Cystic Fibrosis (Muco viscidosis)

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What is cystic fibrosis?

Genetic disease.

Caused by a defective gene.

Body produces mucus that clogs the lungs and leads to infections.

Causes airway mucus to thicken, resulting in chronic respiratory obstruction and infections.

Impairs pancreatic exocrine function.
Complications can decrease lifespan.

What is cystic fibrosis?

- CF is a “multisystem” disease meaning that it affects many body organs. However ,most of the symptoms are to do with the lungs and gut.
- In a healthy person, there is a constant flow of mucus over the surface of the air passage in the lungs. This removes debris and bacteria .In CF patient, this mucus is excessively sticky and cannot perform this role properly. In fact the sticky mucus provides an ideal environment for bacterial growth.
- Cystic fibrosis (CF), a single-gene disease with autosomal recessive inheritance, is caused by a mutation in the *cystic* fibrosis transmembrane conductance regulator (*CFTR) gene.*

- The *CFTR protein is a chloride channel that is critical* to efficient mucus transport.
- Mutations in *CFTR causes*
- *disrupt* chloride secretion,
- sodium reabsorption,
- water transport,
- leading to mucus hyperconcentration,
- and decreased mucociliary clearance.
- Dehydrated mucus secretions lead to bronchial infection with bacteria and exaggerated inflammatory response, which result in the development of severe bronchiectasis rather than fibrosis and, eventually, respiratory failure.

- People with CF are at the risk of bacterial chest infections like pneumonia etc (*Staphyloccus aurens* and *Psuedomonas aeruginosa*).
- If they are not treated early and properly ,these are very difficult to treat.
- If people with CF do not have proper treatment ,they will continue to have oily bowel movements, abdominal pain and problems putting on weight. Constipation is also frequent symptom.
- Occasionally the gut become completely blocked resulting in extreme stomach pain.
- Age of onset early childhood. Variable symptoms. Life expectancy now 20-35 years

Symptoms

Chronic pulmonary disease

Pancreatic insufficiency.

Increase in sweat

Breathing problems.

Thick secretions in airway passages

Salty-tasting skin.

Coughing or wheezing

Excessive appetite but poor weight gain.

Greasy, bulky stools .

Prevalence

- The estimated prevalence rate
- in whites is 1:2,500;
- In Ashkenazi Jew populations is 1:2,500
- in Hispanics 1:3,500;
- in African Americans 1:15,100;
- in Asians, native Hawaiians and Pacific Islanders 1:31,000 to 1:100,000.
- Carrier frequency: 1 in 25
- Equally common in boys and girls

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Name of Gene Product: Cystic fibrosis transmembrane conductance regulator (CFTR)

- CFTR is an ABC (ATP-binding cassette) transporter or traffic ATPase.
- These proteins (ABC) transport molecules such as sugars, peptides, inorganic phosphate, chloride, and metal cations across the cellular membrane.
- CFTR transports chloride ions (Cl^-) ions across the membranes of cells in the lungs, liver, pancreas, digestive tract, reproductive tract, and skin.

CFTR protein

- **Structure of CFTR protein**
- The CFTR gene encodes a 1494-amino acid long transmembrane protein with a molecular mass of approximately 170 kDa when fully maturated .
- Protein has a symmetrical structure with two transmembrane domains (TMD1TMD2) contributing to the channel pore, two nucleotide binding domains (NBD1, NBD2) for binding ATP, a large hydrophilic regulatory domainRdomain) rich in protein kinase A (PKA) and protein kinase C phosphorylation sites.
- *These features are* characteristic of a large family of ATP–binding cassette transporters (ABC transporters) found in eukaryotes and prokaryotes. However, the presence of the large hydrophilic R-domain after the NBD1

