Molecular Aspects of Cystic Fibrosis

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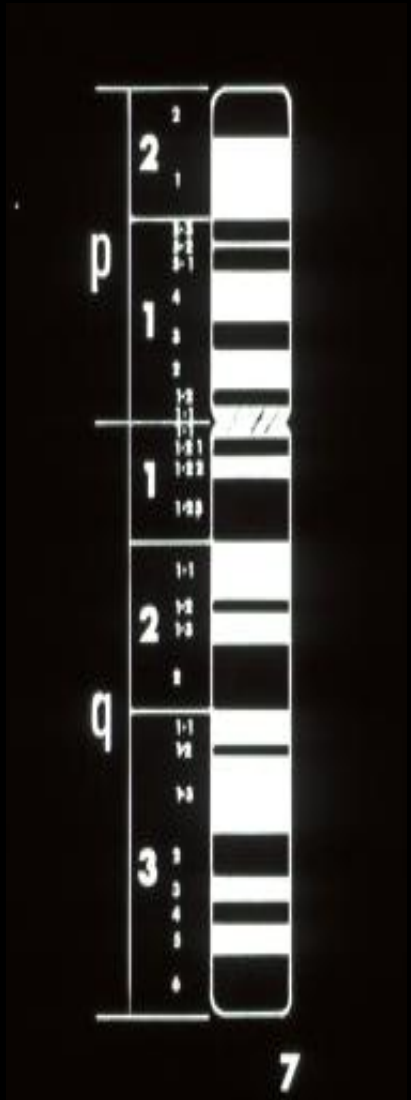
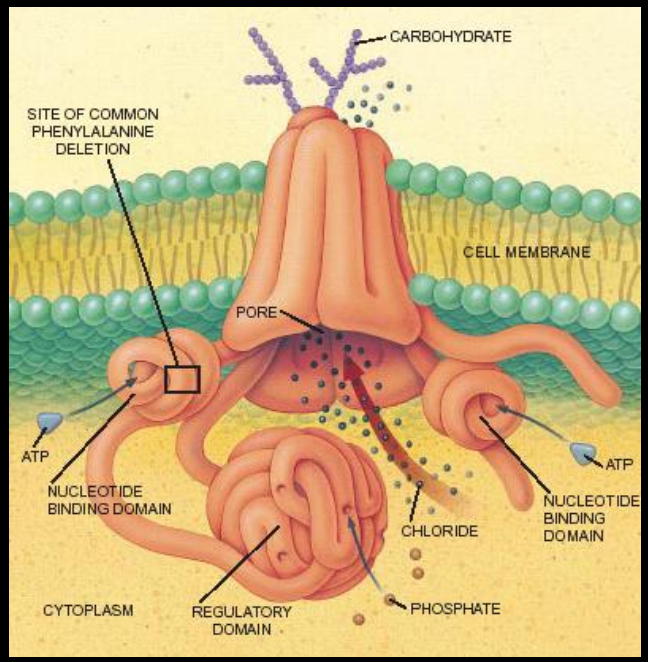
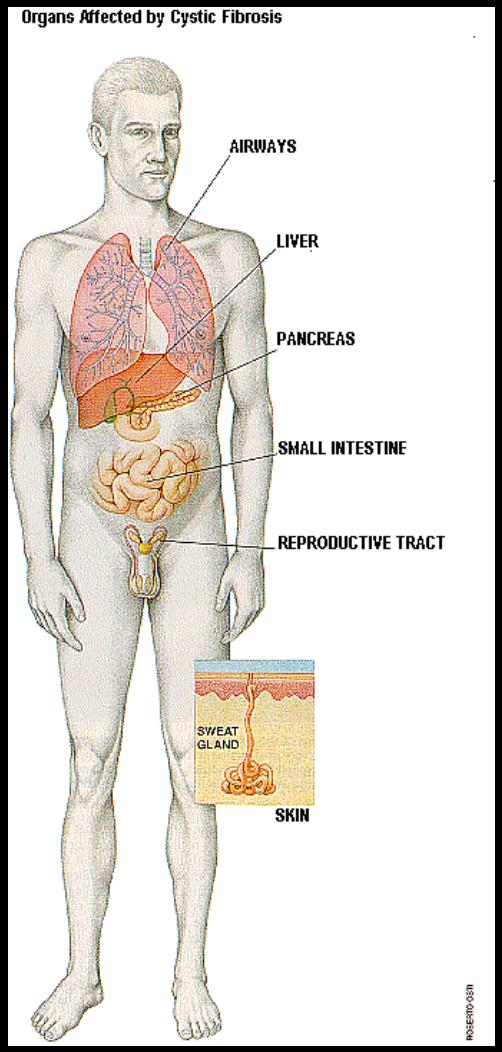
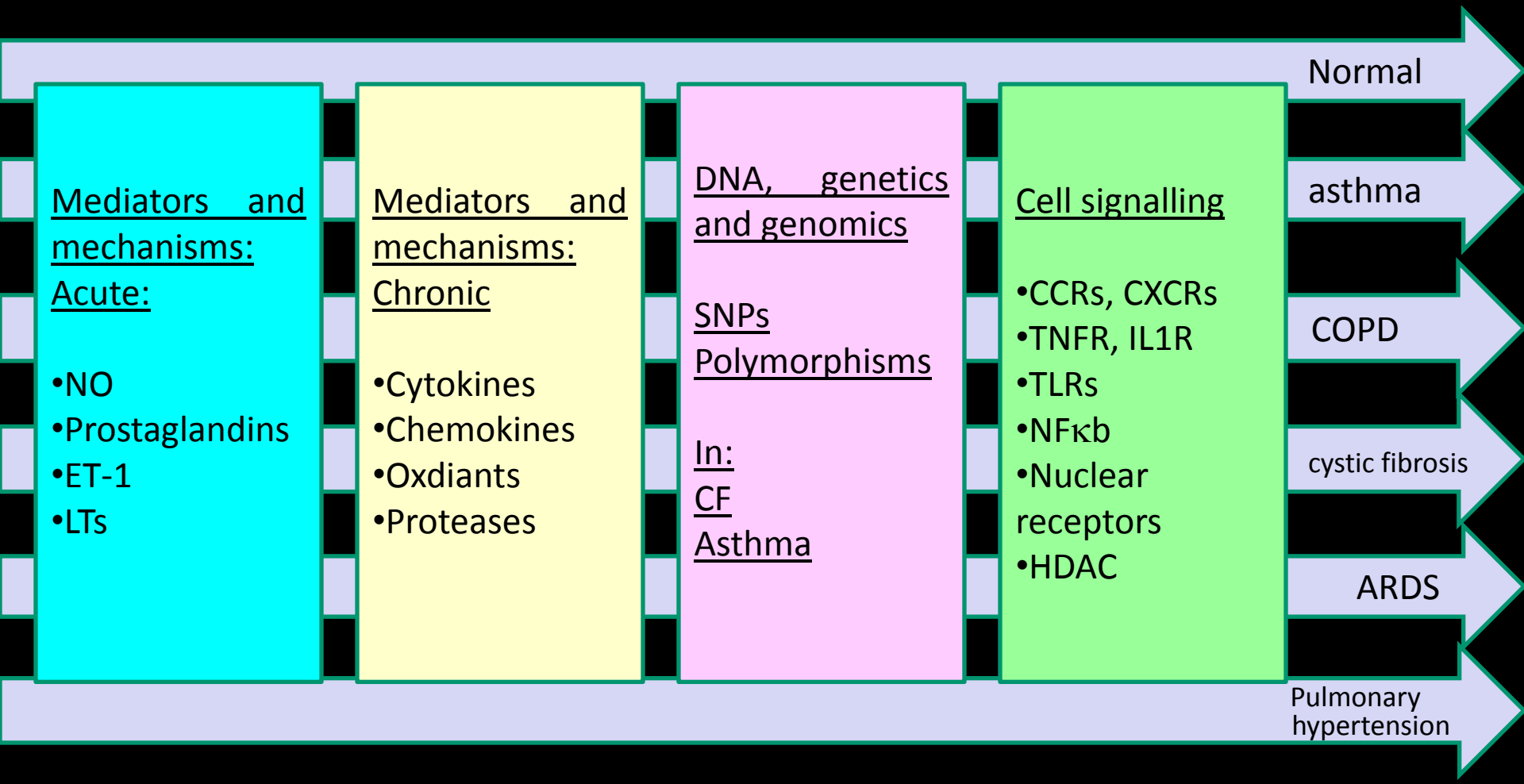




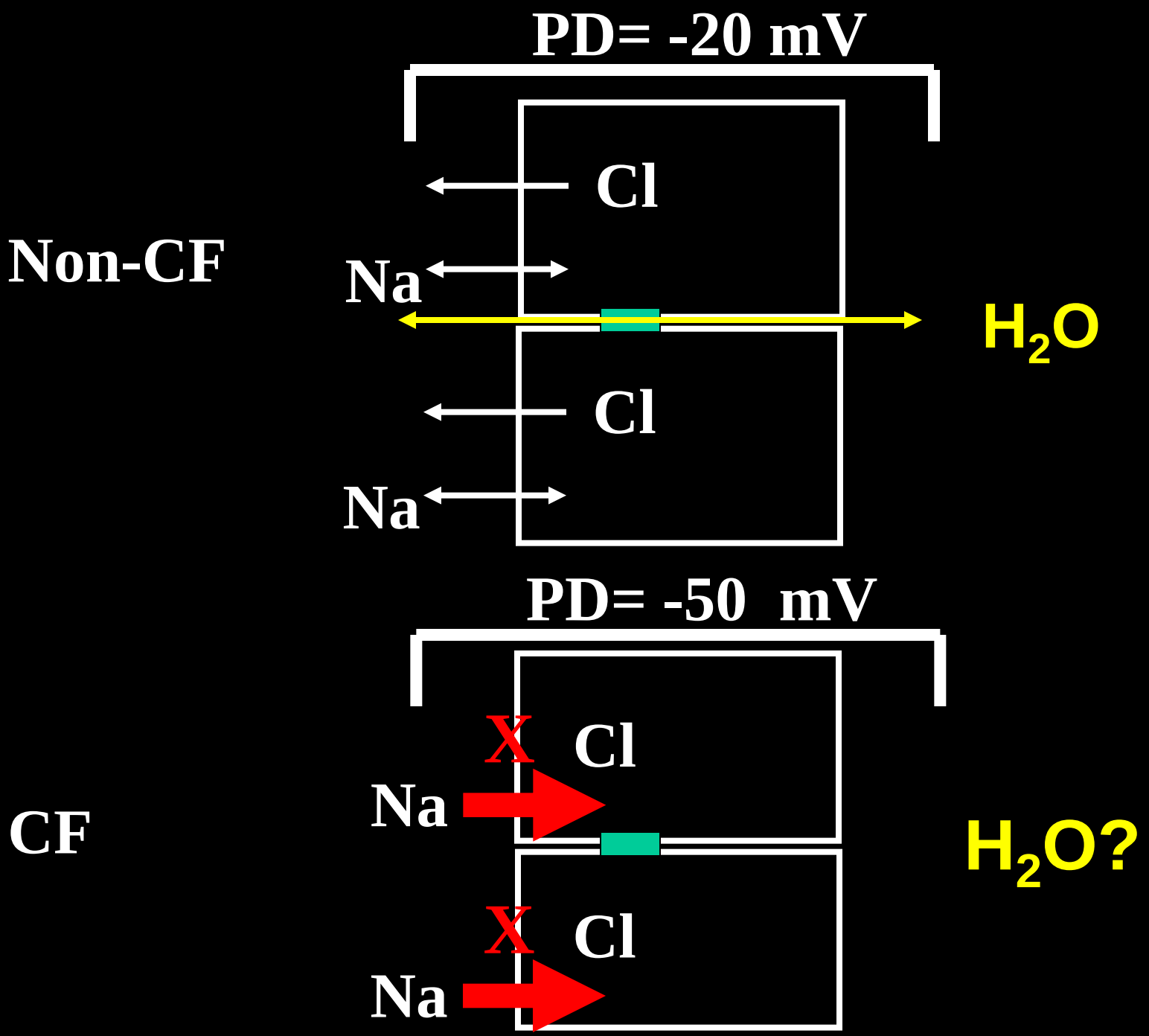


Module 2: Molecular Cell Biology of the Lung



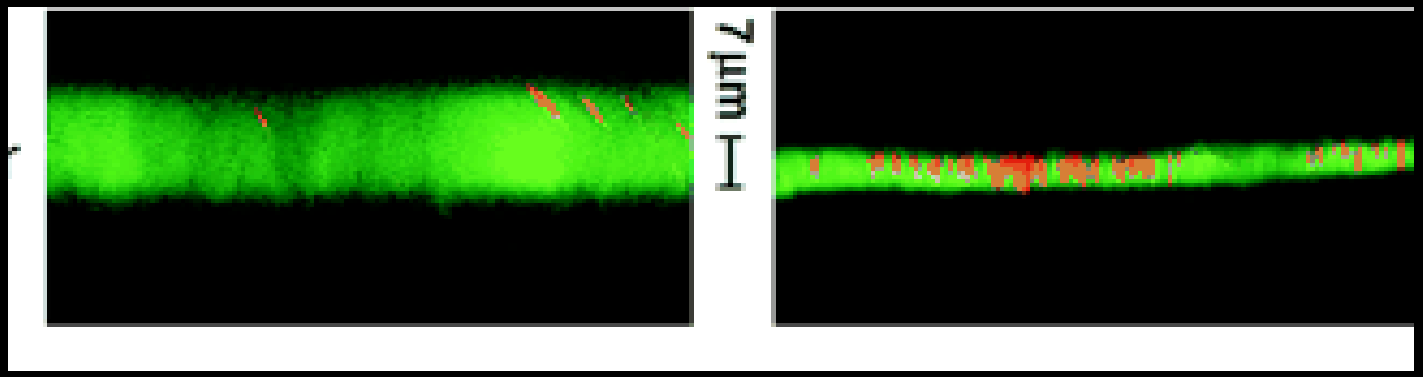
Learning Objectives

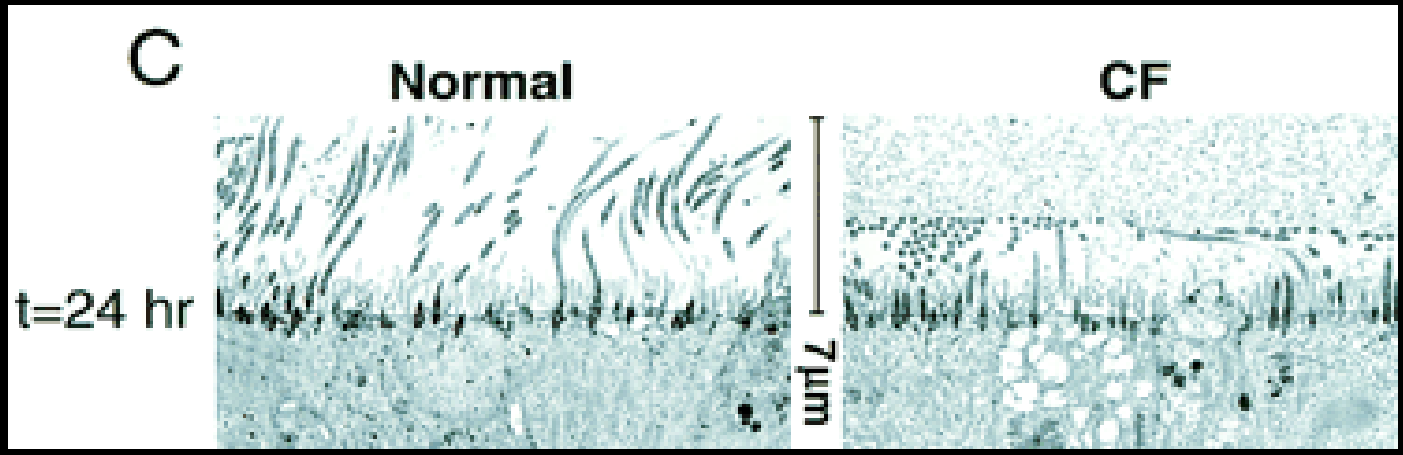
1. CF Genetics
2. Classes of CFTR mutations
3. Other factors affecting disease
4. Mutation specific treatments
5. (Animal models)


PD= -20 mV
Non-CF
Cl
Na
H2O
Cl
Na
PD= -50 mV
CF
X
Cl
Na
H2O?
X
Cl
Na

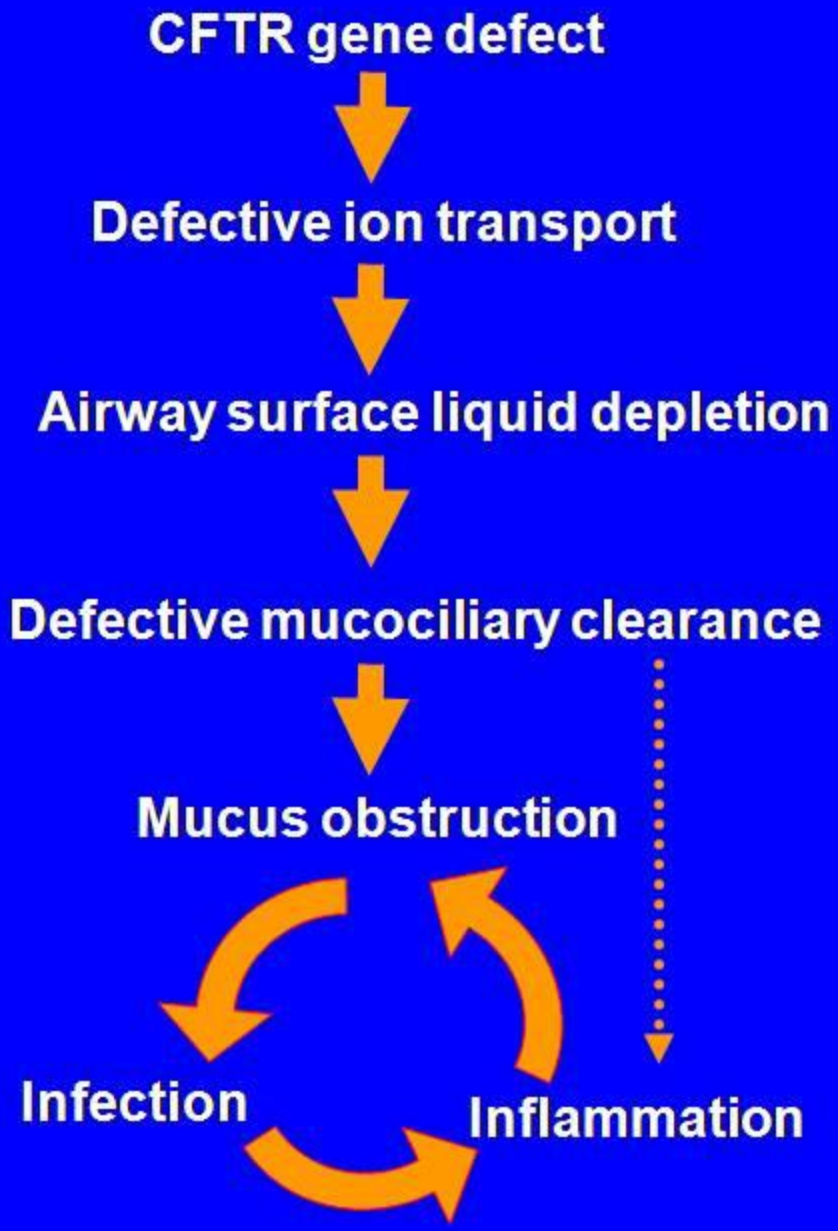
Normal

CF

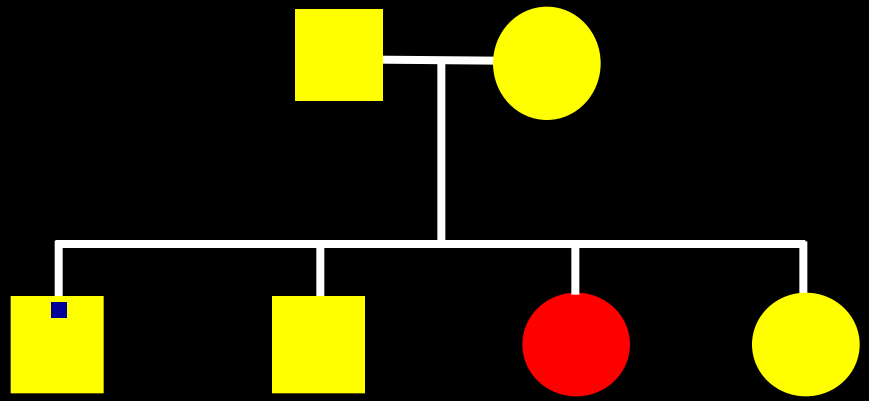




CF Pathophysiology



Autosomal Recessive Disease



Ethnic Background	Risk of CF Mutation	Risk of Child with CF
Caucasian	1 in 29	1 in 3300
Ashkenazi Jewish	1 in 29	1 in 3300
Hispanic	1 in 46	1 in 8000-9000
African-American	1 in 65	1 in 15,300
Asian	1 in 90	1 in 32,100

Heterozygote Advantage

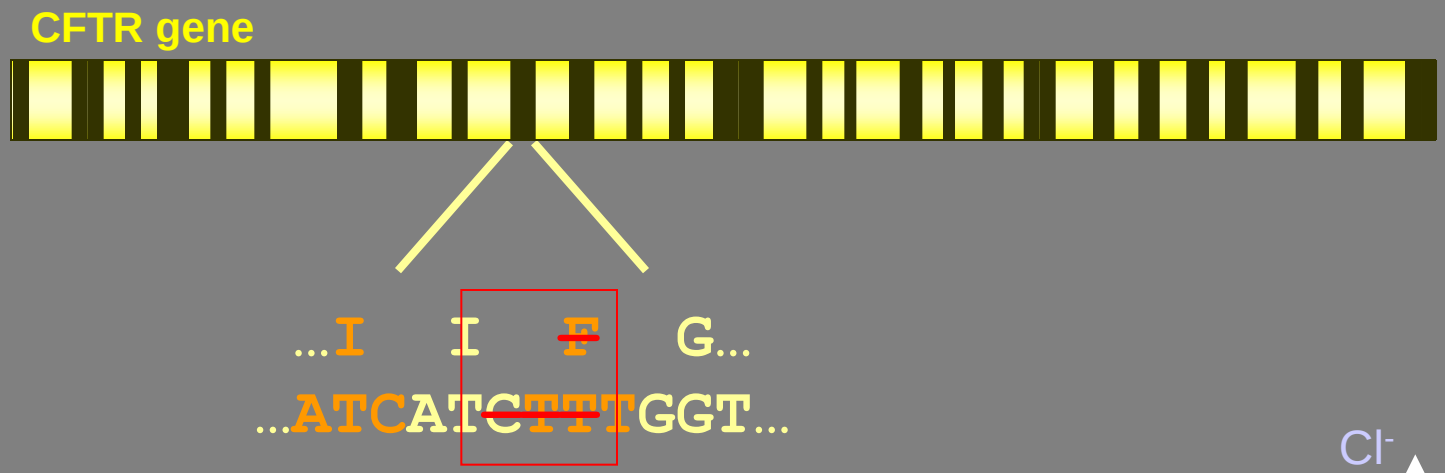
Examples:

1547 mutations currently identified

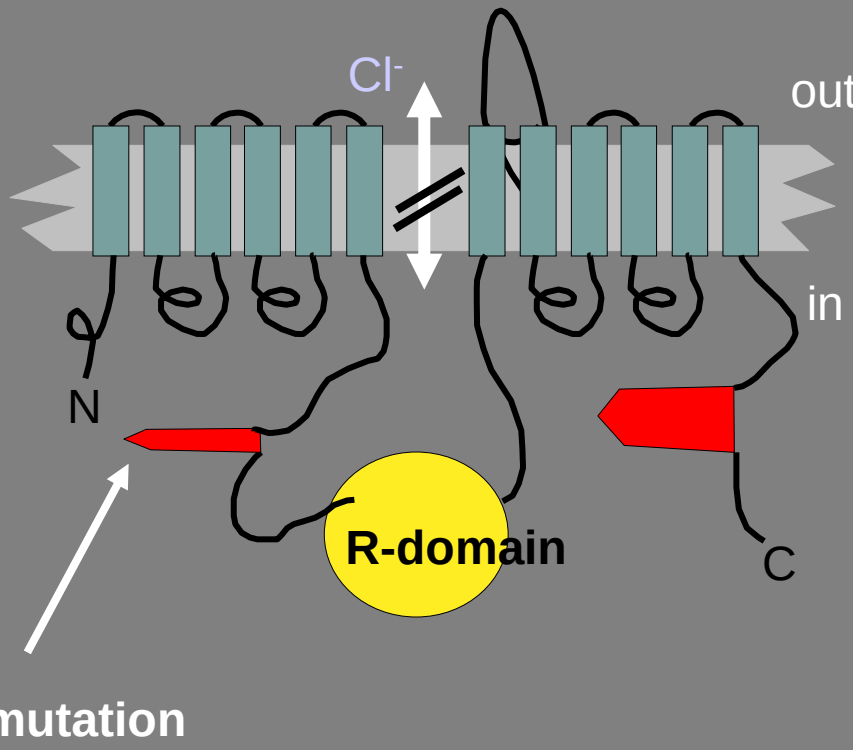
Mutation Type	Frequency %
Missense	42
Frameshift	16
Splicing	13
Nonsense	10
In frame in/del	2
Large in/del	3
Promotor	0.5
Sequence variation	13.5

Varying levels of residual CFTR function

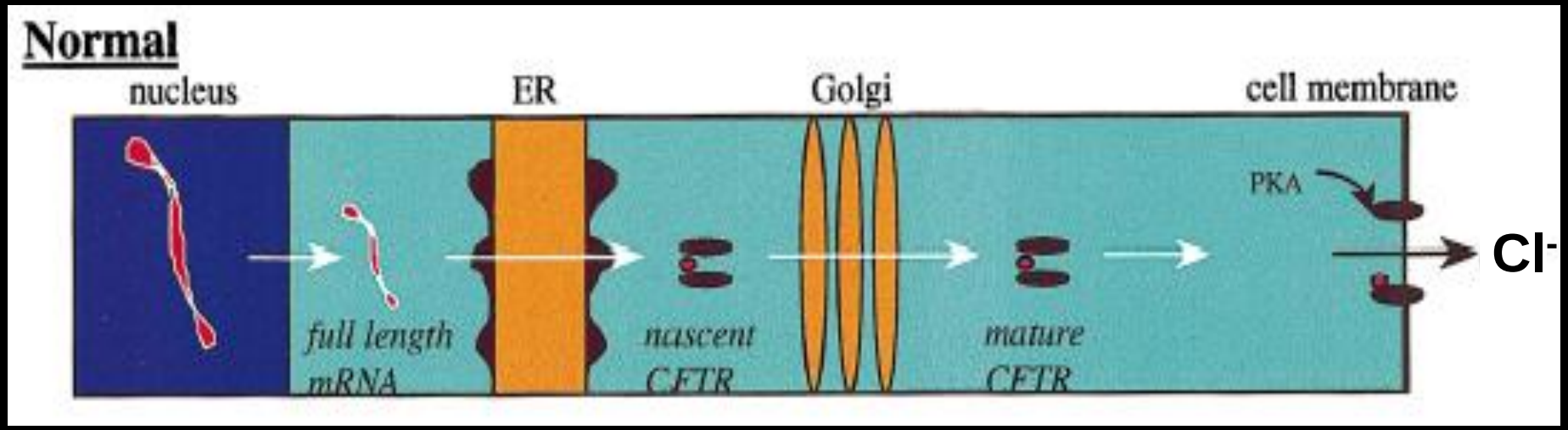
ΔF508 Mutation



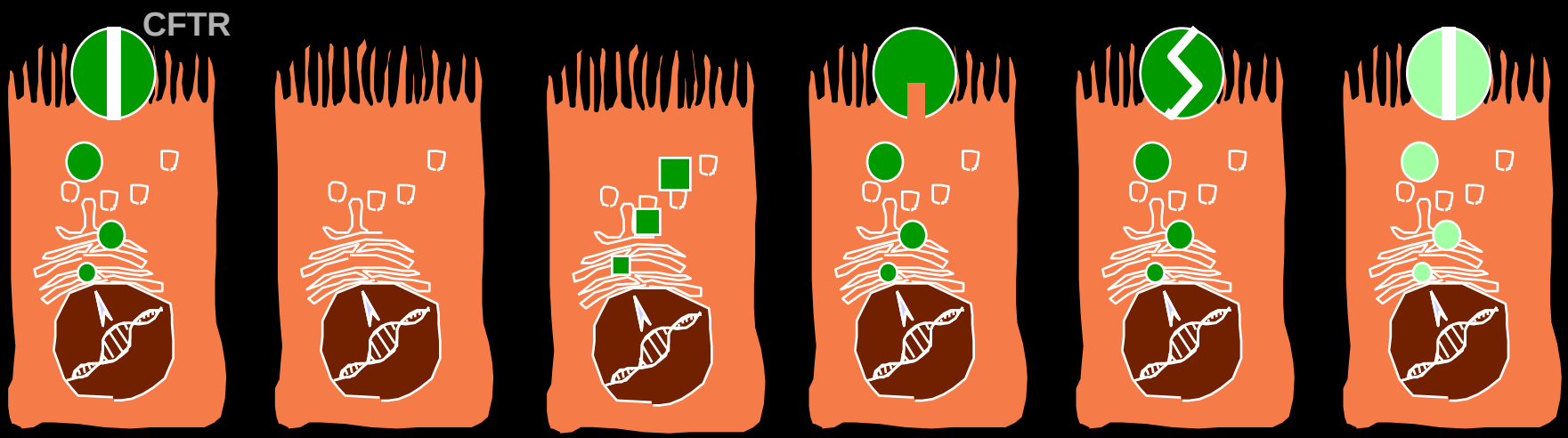
Frame-shift (exon 10)



CFTR Mutations Fall Into Five Functionally Separable Classes



Molecular Consequences of CFTR Mutations



ΔF508

Pancreas disease

I, II, III = severe mutation

IV, V = mild mutation

Causasian Population

Mutation	Prevalence (%)
DF508	79
G551D	2.17
R117H	0.7
621+1 (G>T)	0.5
G542X	0.5
N1303K	0.35
1717-1 (G>T)	0.28
R1162X	0.14
R553X	0.14
3849+10KB (G>T)	0.07
R334W	0.07
W1282X	0.07
TOTAL	84

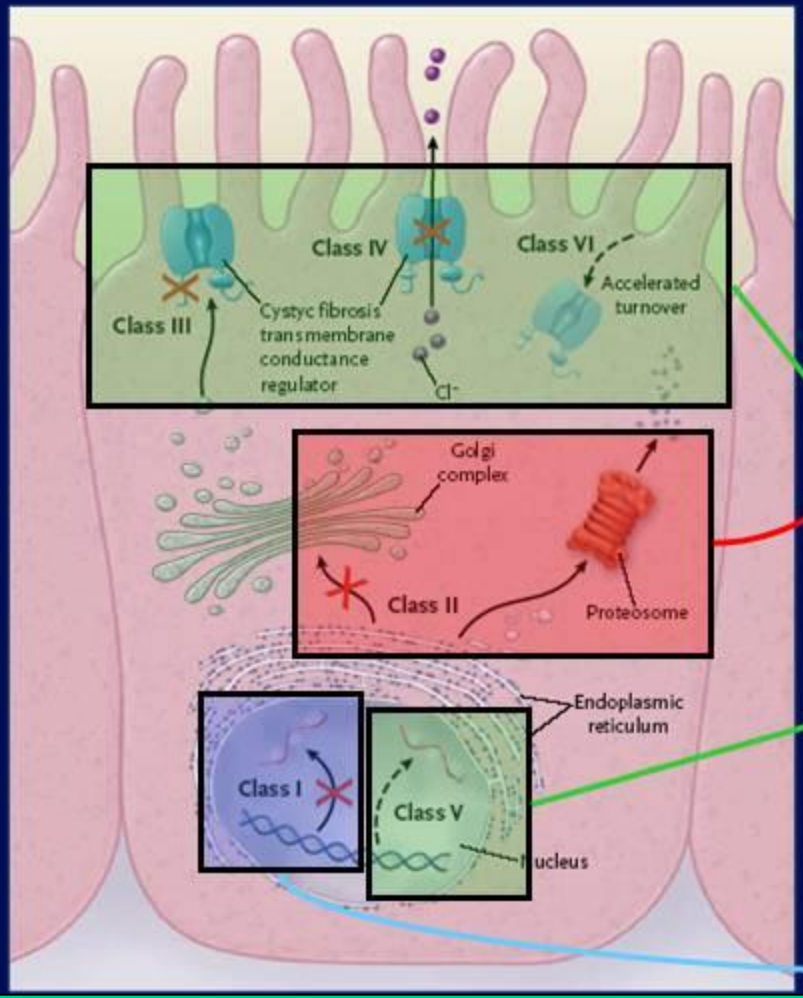
All other mutations << 0.05%

Ashkinazi Jews

DF508 27%

W1282X 51%

Therapeutic Approaches by Class



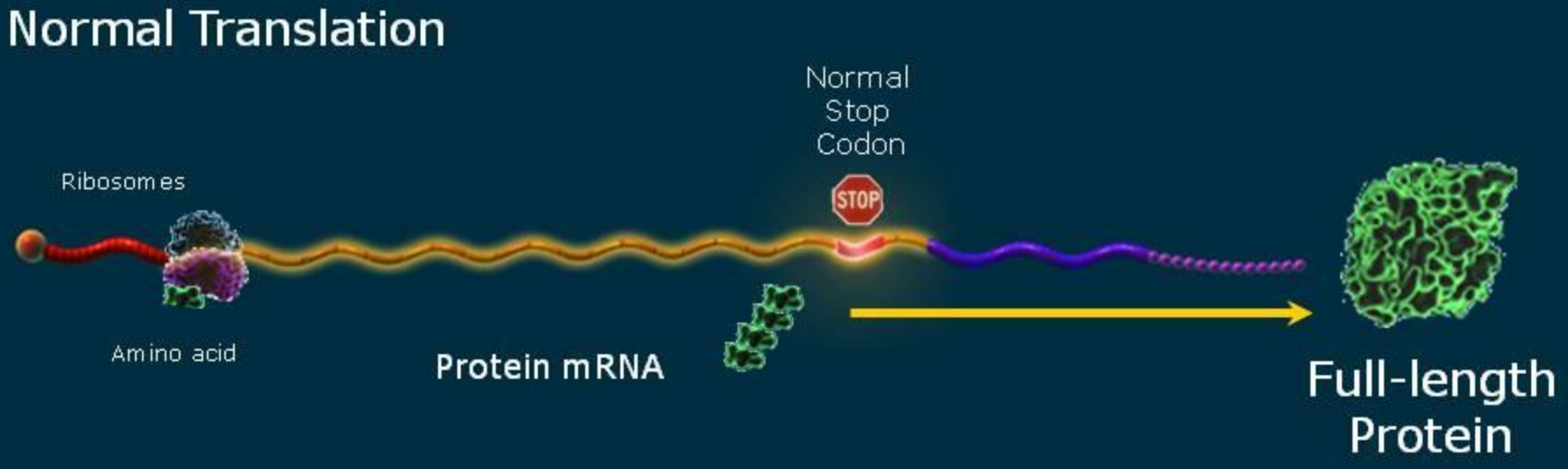
F508del CFTR Processing Corrector

CFTR Potentiators

Translational Readthrough

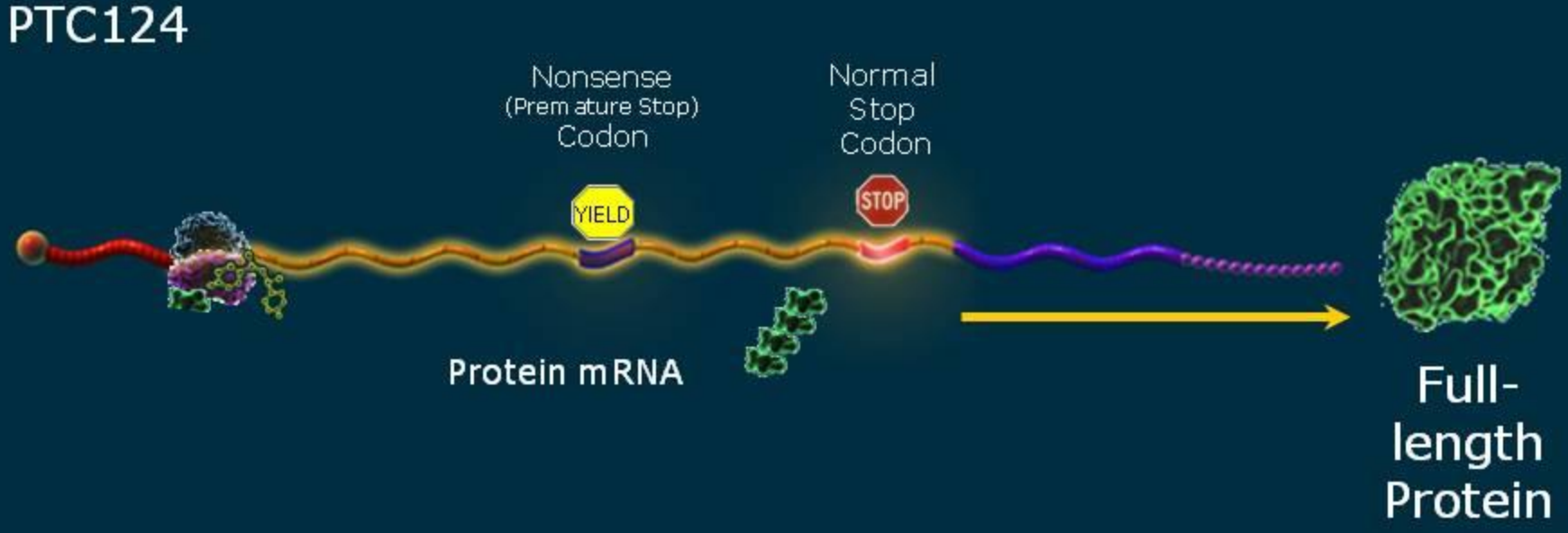
adapted from Rowe et al

Normal Flow of Genetic Information Results in Full-Length Protein Production



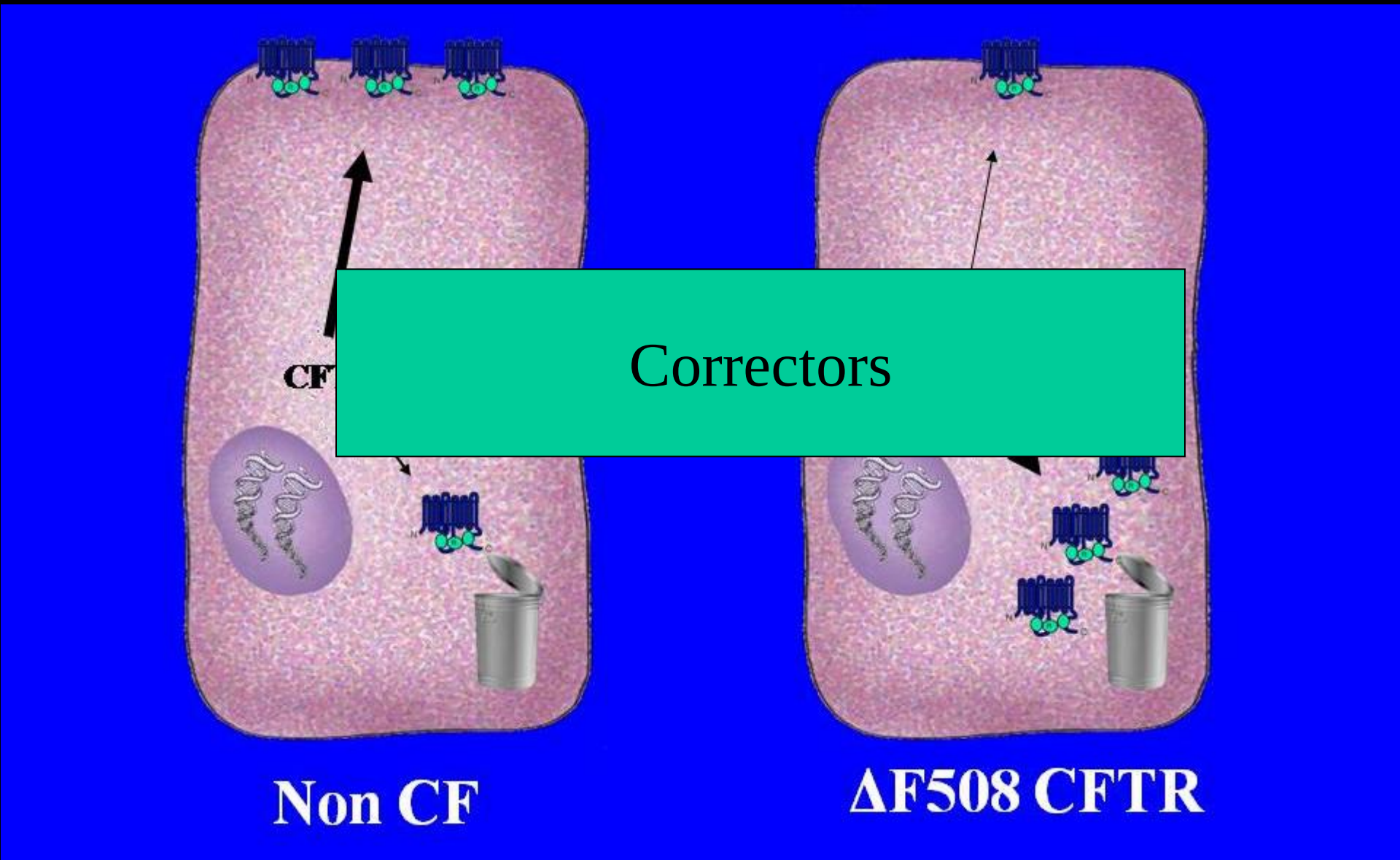
Nonsense Mutation Halts the Flow of Genetic Information and Results in Truncated Protein Production

PTC124 Has Been Designed to Overcome Nonsense Mutations



Overcoming Class II mutations:

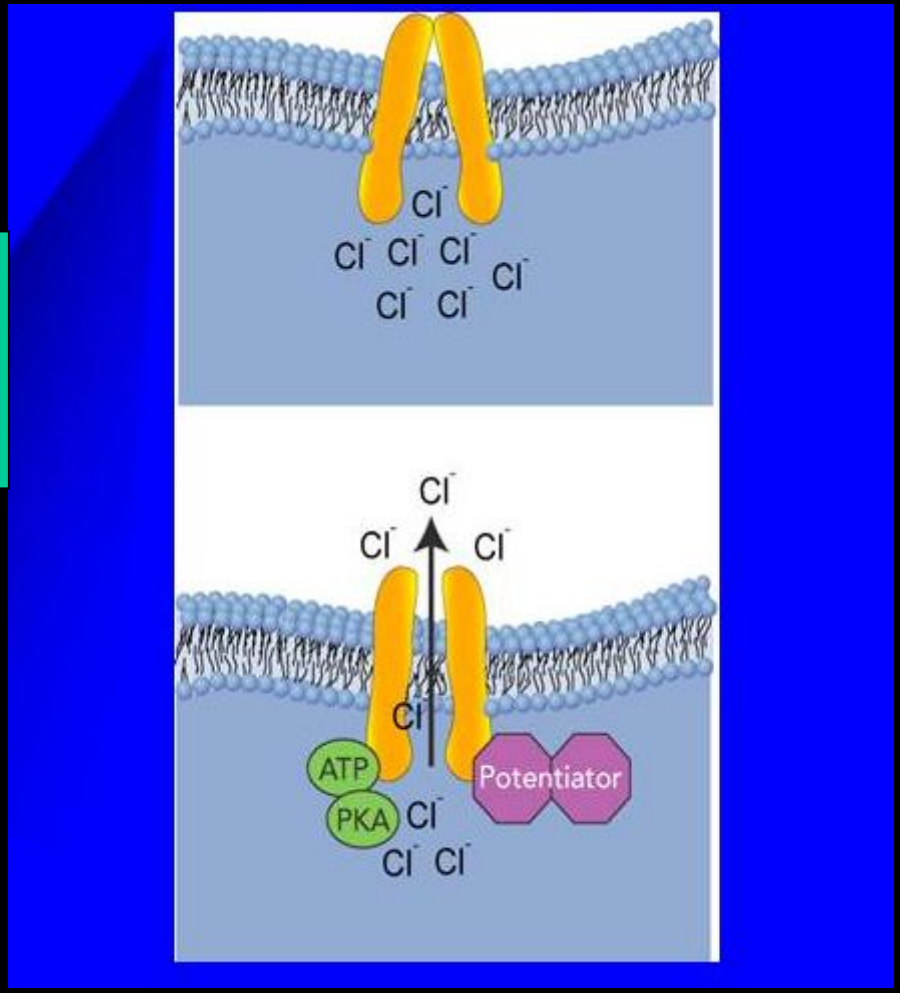
Defective Processing (Delta F508)



Overcoming Class III+IV mutations:

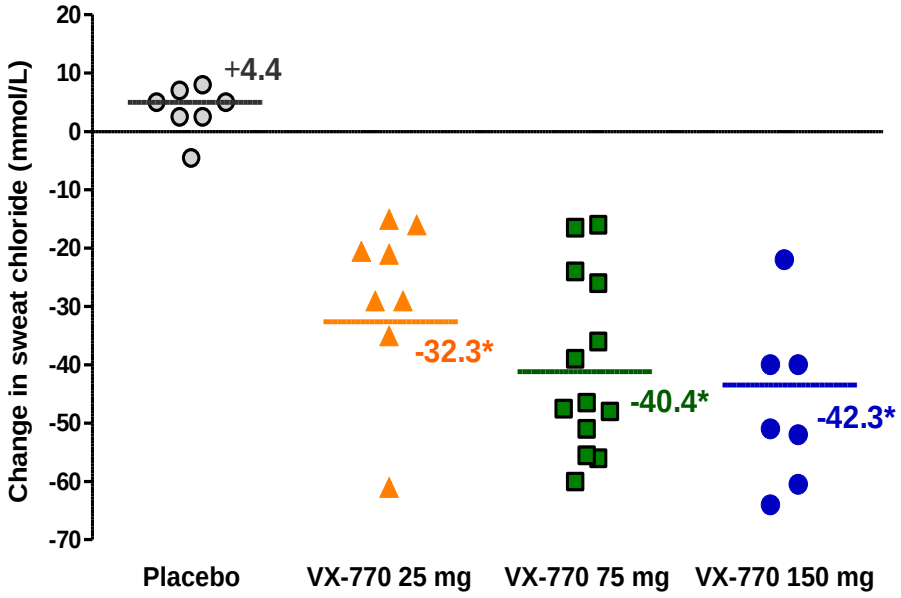
Defective Conductance and Regulation

Potentiators
ie VX770 in patients with G551D mutations



Sweat Chloride Change from Baseline

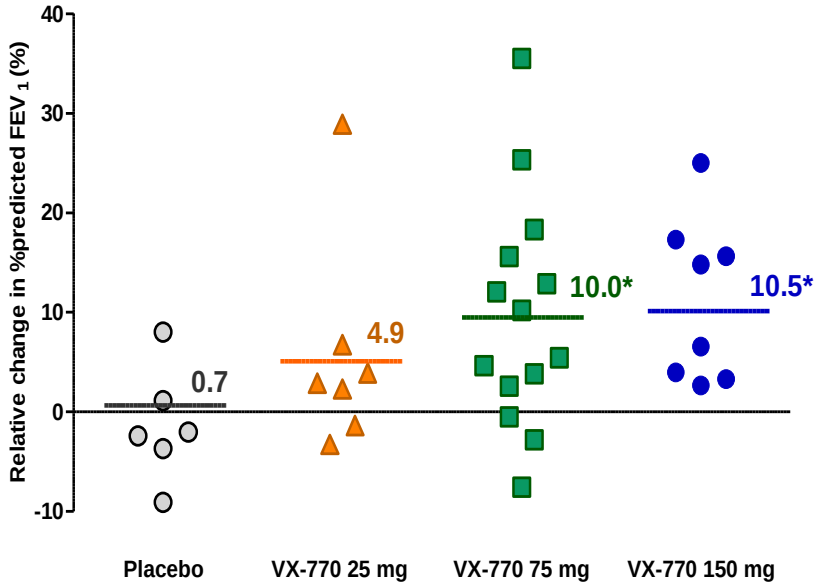
Individual subject response with population mean/median



