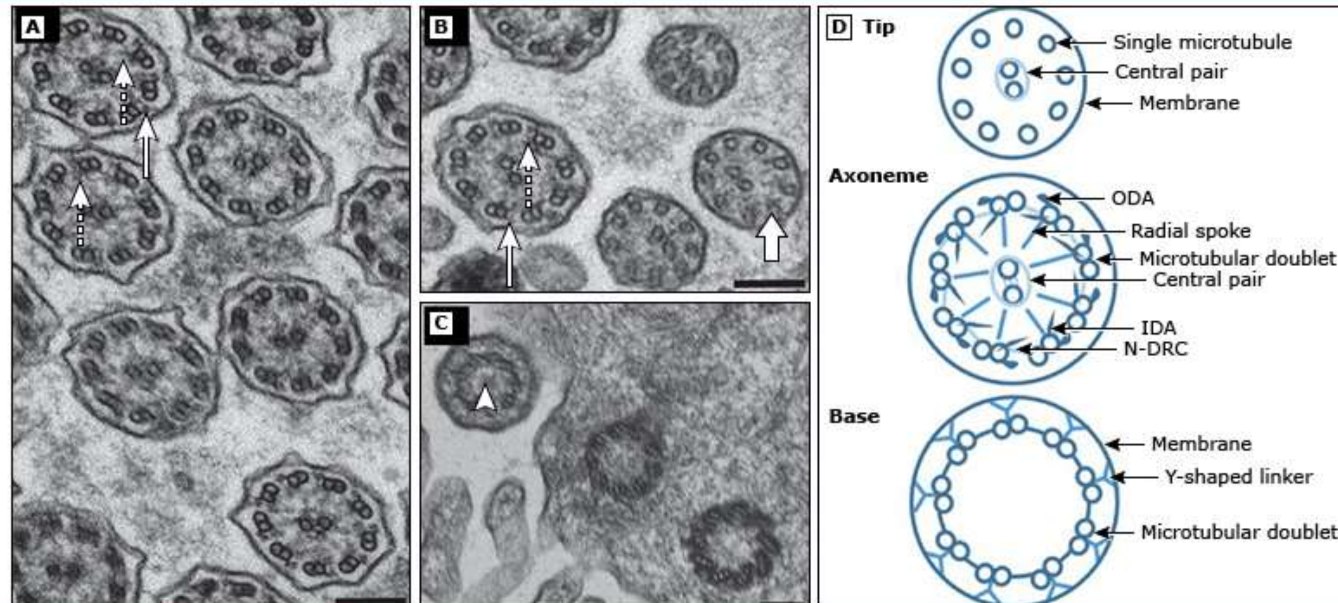
Uptodate 2021

Primary Ciliary Dyskinesia

- Syn: Immotile Cilia Syndrome, Kartagener Syndrome
- Characterized by congenital impairment of mucociliary clearance (MCC)
- Underlying cause is a defect of cilia in the airways, making them unable to beat (ciliary immotility), unable to beat normally (ciliary dyskinesia), or absent altogether (ciliary aplasia).

Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

Electron microscopy of normal ciliary ultrastructure in cross-section



Electron micrographs showing normal ciliary ultrastructure in cross section at (A) the core of the ciliary axoneme with a 9+2 microtubular arrangement; (B) the tip of the cilium with single microtubules (thick arrow); and (C) the base of the axoneme showing absent central pair. Panel (D) gives a diagrammatic representation of normal ciliary ultrastructure in cross sections at the tip, central axoneme, and base. The narrow arrows depict the ODAs and the dashed arrows depict the IDAs. The thick arrow indicates the tip of the cilium with single microtubules (these are a normal part of ciliary ultrastructure and should not be assessed for diagnosis of Class 1 or Class 2 defects). The arrowhead indicates where there is no central pair. Microtubular doublets are often linked to the ciliary membrane with Y-shaped linker, best seen in panel C.

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Genetics of PCD

- Genetically heterogeneous
- Predominantly autosomal recessive disorder
- Caused by biallelic pathogenic mutations in one of the many identified PCD causative genes (39 till date)
- 27 known genes accounting for 50%–60% of PCD cases

Table 1. Genes with mutations linked to primary ciliary dyskinesia. ODA—outer dynein arms, IDA—inner dynein arms.

Gene	Structural Defect
Abnormalities in dynein proteins	
DNAI1	ODA defect (+/− IDA)
DNAH5	ODA defect (+/− IDA)
DNAH11	Beat abnormalities (normal structure)
DNAI2	ODA defect
DNALI1	ODA defect
TXNDC3	ODA defect
ARMC4	ODA defect
Genes coding for proteins responsible for assembly or transport of axonemal proteins	
KTU	ODA and IDA defects
LRRC50	ODA and IDA defects
DNAAF3	ODA and IDA defects
CCDC39	ODA and IDA defects
CCDC40	Axone disorganisation and IDA defect
CCDC103	ODA and IDA defects
CCDC114	ODA defect
HEATR2	Absent ODA
CCDC65	Cilial vibration, normal structure
ZMYND10	Absent ODA + IDA
SPAG1	Absent ODA + IDA
C21orf59	Absent ODA + IDA
Central pair abnormalities	
RSPH9	Central pair defects
RSPH4A	Central pair defects
RSPH1	Central pair defects
HYDIN	Central pair defects
Nexin-dynein complex defects	
DRC CCDC164	Nexin link missing
CCDC65	Beat abnormalities
Genes causing PCD with associated syndromes	
OFD1	Unknown
RPGR	Variable

Collins et al, J Clin Med.2014;3(2):491-503

- Each PCD diagnostic test carries limitations, and those tests dependent on respiratory mucosal (ciliary) biopsy (TEM, ciliary beat frequency [CBF], and HSVM) are encumbered by the need for on-site high-quality specimen sampling, processing, and analysis

- Widespread lack of local expertise and resources in ciliary biopsy testing has made molecular genetic testing an attractive alternative

Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

- Role of genetic testing for PCD diagnosis is evolving with the increasing availability of extended panel tests (>12 genes)

- 2018 ATS guidelines advise use of extended panel genetic testing over TEM ciliary testing and/or standard genetic panel test testing for PCD diagnosis <u>(conditional recommendation, very low certainty in the overall evidence)</u>

Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

- Among 205 children (age 0 to 18 years) who were thought to have definite PCD, a standard genetic panel identified 138 pathogenic variants, while an extended panel identified 26 additional genetic variants.

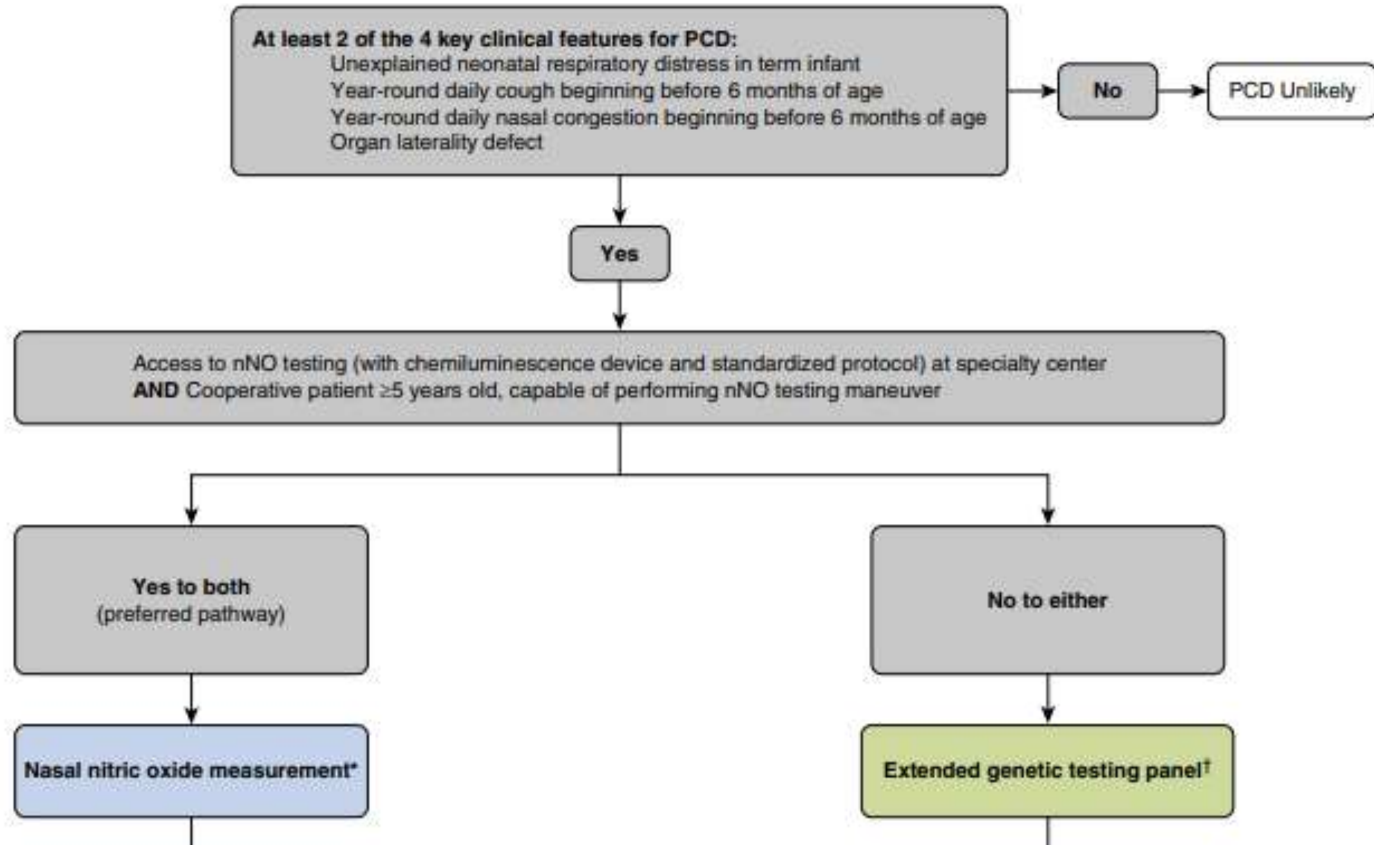
- Sensitivity for the diagnosis of “definite PCD” by an extended genetic panel (>12 genes) in this study was 80%, indicating that 20% of patients were diagnosed by TEM alone (without a causative PCD gene found).

Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

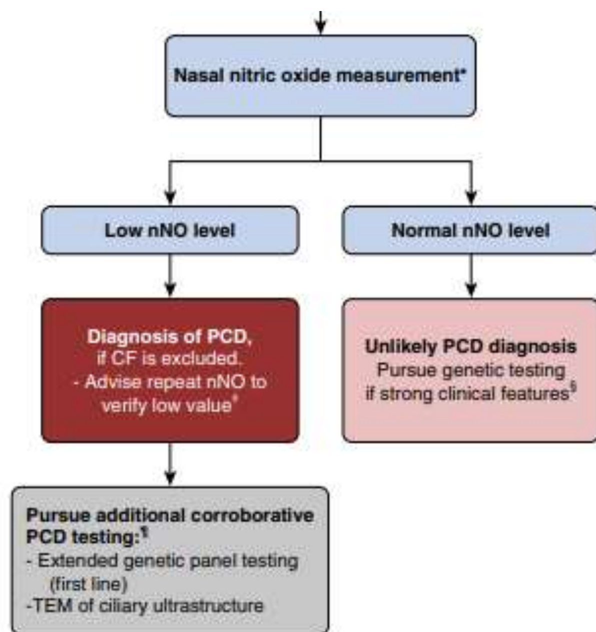
- Also, in four case series studies, sensitivities of each published extended genetic panel were calculated by the author compared with the prespecified reference standard
- Sensitivity of the genetic panel test improved with the increasing number of genes tested for PCD
- Sensitivities were 71.9% when testing 12 genes, 73.3% when testing 19 genes, 54.8% when testing 24 genes, 80% when testing 26 genes, and 93.9% when testing 32 genes with deletion/duplication analysis.
- The lower sensitivity of 54.8% with the 24-gene panel may be due to differences in population stratification