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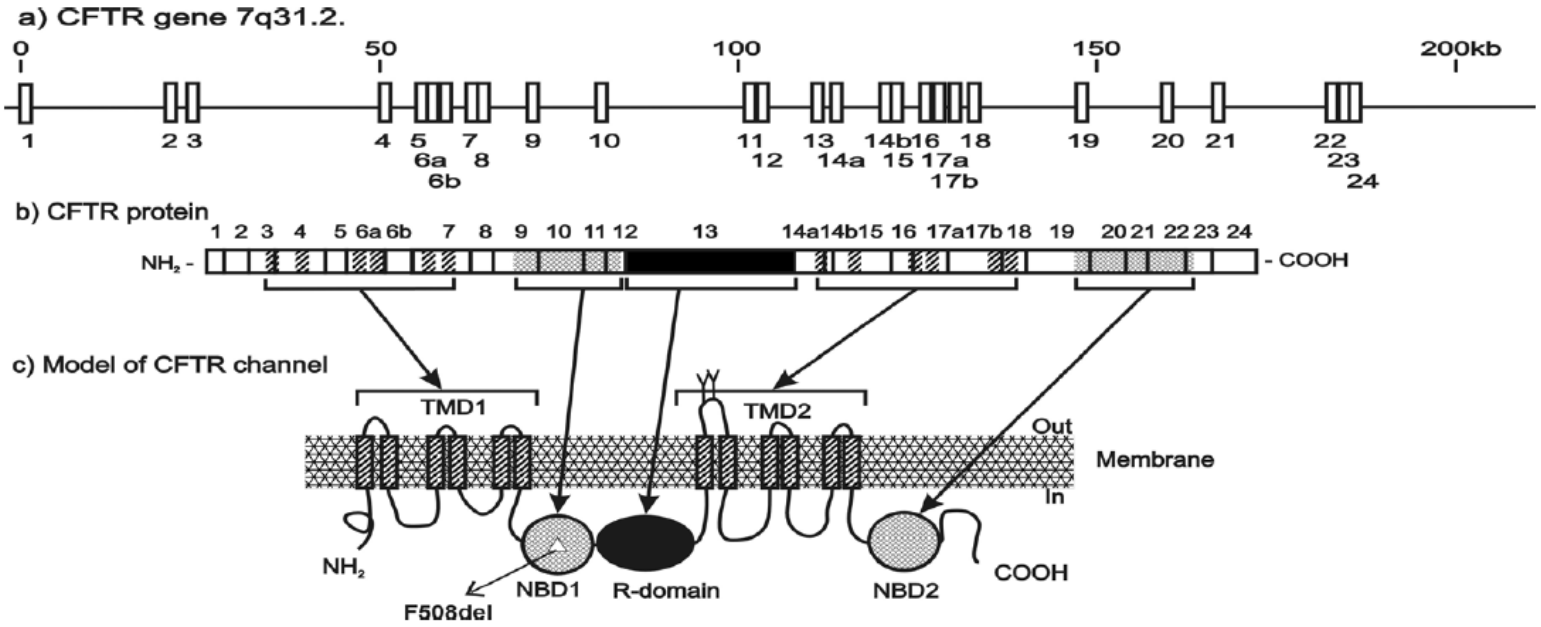
CFTR protein

- There are 6 extracellular and 4 intracellular loops between transmembrane
- segments. It is proposed that 77% of the protein resides in the cytoplasm, 19%
- in the membrane-spanning segments, and 4% in the extracellular loops, which
- (except for first and fourth loops) are very short (Riordan *et al., 1989; Sheppard*
- and Welsh, 1999). The fourth extracellular loop contains 2 *N-linked glycosylation*
- sites at amino acid asparagine N894 and N900 (Sheppard and Welsh.



a) The structure of the CFTR gene; b) the CFTR protein; c) the schematic representation of the CFTR chloride channel. The different domains: transmembrane domain 1 and 2 (TMD1, TMD2); nucleotide binding domain 1 and 2 (NBD1, NBD2); the regulatory domain (R-domain); the N-terminal (NH2) and C-terminal (COOH) ends; two glycosylation sites (YY) present on the fourth extracellular loop (N894 and N900) and the main CFTR mutation F508del (Δ) are indicated (modified from Welsh *et al.,* 1995).