*** *P* < 0.001 within-group and vs. placebo**
† *P* < 0.05 within-group and vs. placebo

Accurso et al N Engl J Med 2010

Relative Change in FEV_1 % pred

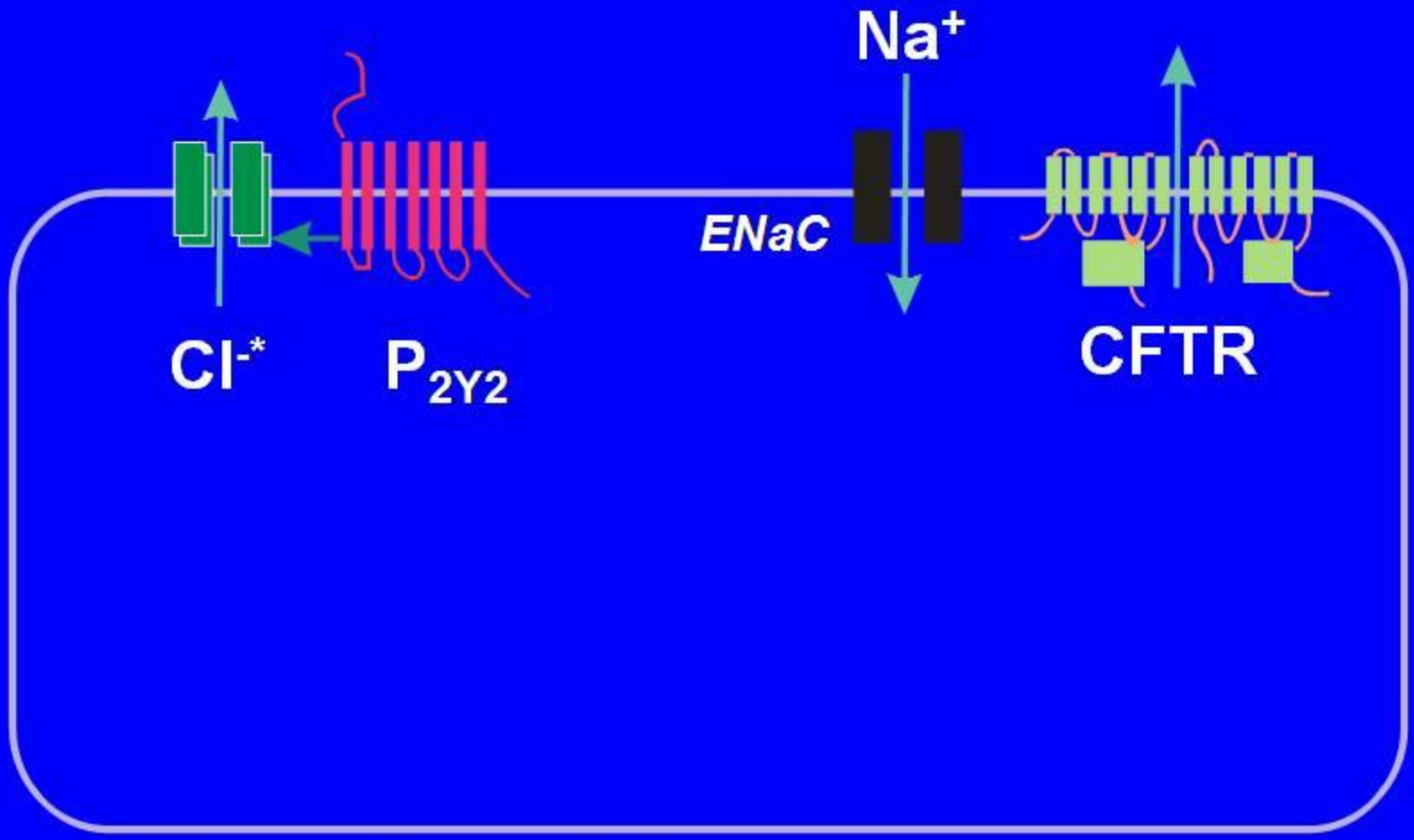


*$P < 0.01$ within-subject
†$P < 0.05$ within-subject

Accurso et al N Engl J Med 2010

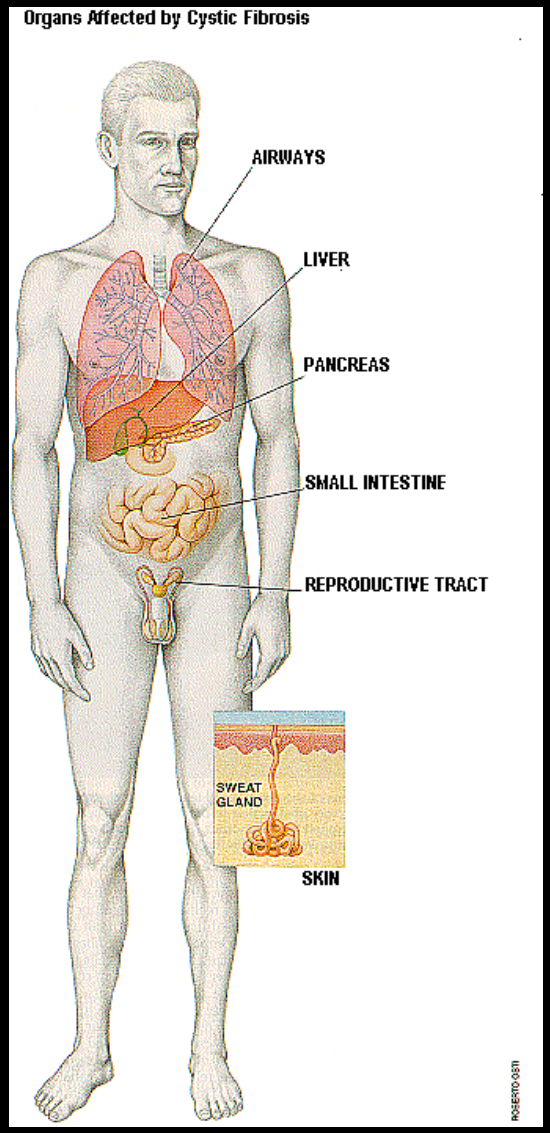
Improving Ion Transport

It's Not Just CFTR



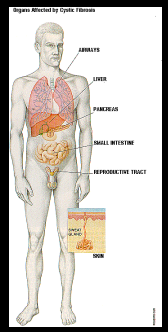
*Calcium activated chloride channel

Genotype/Phenotype Correlation



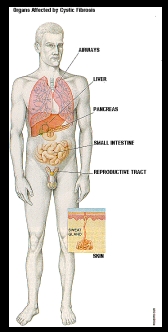
Genotype

Good

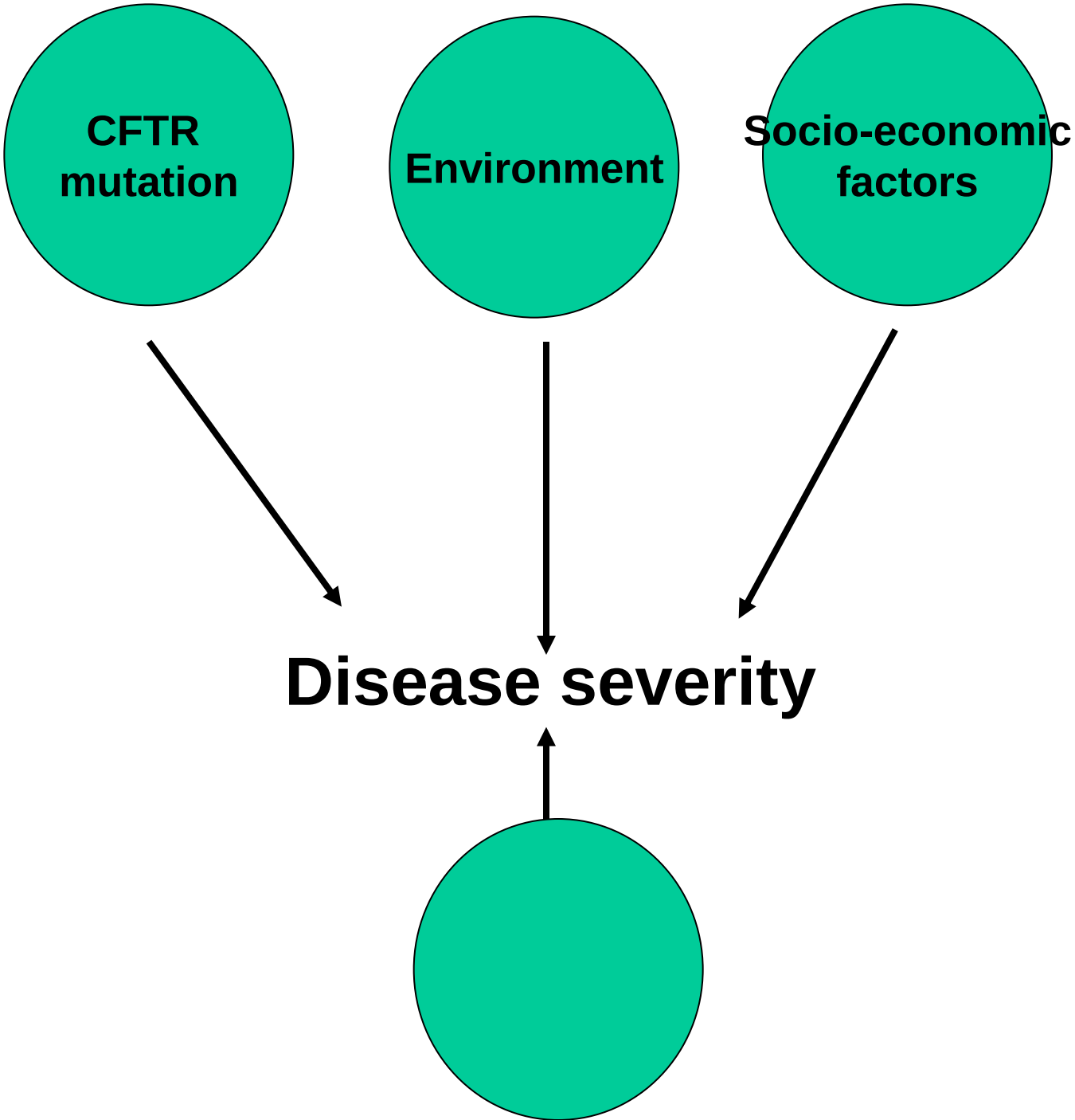


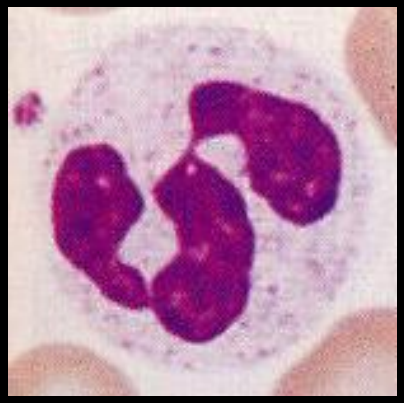
Pancreas

Poor

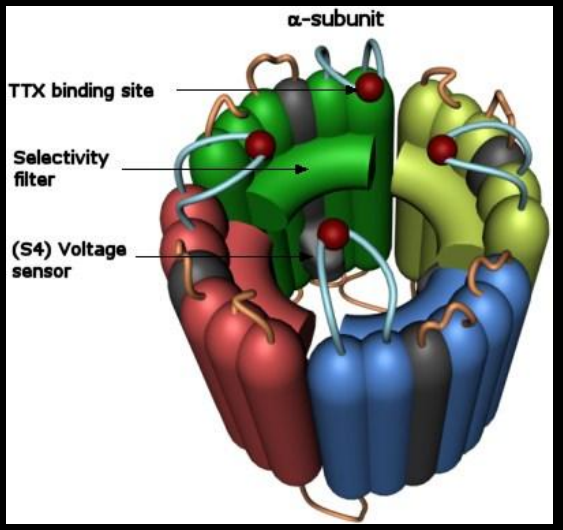


Lung


CFTR mutation
Environment
Socio-economic factors
Disease severity



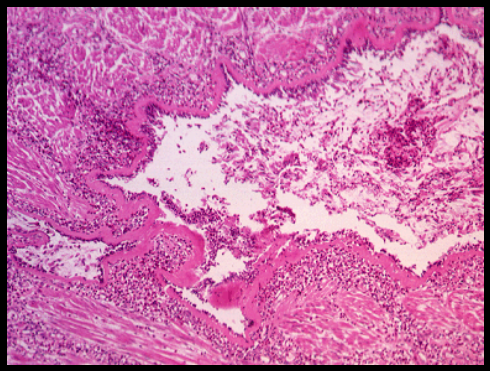
HOST DEFENCE
Neutrophil function
Defensins
Innate immune proteins
Cytokines
Anti-oxidants

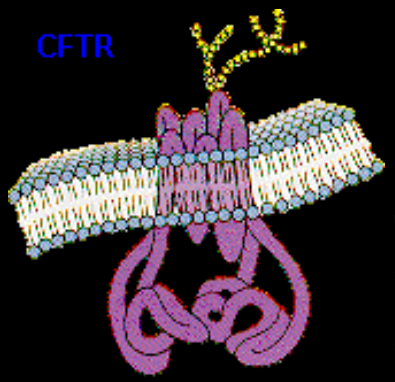


ION CHANNELS
ENaC
Ca^{++} mediated Cl^{-}

Putative Modifiers

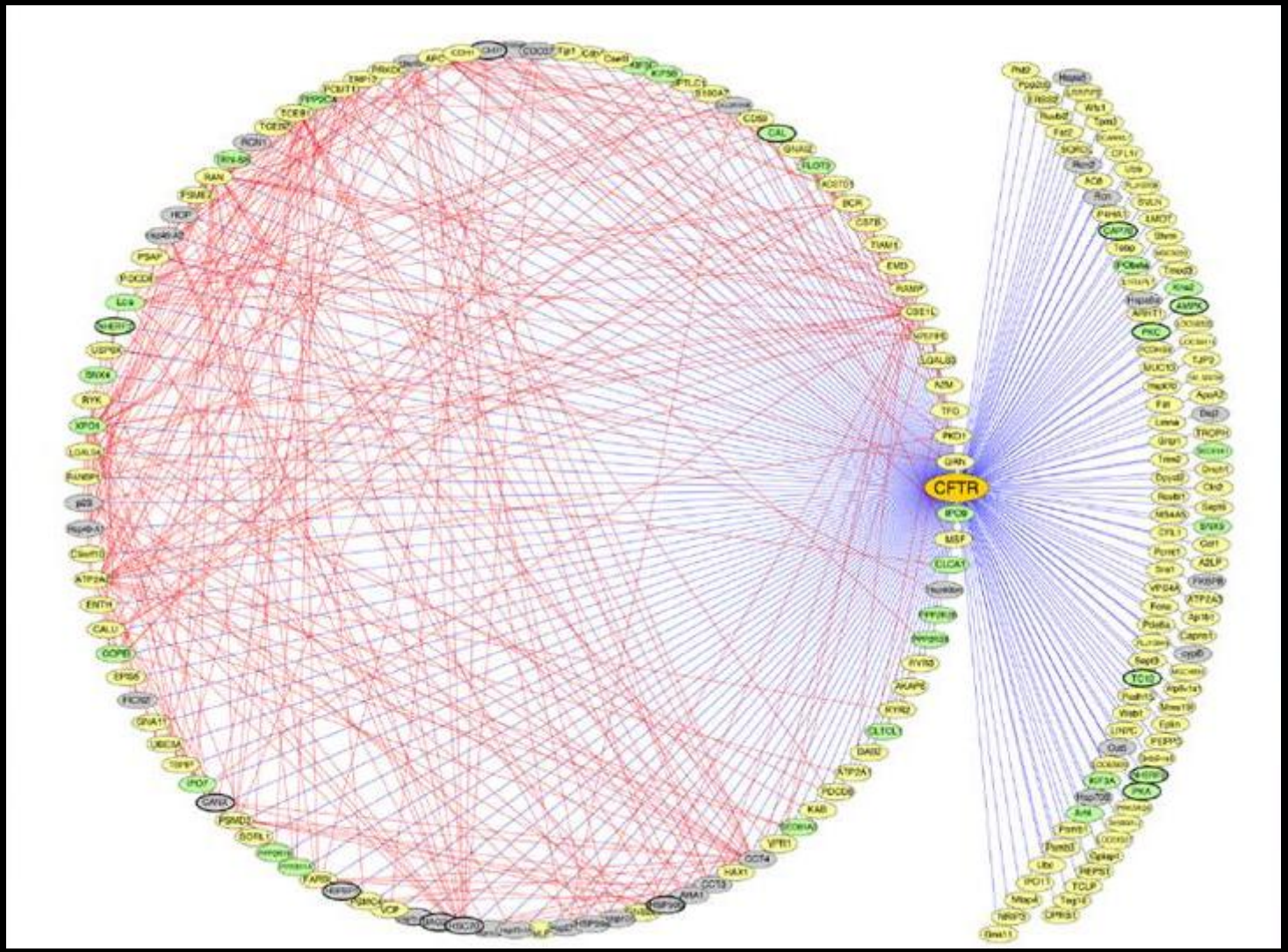
AIRWAY FUNCTION
MUC genes
β_2 adrenergic receptors





CFTR FUNCTION
Chaperones
CFTR polymorphisms

CFTR Interactome



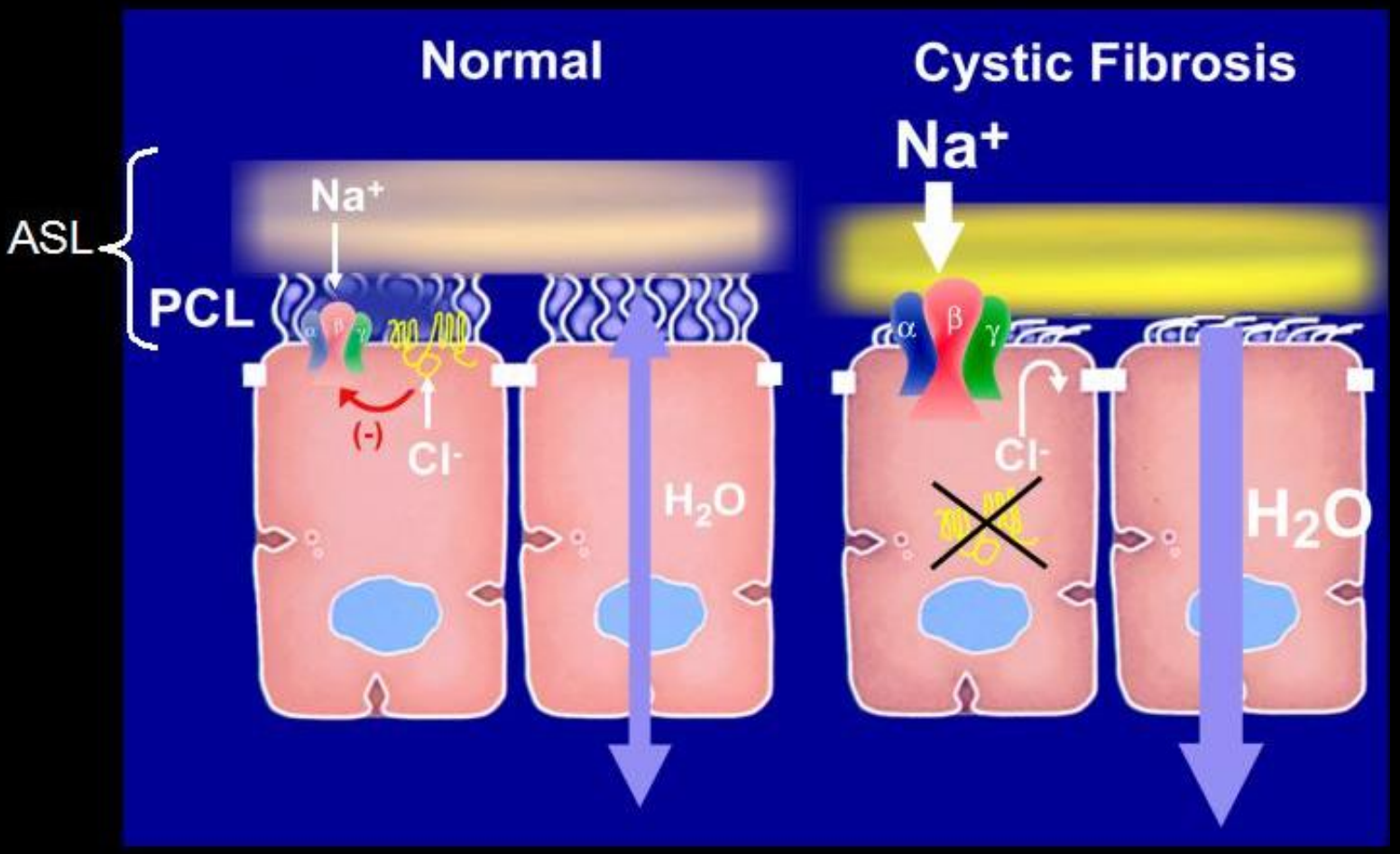
Modifier Gene Studies

- Many studies done
- Often small numbers (10s)
- Often not reproducible

- More recently larger studies (1000s)

Candidate gene approach
Genome wide screening/sequencing

Epithelial Sodium Channel (ENaC)



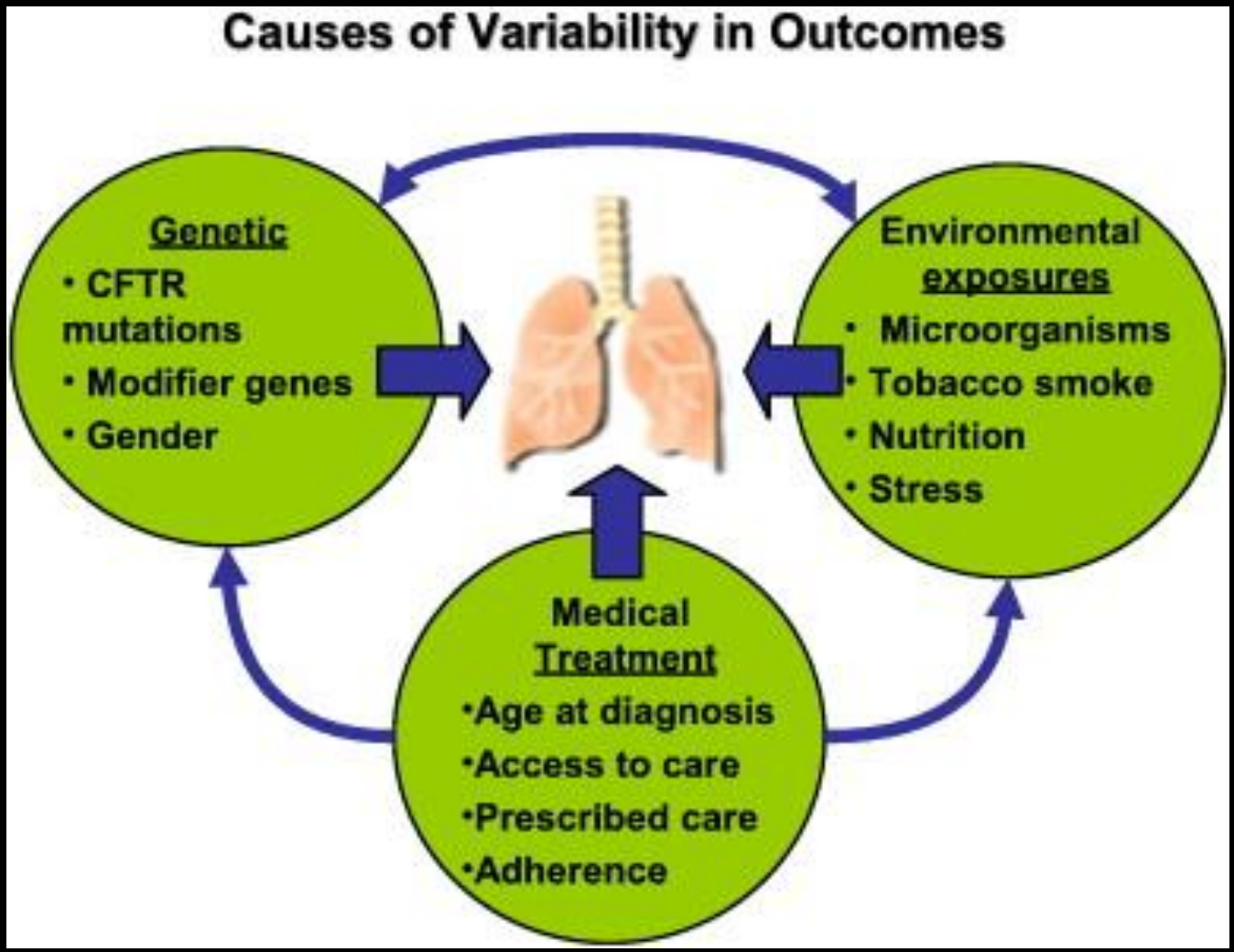
Human Molecular Genetics, 2005, Vol. 14, No. 22 **3493–3498**
doi:10.1093/hmg/ddi374
Advance Access published on October 5, 2005

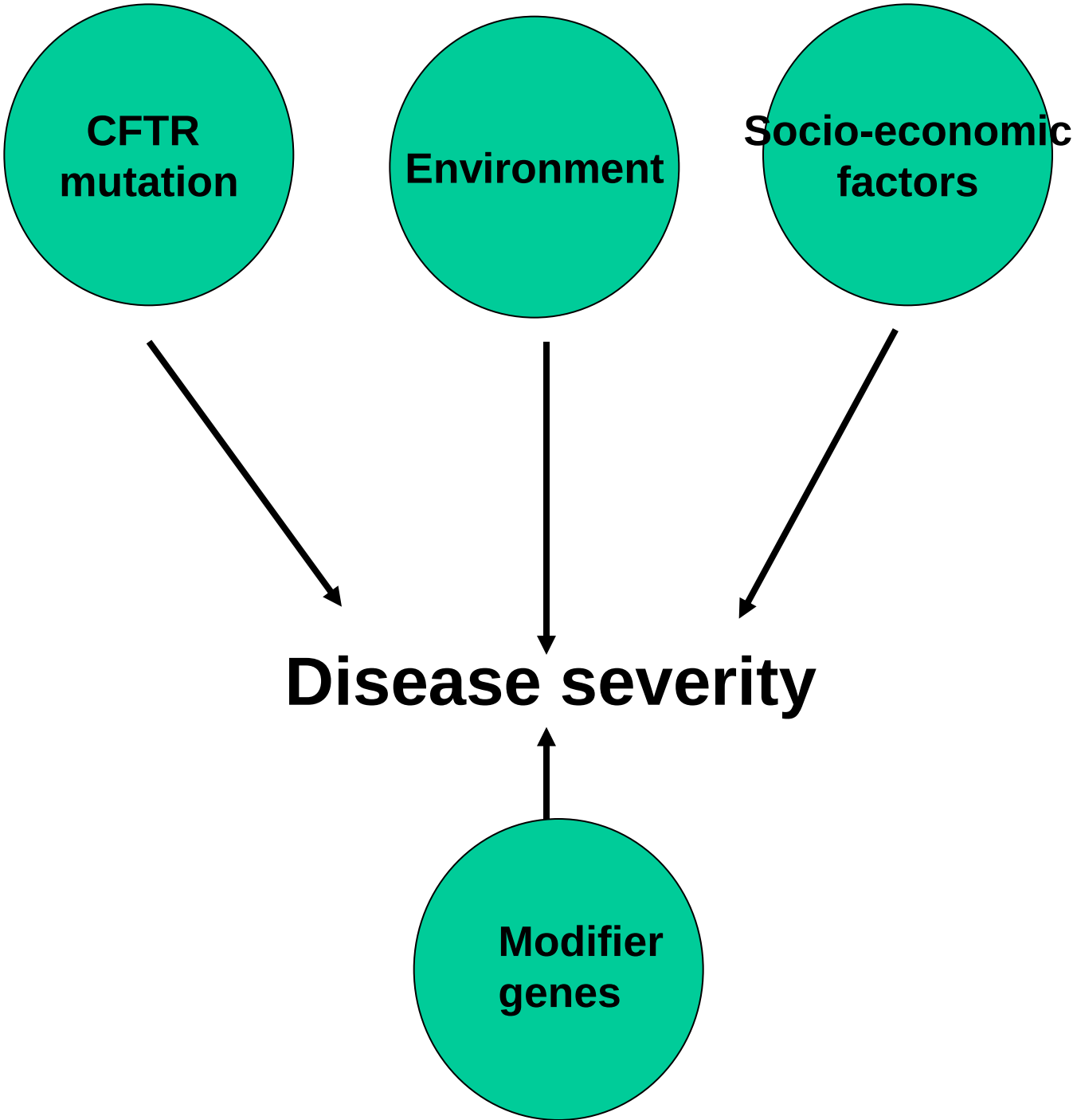
Mutations in the beta-subunit of the epithelial Na^+ channel in patients with a cystic fibrosis-like syndrome

Molly B. Sheridan[1], Peying Fong[2], Joshua D. Groman[1], Carol Conrad[3], Patrick Flume[4], Ruben Diaz[5], Christopher Harris[6], Michael Knowles[7] and Garry R. Cutting[1,*]

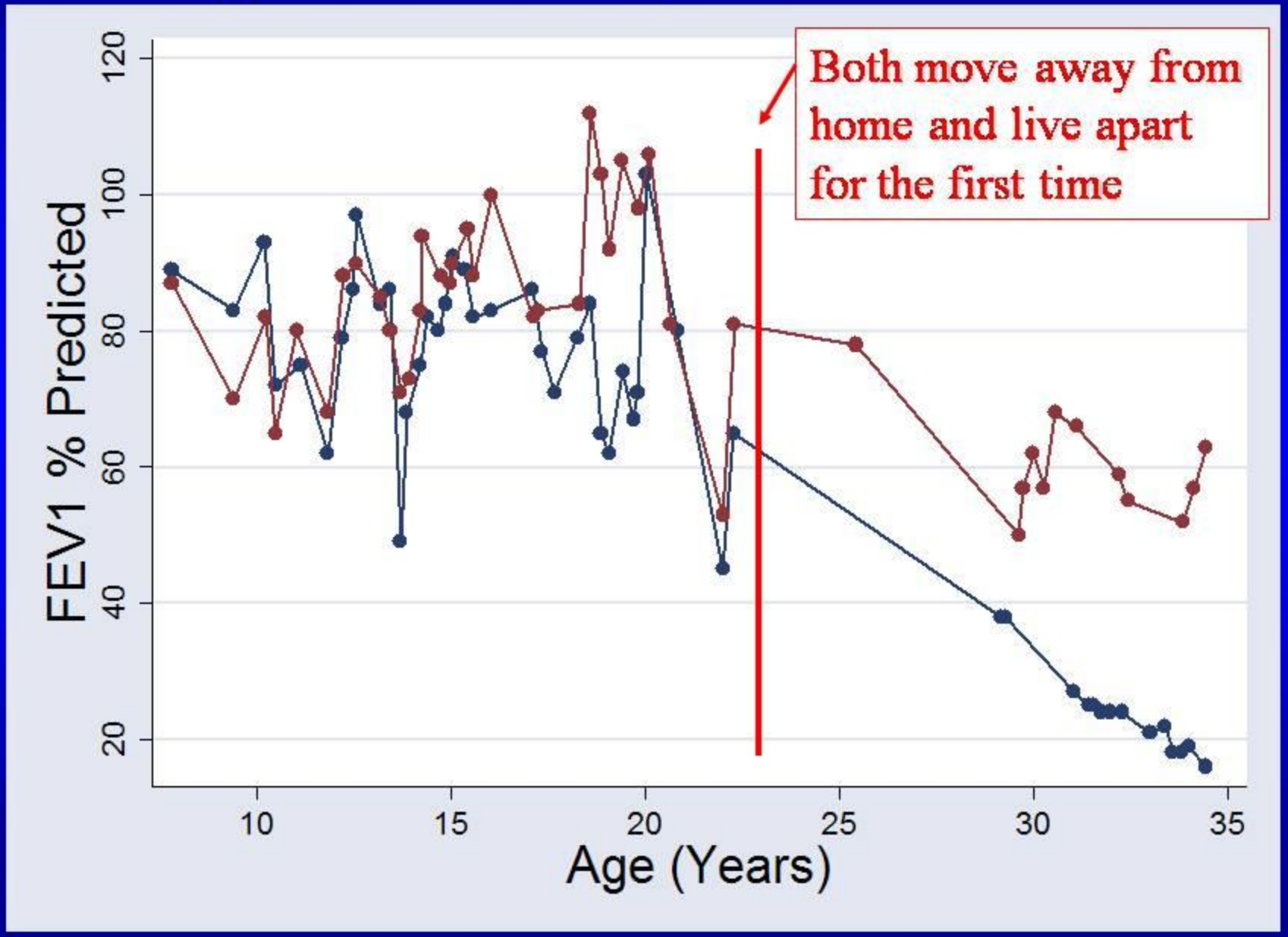
2 patients with CF-like disease
No CFTR mutations

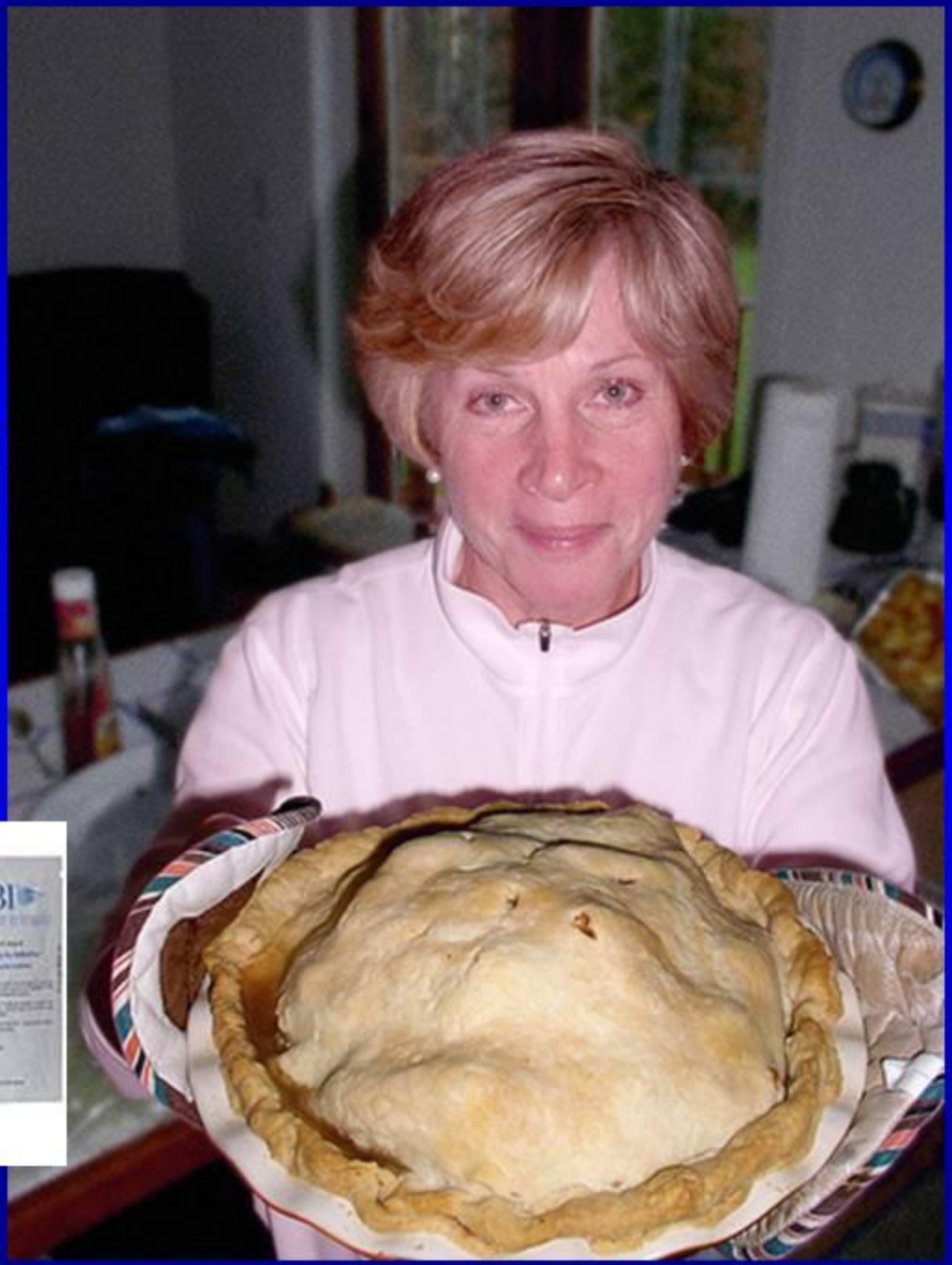
Causes of Variability in Outcomes



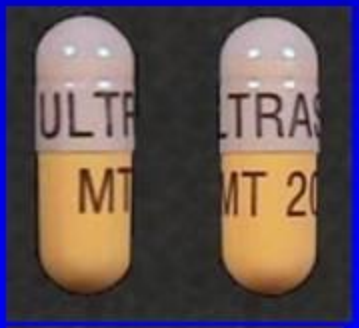

CFTR mutation
Environment
Socio-economic factors
Disease severity
Modifier genes

Best FEV_1 % Predicted per Year in a MZ Pair






DORNASE ALFA
PULMOZYME
INHALATION SOLUTION
Genentech, Inc.




SOLVAY


TOBI


Hyper-Sal 3.5%
Sodium Chloride Solution USP 3.5%

Animal Models

CF knockout mice

- **gut, but not lung disease**
- **alternative chloride channel in lung**

Lung disease

Summary

- Mutant CFTR affects ion and fluid transport across the epithelial membrane, which impairs mucociliary clearance and encourage bacterial colonization of the airways.
- Genetics of CF are complicated (>1500 mutations identified)
- Understand of the genetics has contributed to development of mutation specific treatments
- In addition to mutant CFTR, environmental factors and genetic modifiers also contribute to the pathophysiology of CF disease.

Recommended Reading

Cystic fibrosis: Exploiting its genetic basis in the hunt for new therapies, James L. Kreindler Pharmacol Ther. 2010

Update in Cystic Fibrosis 2010 Am J Respir Crit Care Med.
Peter J. Mogayzel, Jr. and Patrick A. Flume

Pharmacological therapy for CF: From bench to bedside, Becq F et al, Journal of CF 2011

CF Discussion (Nov 17)

4 groups

6/group

1 paper/group + questions

Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth.

Stoltz DA, Meyerholz DK et al

Sci Transl Med. 2010 Apr 28;2(29):29ra31.

Questions:

discuss content of the paper:

1. How well does the pig model mimic human CF disease?
2. How does the characteristic CF lung pathology develop in the pig?
3. What comes first infection or inflammation and why is it important to know this?

Hartl D et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. Nat Med. 2007 Dec;13(12):1423-30.

Questions:
discuss content of the paper:

1. Why do the results presented in the paper stimulated interesting discussion about CF pathophysiology?
2. Briefly discuss novel treatment approaches that may arise from this study

European best practice guidelines for cystic fibrosis neonatal screening.
Castellani C et al
J Cyst Fibros. 2009 May;8(3):153-73.

Questions:
discuss content of the paper:

1. What is the current state of newborn screening for CF in the UK? What tests are used?
2. What are the advantages of screening?
3. Are there disadvantages?

Gender differences in the Scandinavian **cystic fibrosis** population.

Olesen HV, Pressler T, Hjelte L, Mared L, Lindblad A, Knudsen PK, Laerum BN, Johannesson M; Scandinavian **Cystic Fibrosis** Study Consortium.

Pediatr Pulmonol. 2010 Oct;45(10):959-65.

Questions:

Discuss content of the paper:

1. Evidence for and against gender gap?
2. What might explain the gender gap?
3. What might be done to close the gender gap?