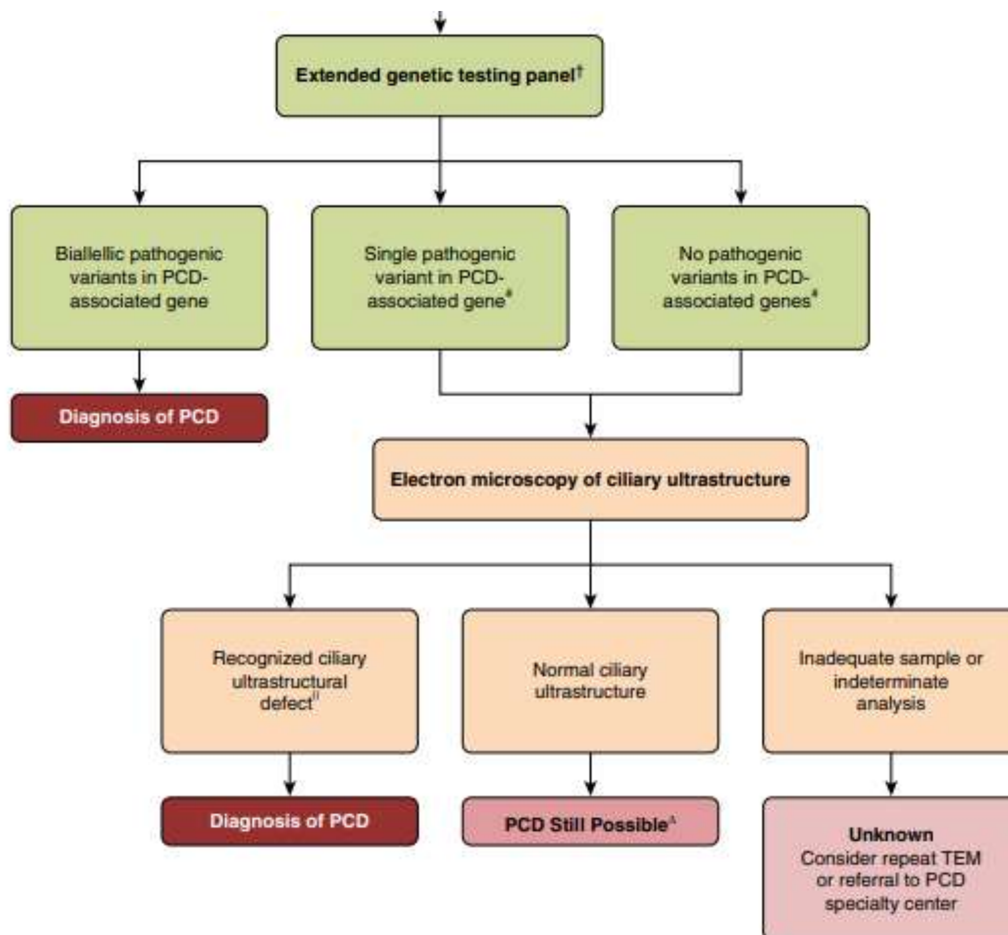
Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

Diagnostic algorithm: ATS 2018



Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39





Shapiro et al, Am J Respir Crit Care Med. 2018;197(12):e24-e39

Population	**• Multicentre, double-blind,** **• RCT Phase 3** **• Patients with diagnosis of PCD, aged 7-50 years old, and predicted FEV1 greater than 40%** **• n = 90**
Intervention	Azithromycin 250 mg or 500 mg as tablets according to bodywt (</≥ 40 kg) or identical placebo, 3x a week for 6 months
Outcome	Primary end point: Number of respiratory exacerbations over 6 months
Results	A zithromycin maintenance therapy for 6 months was well tolerated and halved the rate of respiratory exacerbations. Loose stools or diarrhoea more common in the azithromycin group than in the placebo group(11 [23%] vs two [5%])

BEST-CILIA. Helene E Kobbernagel et al
Lancet Respir Med 2020 May;8(5):493-505

Alpha-1-antitrypsin deficiency

- Whether alpha-1-antitrypsin (AAT) deficiency per se is associated with bronchiectasis is controversial

- One study examined 74 patients with the Protease Inhibitor ZZ (PiZZ) genotype – the most common genotype that results in frank AAT deficiency – and found that 70 (95%) had bronchiectatic changes on CT scan involving an average of 3.7 lobes and 20 (27%) had "clinically significant bronchiectasis," defined as bronchiectasis affecting ≥ 4 lobes and "regular sputum production

Chan et al, Respiratory Medicine, 2019

- Since the “Z” isoform of AAT may polymerize in the lung and act as a chemoattractant for neutrophils, which can then release inflammatory mediators and elastase that incite airway damage, this is a plausible mechanism by which an abnormal AAT protein may predispose to bronchiectasis

- Caution must be exercised in ascribing bronchiectasis to AAT deficiency as chronic obstructive pulmonary disease (COPD) itself may be associated with bronchiectasis

- Another indirect mechanism for AAT deficiency-associated bronchiectasis is that anomalous AAT may predispose to NTM infection, which can secondarily cause bronchiectasis

Chan et al, Respiratory Medicine, 2019

- Only the presence of lower lobes emphysema or early onset airways' obstruction could represent an indication to screen for AATd
- Undefined role for genetic testing

Polverino et al, Eur. Respir. J. 2017

Immunodeficiency

- Humoral immune deficiencies account for approximately 70% of all primary immune deficiencies and the majority of primary immune deficiency-associated causes of bronchiectasis

- Notable primary immunodeficiency associated with bronchiectasis include:
  - Common variable immune deficiency, the most commonly diagnosed immune deficiency characterised by substantial reductions in IgG and sometimes reductions in IgA or IgM, or both, and presentation later in life with recurrent pyogenic sinopulmonary infections

  - X-linked agammaglobulinaemia is caused by mutations in the Bruton's tyrosine kinase gene and can result in a profound humoral immune deficiency

Flume et al, Lancet 2018;392(10150):880-90

Genetic defects associated with primary humoral immunodeficiencies

- Other genetic primary immunodeficiencies can manifest with bronchiectasis including those associated with phosphoinositol-3-kinase (PI3K) gain-of-function (activated PI3K delta syndrome or APDS), hyper-IgE syndrome (STAT3 loss-of-function, DOCK8 and Tyk2 deficiency), CTLA4 haploinsufficiency, LRBA deficiency.

Chan et al. Respiratory Medicine: X. 2019;1:100006.

Recent diagnostic criteria for CVID

Table 1. Proposed definition of common variable immune deficiency (CVID).

A Must meet all major criteria
- Hypogammaglobulinaemia: IgG below 5 g/l for adults [57]
- No other cause identified for immune defect [52]
- Age > 4 years [21]

B Clinical sequelae directly attributable to *in-vivo* failure of the immune system (one or more criteria)
- Recurrent, severe or unusual infections
- Poor response to antibiotics
- Breakthrough bacterial infections in spite of prophylactic antibiotics
- Infections in spite of immunization with the appropriate vaccine, e.g. HPV disease
- Bronchiectasis and/or chronic sinus disease
- Inflammatory disorders or autoimmunity [58]

C Supportive laboratory evidence (three or more criteria)
- Concomitant deficiency or reduction of IgA (<0·8 g/l) and/or IgM (<0·4 g/l) [4,56]
- Presence of B cells but reduced memory B cell subsets and/or increased CD21 low subsets by flow cytometry [59]
- IgG3 deficiency (<0·2 g/l) [60,61]
- Impaired vaccine responses compared to age-matched controls
- Transient responses to vaccines compared to age-matched controls [62]
- Absent isohaemagglutinins (if not blood group AB) [32]
- Serological support for autoimmunity in section B, e.g. positive Coombs' test
- Sequence variations of genes predisposing to CVID, e.g. *TACI*, *BAFFR*, *MSH5*, etc. [11,63]

D Presence of any one of relatively specific histological markers of CVID (not required for diagnosis but presence increases diagnostic certainty)
- Lymphoid interstitial pneumonitis [64]
- Granulomatous disorder [65,66]
- Nodular regenerative hyperplasia of the liver [67,68]
- Nodular lymphoid hyperplasia of the gut [69]
- Absence of plasma cells on gut biopsy [70,71]

Meeting criteria in categories ABC or ABD indicates probable CVID. Patients meeting criteria ABC and ABD should be treated with intravenous immunoglobulin/subcutaneous immunoglobulin (IVIG/scIG). Patients meeting criteria A alone, AB or AC or AD but not B, are termed possible CVID. Some of these patients may need to be treated with IVIG/scIG. Patients with levels of immunoglobulin (Ig)G > 5 g/l, not meeting any other criteria are termed hypogammaglobulinaemia of uncertain significance (HGUS). A diagnostic algorithm is shown in Fig. 1. HPV: human papillomavirus.

Ameratunga et al. Clin Exp Immunol 2013;174(2):203-11.

- Options: targeted sequencing, whole exome sequencing and whole genome sequencing

Heimall et al, J Allergy Clin Immunol Pract .2019;7(3):833-38

- Autoimmune diseases, notably rheumatoid arthritis and inflammatory bowel disease, are associated with bronchiectasis

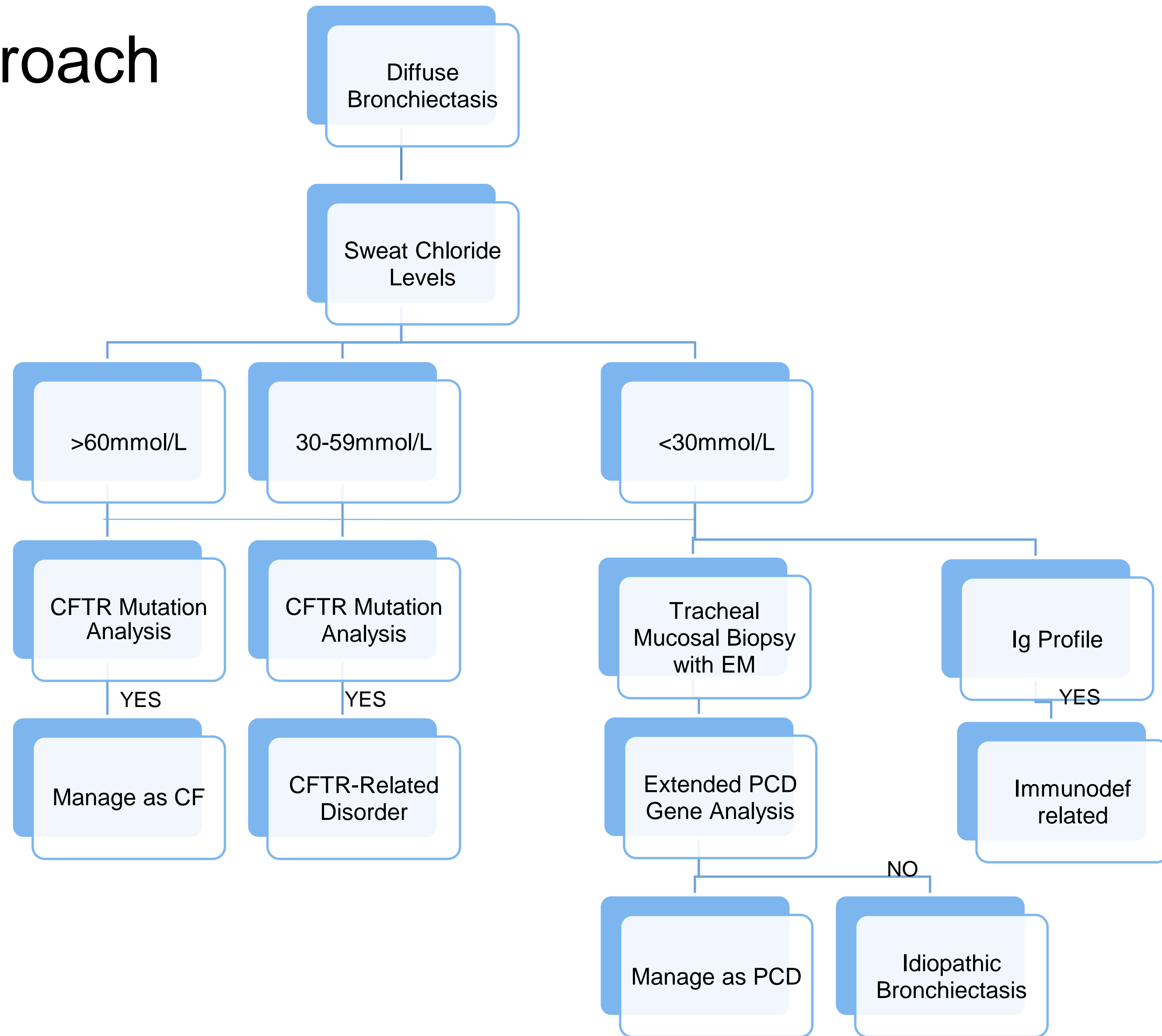
- For inflammatory bowel disease, associated genetic risk loci have been reported including NOD2, ATG16L1, IRGM, IL23R, TNFSF15, and HLAD-QA1

- A study also identified allergic bronchopulmonary aspergillosis-associated single nucleotide polymorphisms in TLR3, IL4R, and IL13.

- Further investigation is needed to establish whether these loci could be helpful in elucidating pathways, evaluation and pointing to more specific therapies

Flume et al, Lancet 2018;392(10150):880-90

An Approach



Courtesy Dr Inderpaul S Sehgal

- Genetic testing has critical role in understanding, detecting and managing the disease

- It continues to evolve and new methodologies are being developed

- It has already been in clinical practice and may be utilised more commonly in clinical practice