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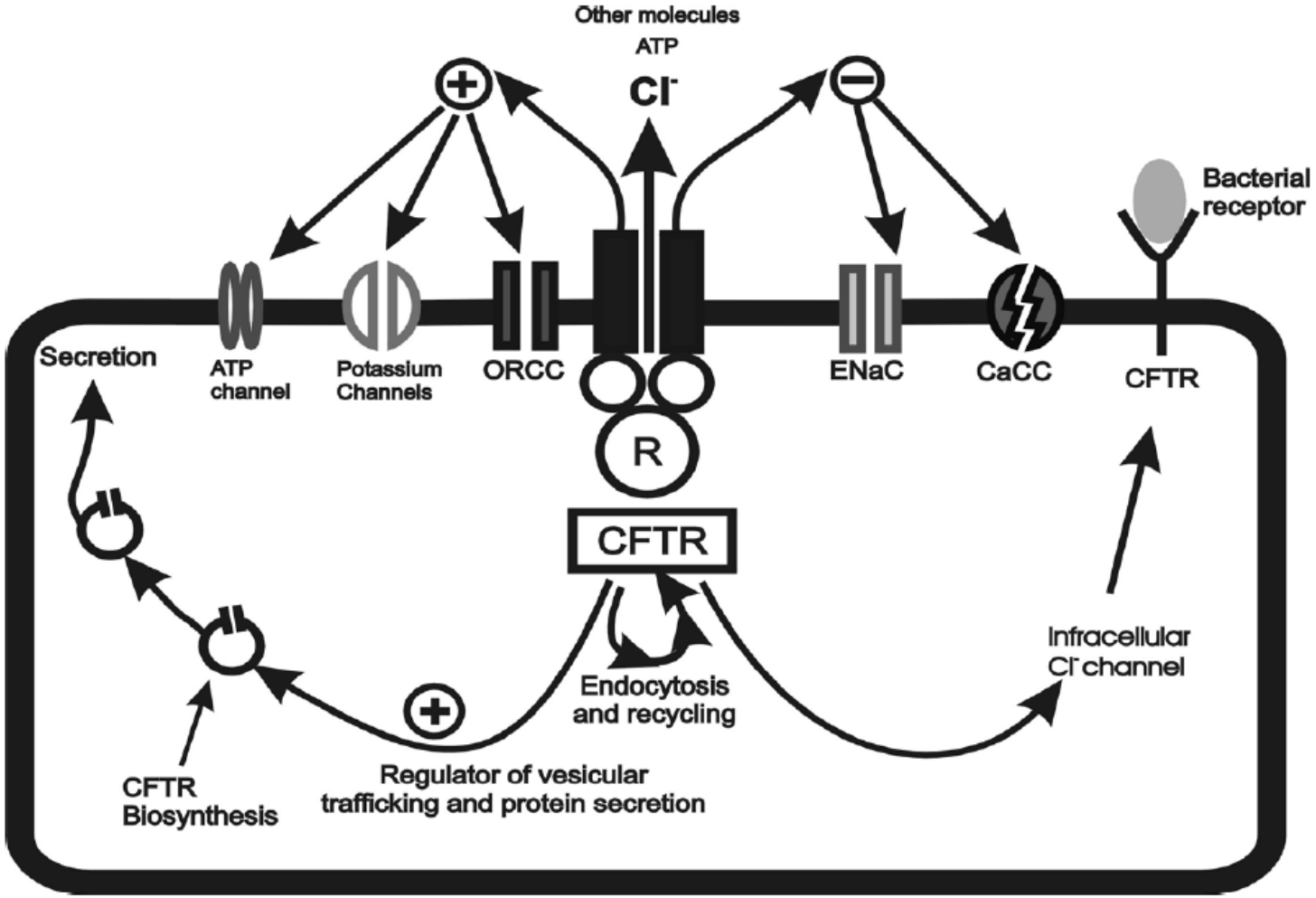

Possible cellular functions of CFTR. In addition to its well-known role as cAMP regulated chloride channel, CFTR may have pleiotropic roles, including: a transporter of ATP and other molecules, positive regulation of the outwardly rectifying chloride channels (ORCC), potassium channels and distinct ATP channels; negative regulation of the epithelial sodium channel (ENaC) and Ca2+ sensitive chloride channel (CaCC). It may also function as a regulator of vesicular traffic and as an intracellular chloride channel, and could also be a receptor for *Pseudomonas aeruginosa*.
R, regulatory domain of CFTR (modified from Schwiebert *et al., 1998).*

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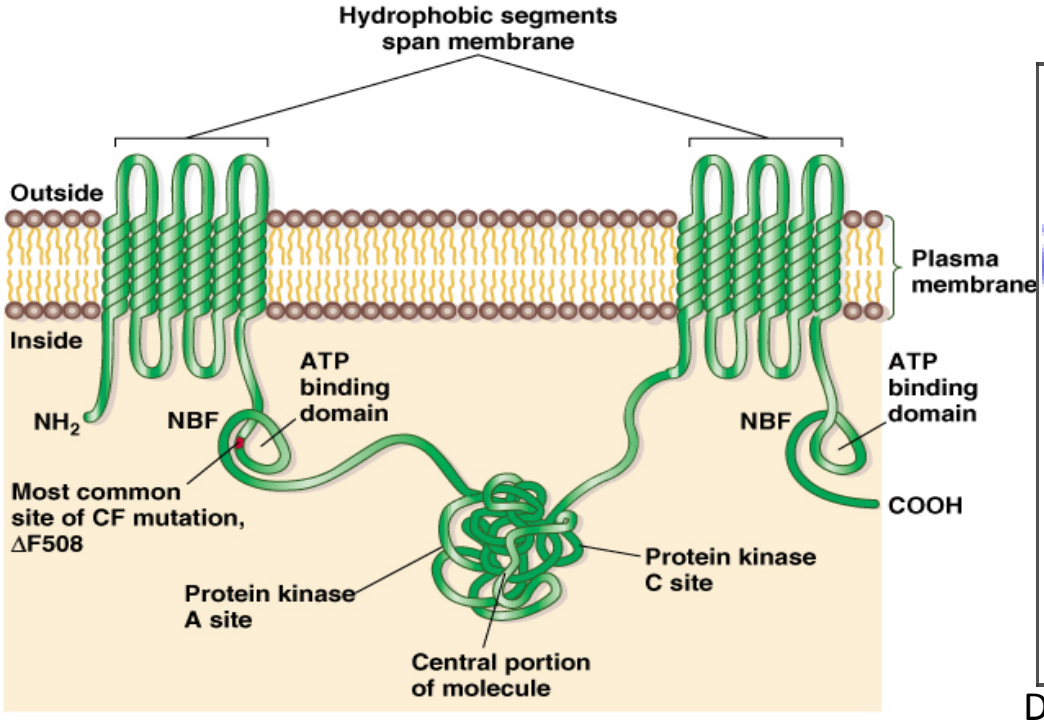
CFTR protein: a multifunctional protein

- In fact ,the CFTR protein regulates other channels also, the outwardly rectifying chloride channel (ORCC),epithelial Na+ channel(ENaC) and at least two inwardly K+ channels ROMK1 and ROMK2.
- Besides being a channel regulator , it also play a role in transport of ATP, modifying the phenomenon of exocytosis/ endocytosis,

regulation of pH of intercellualr organelles.

Proposed structure for cystic fibrosis transmembrane conductance regulator (CFTR)



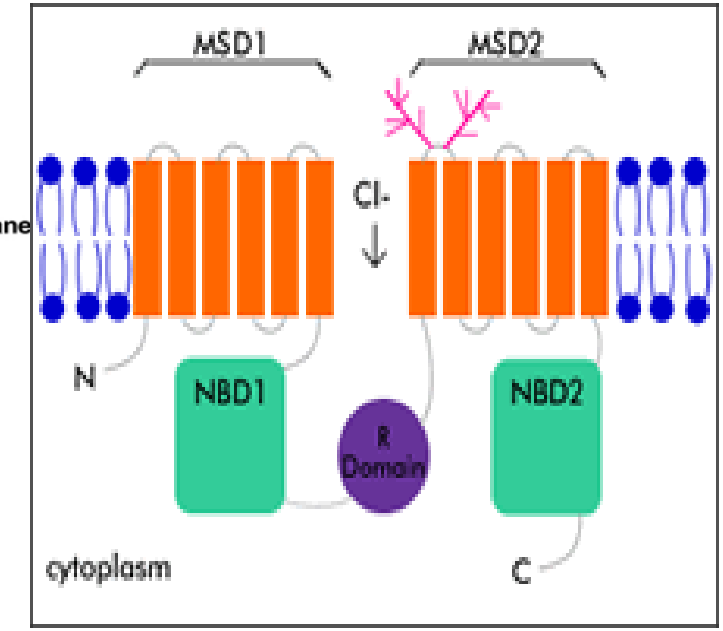
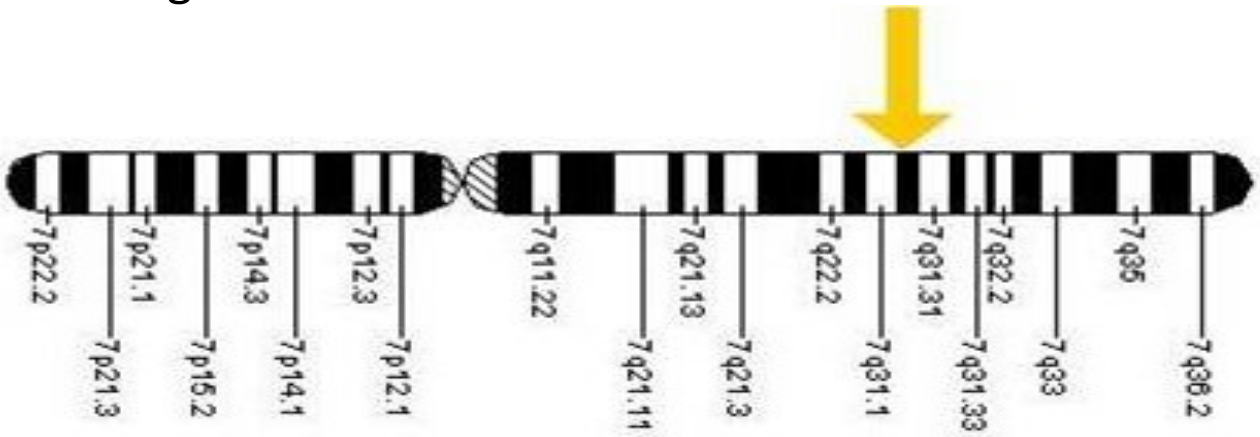


Diagram depicting the five domains of the CFTR membrane protein (Sheppard 1999).

CFTR gene

- CFTR gene encodes a Chloride- channel. Cystic Fibrosis is caused by defects in the CFTR gene which results in either a decrease in its Cl- transport capacity or its level of cell surface expression
- **Name of Gene Product: C**ystic fibrosis transmembrane conductance regulator (CFTR)
- CFTR gene is located on chromosome 731.31



Discovery of CFTR gene

-1989 by Francis Collins and Lap-Chee Tsui by positional cloning

Structure of CFTR gene

- The CF gene spans over 200 kb of genomic DNA; initially 24 exons were identified *and later 3 more were recognized: 6b, 14b, 17b, bringing the current count to 27 exons.*
- *The* encoded mRNA is 6128bp long and can be detected in a variety of tissues, mainly at the apical border of the epithelial cells that line most exocrine glands.
- DNA sequence analysis of the CFTR promoter region in humans and in rodents has revealed high GC content, typical to the so-called “house-keeping” genes, but lacks a TATAbox, an RNA polymerase recognition site characte ristic of genes that exhibit tissue-specific expression.
- This region does, however, contain consensus sequences for binding sites

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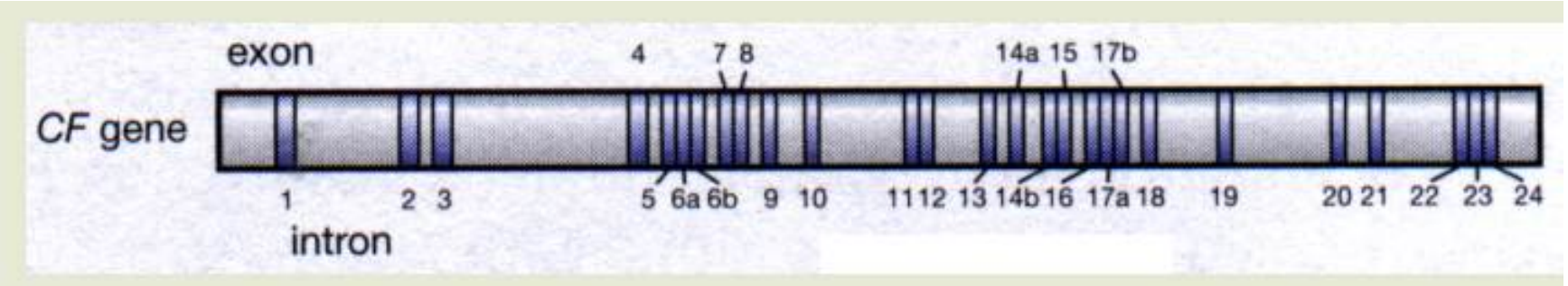
CFTR/CF Gene

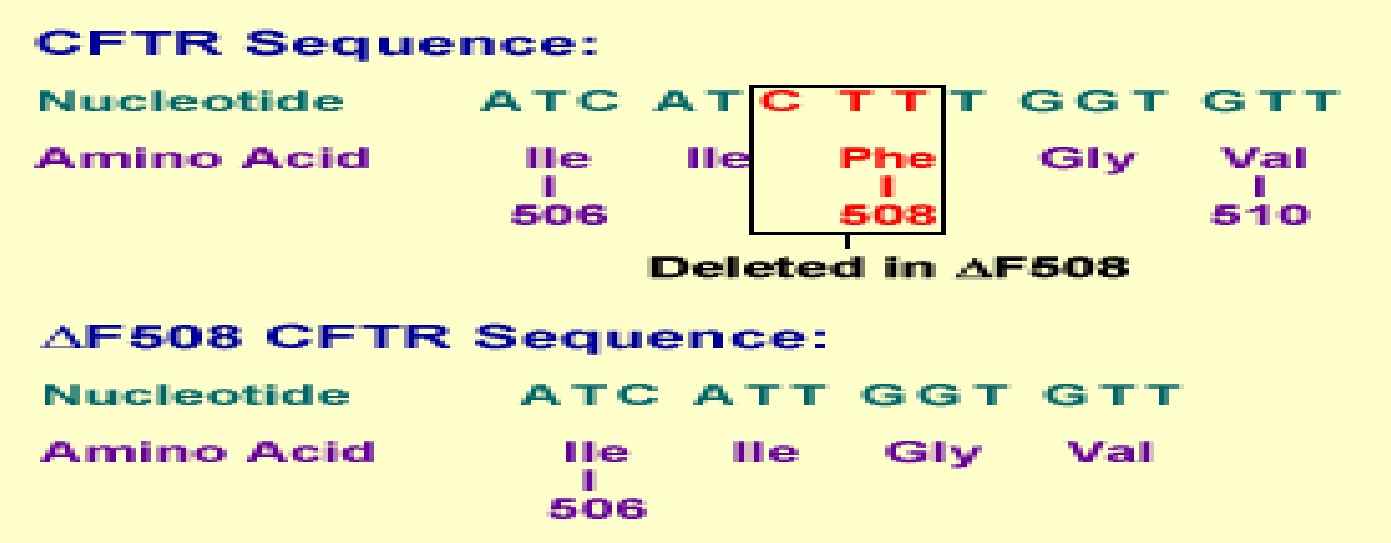


The genetic analysis showed that this gene, which is responsible for this disorder, contains 27 exons spreading over 250 kb of chromosome 7 (7q31) and encodes an mRNA of 6.5 kb.

The protein is composed of 1480 amino acids. Molecular weight of CFTR protein is 168 kDa.

- Most common mutation is the deletion of phenylalanine ΔF508 (del C508)

  -Deletion of three consecutive base pairs in the ATP –binding nucleotide –binding fold(NBF),cause of defective folding of CFTR in the endoplasmic reticulum which prevent it from moving efficiently through the Golgi Complex.



CFTR has 3-bp deletion leading to Del508 (Phe) in 1480 aa protein (epithelial Cl^- channel) – the protein is degraded in the Endoplasmatic Reticulum (ER) instead of being inserted into cell membrane.

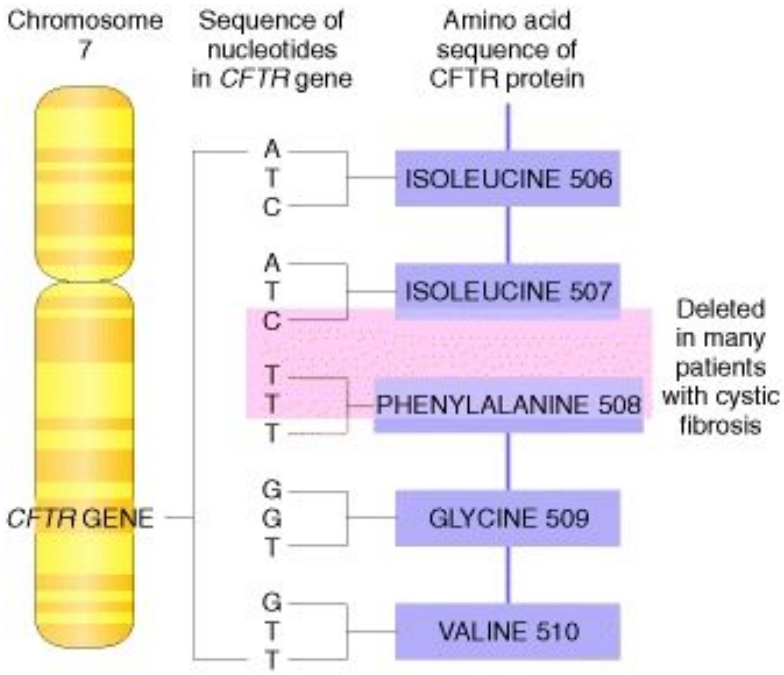
The isoleucine (Ile) at amino acid position 507 remains unchanged because

Cystic Fibrosis Genotype/Phenotype

- The functional consequences of the *CFTR mutations* may be grouped into 6 classes that represent alterations of normal protein production, trafficking, and function at the epithelial cell membrane, or a combination of these abnormalities.
- These classes of mutations give rise to a range of phenotypes extending from classic CF to single-organ involvement
- Class I mutations cause a total or partial lack of production of a functional *CFTR protein, most*
- commonly as a result of a premature termination codon.
- Class II mutations lead to misfolding of the protein and failure of trafficking to the cell surface.
- Class III are mutations where the gating mechanism fails to open in response to intracellular signals.
- The majority of patients in the Cystic Fibrosis Foundation Patient Registry are in the mutation class I–III grouping, and these mutations characteristically have little

Cystic Fibrosis Genotype/Phenotype

- Although the prevalence of *CFTR mutations differs* among populations, the most common *CFTR mutation is* the p.F508del; 85.8% of individuals in the 2017 Cystic Fibrosis Foundation Patient Registry have at least one copy of this mutation with a homozygous prevalence of 45.3%.
- There are 1,800 *CFTR variants reported, although not* all cause disease.
- The CFTR2 database only includes information about 322 of the most common *CFTR variants.*
- It is important to note that not all patients with the same variants have the same outcome.
- Classes IV–VI mutations retain some residual *CFTR* function and are more likely to be associated with pancreatic- sufficient CF.
- Lung function has a less clear correlation between



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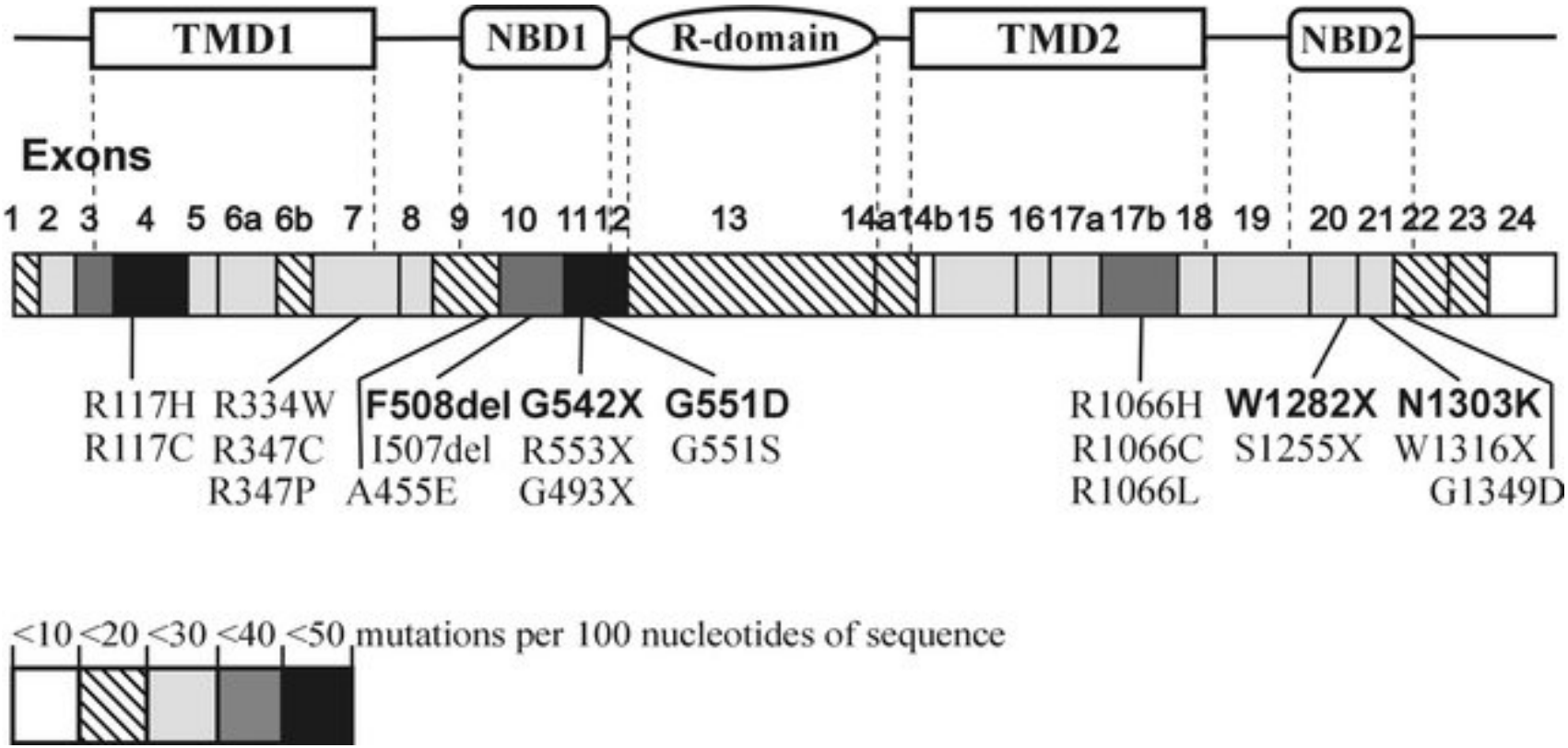
- The most common mutant allele is the F508del mutation in exon 10, *which is a deletion of three nucleotides resulting in the loss of a* phenylalanine residue at codon 508 and subsequent defective intracellular processing of the CFTR protein.
- Mutant CFTR is unable to adopt a proteaseresistant mature conformation*, remains* interacted with the chaperones calnexin and heat shock protein 70 *and is prematurely degradated by the ubiquitinproteasome* pathway in a pre-Golgi compartment.
- *Reduction of temperature or the addition of chemical chaperones* such as glycerol or trimethylamine-*N-oxide* can overcome impediments to the folding and allow proper targeting.
- However, at the cell surface the chloride channel formed therefrom has shown a decreased half-life and reduced open probability and sensitivity

The most common mutation in the CFTR gene



Density of mutations in different exons of CFTR gene. Localization of 5 more common CFTR mutations are given in bold (CFMDB, Available from http://www.genet.sickkids.on.ca/cftr). Some important mutations linked to disease and their approximate location within the CFTR gene and protein are shown (Schwiebert et al., 1997). TMD1 and TMD2, transmembrane domain 1 and 2; NBD1 and NBD2, nucleotide binding domain 1 and 2; R –domain, regulatory domain of the CFTR protein.

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Correlation of CFTR mutations with the chloride channel function

- The molecular anomalies have variable effects on the CFTR protein and its function. Wesh and Smith have proposed a classification of these anomalies in relation to the Cl- channel function(Wesh and Smith ,1993)
- **Class1:**mutations altering the production of the protein. These mutations result in the total or partial absence of the protein. This class includes the nonsense mutations and those that produce a premature stop codon(anomalies of splicing and frame-shift mutations).In certain cases the mutated mRNA is unstable and does not produce protein.
- In other cases, abnormal protein produced will probably be unstable and degrade rapidly. This is what produces the truncated protein or

- Class2: mutations altering the cellular maturation of the protein.A number of mutations alter the maturation of the protein and thus the transport of these proteins to the plasma membrane.In this way,the protein is either absent from the membrane or present in a very small quantity.
- The mutations of this class represent the majority of CF alleles(DF508).
- Class3: mutations disturbing the regulation of Cl- channel.
- These mutations are frequently situated in the ATP binding domain(NBF1 and2).

- Class4:mutations altering the conductance of Cl- channel .Certain segments of membrane spanning domains participate in the formation of an ionic pore.The missense mutations situated in these regions produce a correctly positioned protein that has a cAMP dependent Cl- channel activity.But the characteristic of these channel is different from those of endogenous CFTR channel with a diminution of ion flux and modified selectivity.
- Class5:mutations altering the stability of mRNA.
- Class6:mutations altering the stability of mature CFTR protein.

Regulation of CFTR gene

- Regulation of expression of the CFTR gene is complex. Both CFTR mRNA and protein are highly conserved within a wide range of vertebrate species.
- *They show patterns of expression that seem* to be tightly regulated both developmentally *and in a tissue-specific manner, but with differences depending on the* species.
- *Expression of CFTR is mainly restricted to* epithelial cells in the lung, intestine, pancreas, liver, gall bladder, kidney, salivary and sweat glands, testis and uterus.
- In each of these tissues only a subpopulation of specified cells is involved in CFTR expression.
- In humans, the pancreas and intestinal tract are the locations of the most

Function of CFTR protein as chloride channel

- CFTR is an epithelial anionic channel stimulated by ATP and cyclic AMPdependent PKA.
- *CFTR Cl– channel* activity may also be regulated by membrane-associated phosphatases in an inhibitory manner and by protein kinase C isoforms in an either stimulatory or inhibitory manner.
- *CFTR channel is* characterised by small conductance (6–10 pS) with an ability to transport passively 106 –107 ions/s.
- *The channel has a linear current* voltage relationship in Cl– solutions.
- It is selective for anions over cations and exhibits the following anion permeability sequence: Br– ≥ Cl– > I– > F–.
- *These features are* conferred on CFTR Cl– channels by the function of the TMDs. the NBDs. and the R-domain.

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Function of CFTR protein as chloride channel

- