Medicines for Cystic Fibrosis

pharmacogenetics

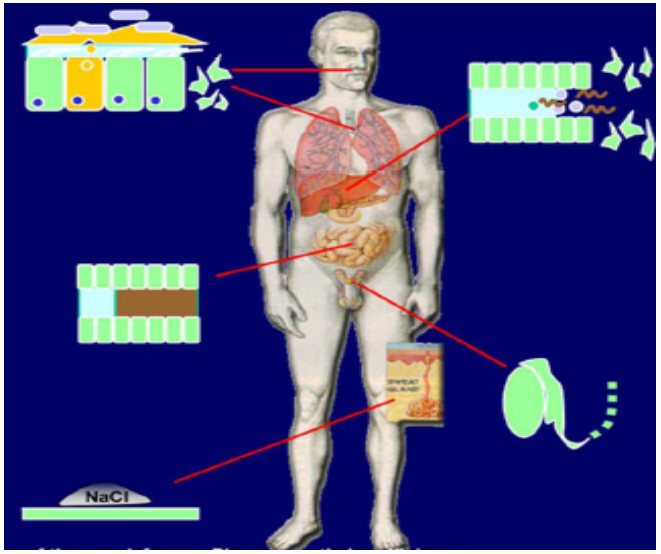
versus

genetic medicines

Cystic fibrosis is a multiorgan disorder

Lungs:
Bronchiectasis
Formation of mucus plaque
Chronic bacterial and fungal infections

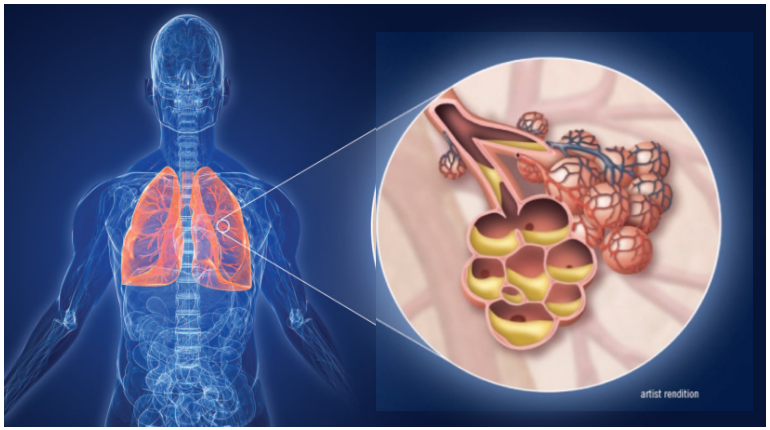
Small Bowel:
Meconium ileus
Distal intestinal obstruction syndrome



Pancreas & liver :
Exocrine insufficiency
Malabsorption
Steatorrhoea
Pancreatitis
Diabetes
Cirrhosis

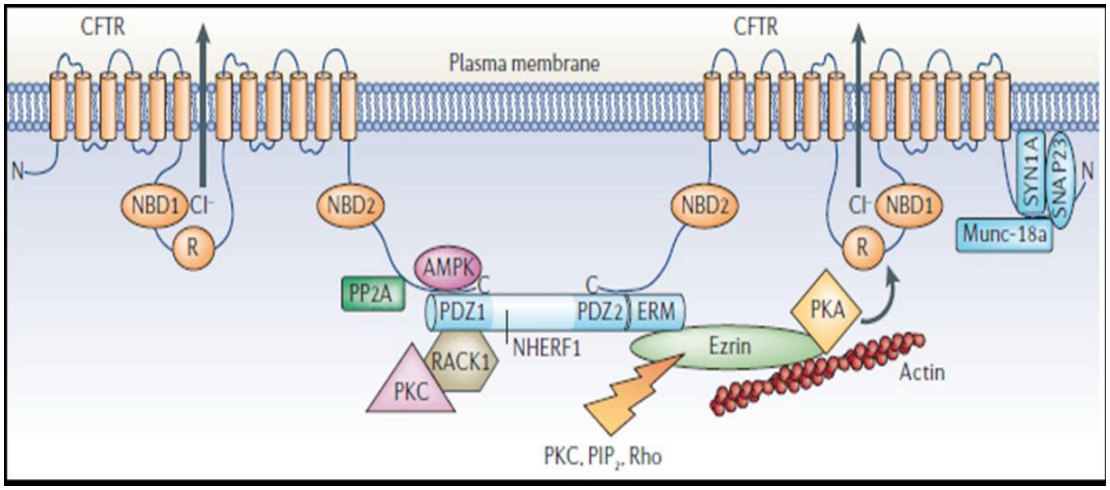
Reproductive tract:
Infertility

but the lung is the most affected organ!



Cystic Fibrosis

- CF is the most common lethal autosomal recessive condition in Europe (1/2500 live births)
- It is caused by a mutation in ***Cystic Fibrosis Transmembrane Conductance Regulator*** gene (*CFTR*), located on the long arm of chromosome 7.
- *CFTR* gene encodes a 1480 amino acid membrane protein (**CFTR**)



- CFTR is localized on the apical plasma membrane and functions as a regulated chloride channel.

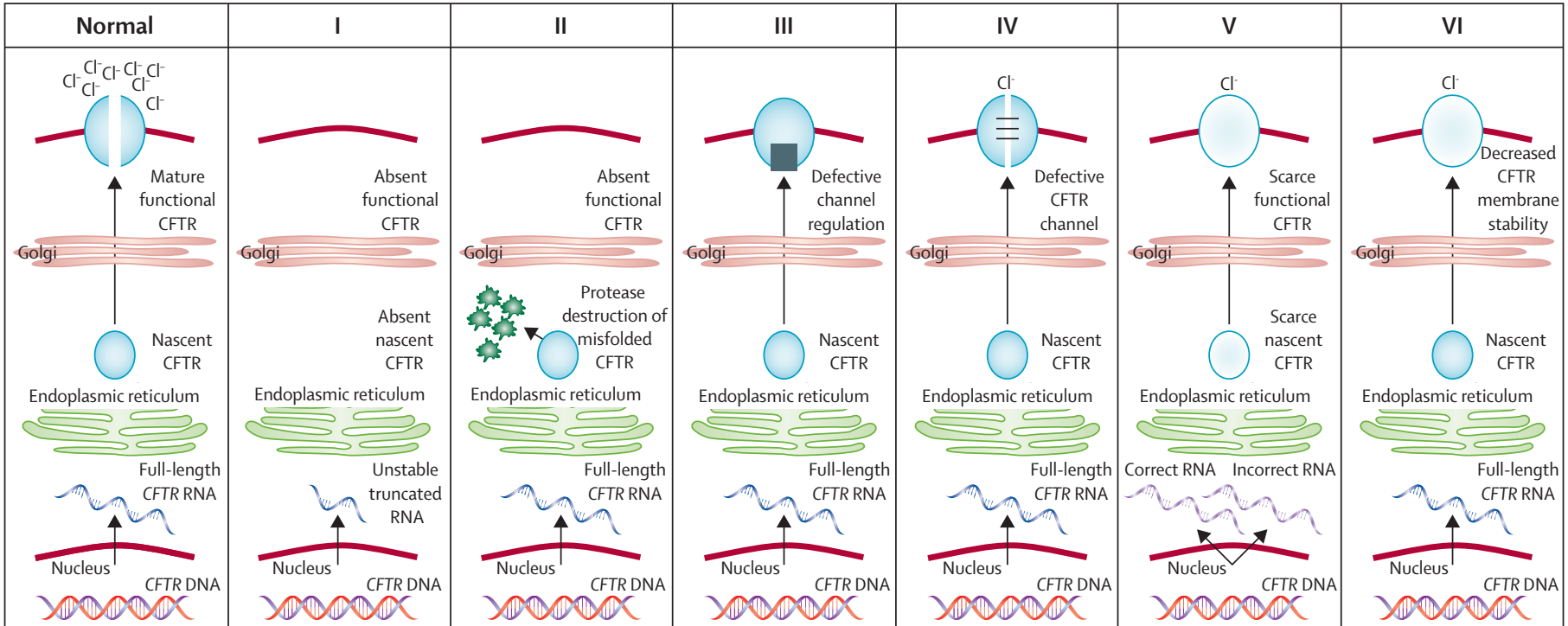
CF mutations databases

At present, 2009 mutations in the *CFTR* gene have been reported including: missense (39,6%); frameshift (15,6%); splicing (11,4%); nonsense (8,3%); in frame deletions or insertions (2%), large deletions or insertions (2,6%); promoter mutations (0,7%); sequence variation (13,4%) and unknown (6,42%).

CFTR mutations are collected in the Cystic Fibrosis Mutation Database that relates to the details of discovery of specific mutations (CFTR1; http://www.genet.sickkids.on.ca/).

The Clinical and Functional TRanslation of CFTR (CFTR2, www.cftr2.org) collects clinical and experimental data of the most common 276 mutations: CF-causing (242); non-CF causing (12); mutations or mutations of varying clinical consequences (19); mutations of unknown significance (3). The CFTR2 website uses data from the 88.000 patients included in the database to provide information about the clinical signs and symptoms associated with specific mutations and the different mechanisms by which mutations cause CF disease

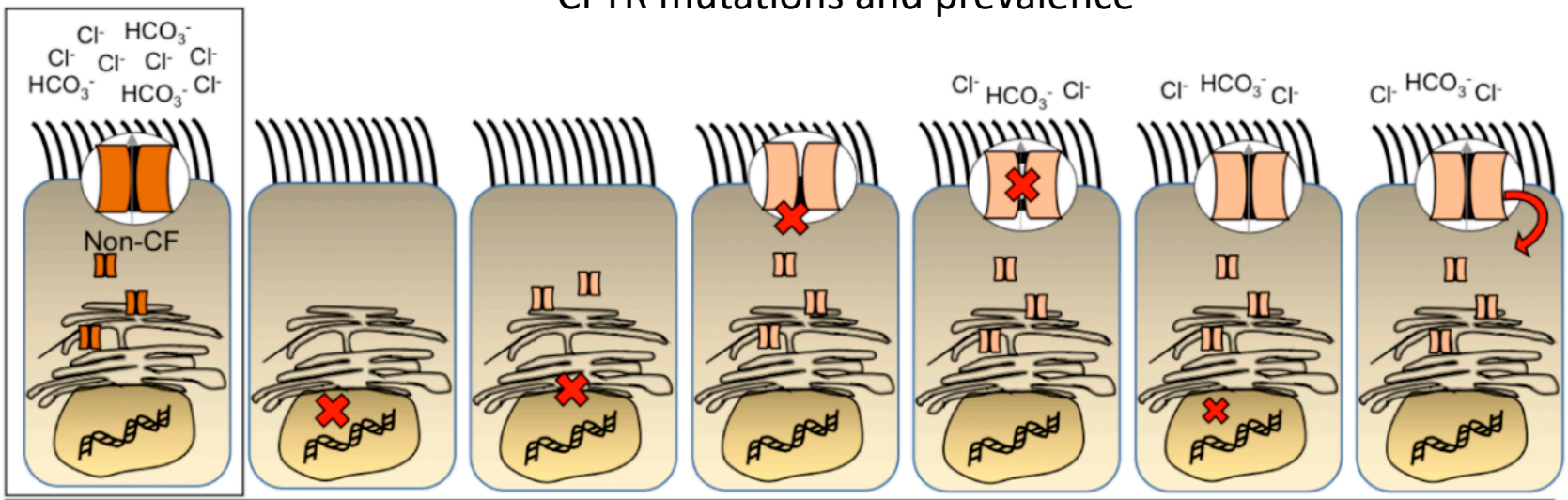
Classes of *CFTR* mutations



	Normal	I	II	III	IV	V	VI
CFTR defect		No functional CFTR protein	CFTR trafficking defect	Defective channel regulation	Decreased channel conductance	Reduced synthesis of CFTR	Decreased CFTR stability
Type of mutations		Nonsense; frameshift; canonical splice	Missense; aminoacid deletion	Missense; aminoacid change	Missense; aminoacid change	Splicing defect; missense	Missense; aminoacid change
Specific mutation examples		Gly542X Trp1282X Arg553X 621+1G→T	Phe508del Asn1303Lys Ile507del Arg560Thr	Gly551Asp Gly178Arg Gly551Ser Ser549Asn	Arg117His Arg347Pro Arg117Cys Arg334Trp	3849+10kbC→T 2789+5G→A 3120+1G→A 5T	4326delTC Gln1412X 4279insA

Mutations in the CFTR gene can be divided into six classes. Class I mutations result in no protein production. Class II mutations (including the most prevalent, Phe508del) cause retention of a misfolded protein at the endoplasmic reticulum, and subsequent degradation in the proteasome. Class III mutations affect channel regulation, impairing channel opening (eg, Gly551Asp). Class IV mutants show reduced conduction—ie, decreased flow of ions (eg, Arg117His). Class V mutations cause substantial reduction in mRNA or protein, or both, Class VI mutations cause substantial plasma membrane instability and include Phe508del when rescued by most correctors (rPhe508del)

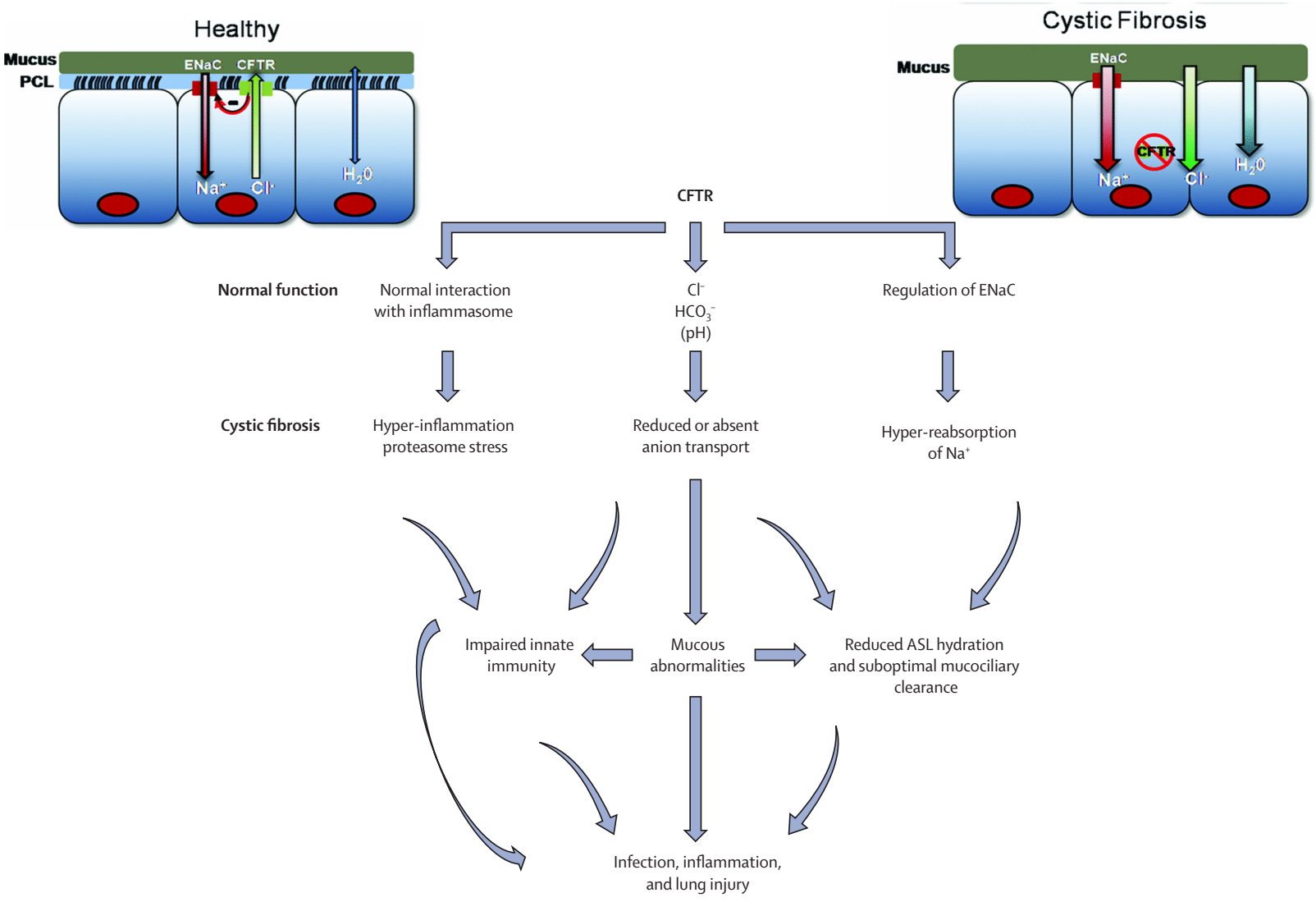
CFTR mutations and prevalence



Class of CFTR mutation	I	II	III	IV	V	VI
Protein output	No synthesis	Block in processing	Block in regulation	Reduced conductance	Reduced synthesis	Reduced half-life
Mutation example	G542X	F508	G551D	R117H	3489+10kb C>T	4326delTC
% of people with CF who have at least one mutation*	22%	88%	6%	6%	5%	5%
Small molecules**		VX-809,VX-661 correctors	VX-770 potentiator			

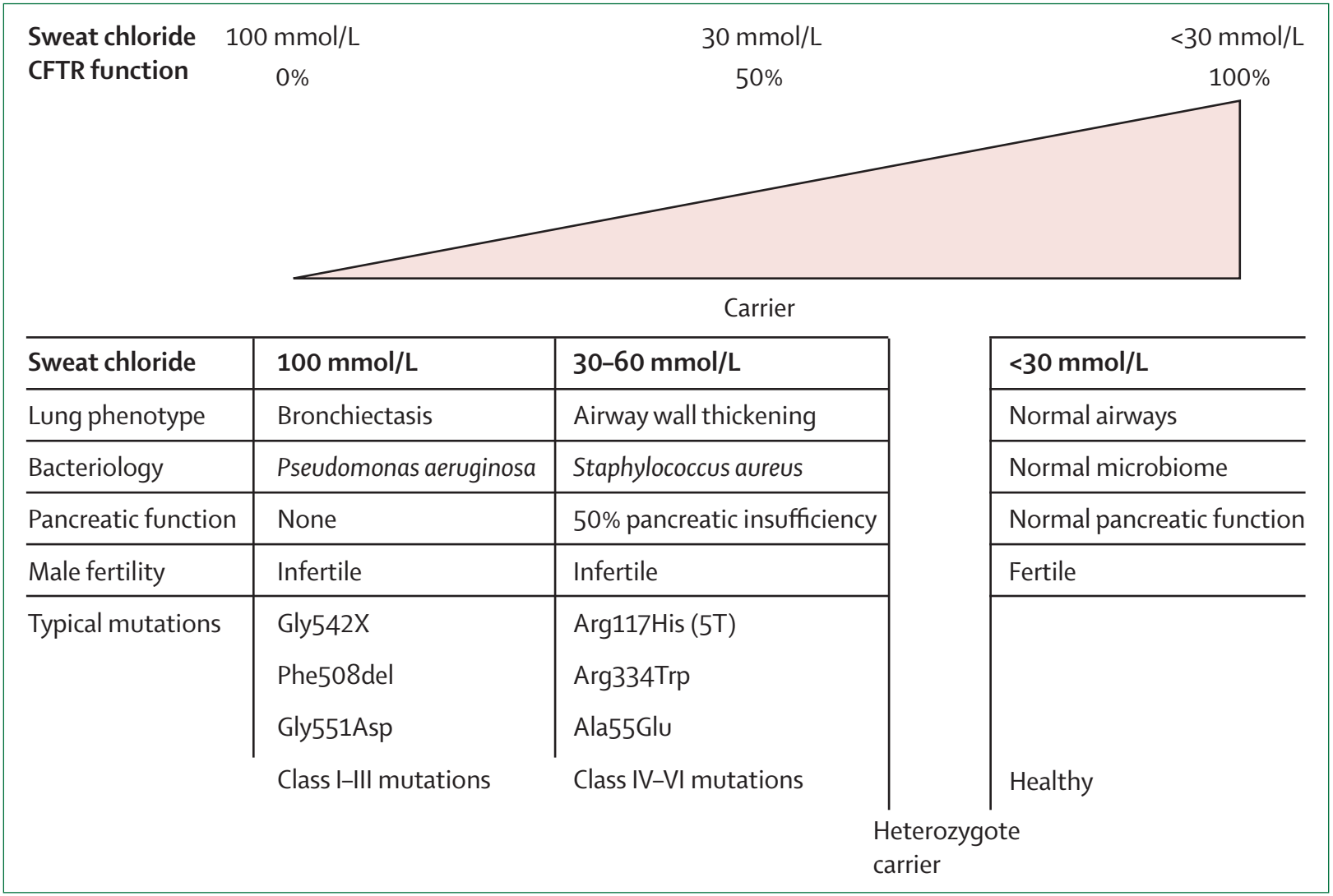
Figure 1. Classes of cystic fibrosis transmembrane conductance regulator (CFTR) mutations. Cystic fibrosis transmembrane conductance regulator mutations are categorized into 6 classes based on the mutation function or protein output [5]. A red "x" or arrow indicates where each CFTR mutant protein is affected. A common mutation example is listed for each class. * People with CF can have more than one mutation; thus, the percentage is representative of the entire population and does not add up to 100. Percentages acquired from the Cystic Fibrosis Foundation (U.S., 2017). ** Potentiators and correctors provide relief to some people with CF in these classes. Additional mutations have been approved for use of CFTR modulators.

Efects of CFTR dysfunction



ASL=airway surface liquid. CFTR=cystic fibrosis transmembrane conductance regulator. ENaC=epithelial sodium channel.

Relation between phenotype and genotype



Sweat chloride	100 mmol/L	30–60 mmol/L	<30 mmol/L
Lung phenotype	Bronchiectasis	Airway wall thickening	Normal airways
Bacteriology	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	Normal microbiome
Pancreatic function	None	50% pancreatic insufficiency	Normal pancreatic function
Male fertility	Infertile	Infertile	Fertile
Typical mutations	Gly542X Phe508del Gly551Asp	Arg117His (5T) Arg334Trp Ala55Glu	
	Class I–III mutations	Class IV–VI mutations	Healthy

Relation between phenotype, genotype, and CFTR function in patients with cystic fibrosis, carriers, and healthy individuals. CFTR=cystic fibrosis transmembrane conductance regulator.

current therapies for CF patients

Symptomatic therapies

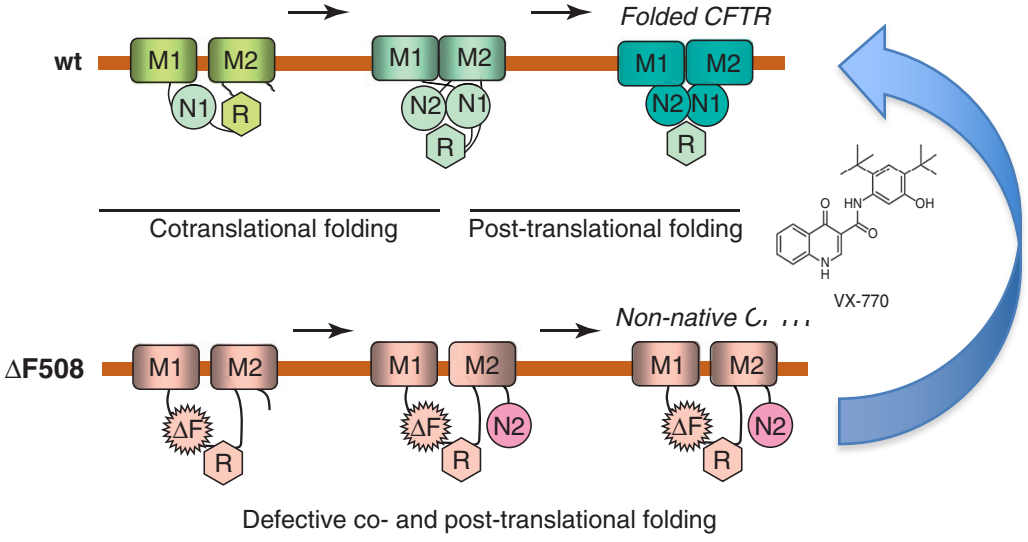
- anti-microbial drugs
- anti-inflammatory drugs
- mucolytic

Restore the activity of dysfunctional CFTR

- correctors
  promote accumulation in the plasma membrane
- potentiators
  normalize defective channel gating

→ correct defective CFTR protein

- gene therapy → provide a *wt* copy of the gene



correctors and potenziators are allele specific treatments which correct specific defects.
very recently the potentiator VX-770 enters the clinic for the treatment of G551D CF patients (about 4% of CF patients in USA, more rare in Italy)

kalydecoTM approved by FDA (Jan 2012) for people age 6 or older with G551D mutation of CF

pharmacogenetics

The improved understanding of cystic fibrosis (CF) pharmacogenetics has led to licensing of drugs that begin to address the molecular defect caused by certain cystic fibrosis transmembrane conductance regulator (CFTR) mutations.

Ivacaftor (kalydeco VX-770) is a corrector identified by high-throughput screening; it is specific for the Gly551Asp mutation

lumacaftor (VX-809) is a potentiator specifically targeting DF508 mutation, it shows some activity in combination with ivacaftor

Ivacaftor and lumacaftor, (orkambi) on going clinical trials

	Class	Drug	Effect on sweat chloride	Clinical response		Reference
				FEV_1	Pulmonary exacerbations	
Gly551Asp	III	Ivacaftor	50% decrease	10% increase	40% decrease	118–122
Gly551Ser, Gly178Arg, Gly1244Glu, Gly1349Asp, Ser549Asn, Ser549Arg, Ser1251Asn, Ser1255Pro	III	Ivacaftor	50% decrease	10% increase	NK	123
Arg117His	IV	Ivacaftor	25% decrease	3% increase (>18 years)	NK	124
Stop mutations (eg, Gly542X)	I	Ataluren	No change	No change	No effect	125, 126
Phe508del	II	Ivacaftor plus lumacaftor	8% decrease	3% increase	30% decrease	127, 128

FEV_1=forced expiratory volume in 1 s. NK=not known.

Table 2: **Summary of clinical trial results of precision medicine-based treatments of CFTR dystinction by small molecules in people with cystic fibrosis, by mutation**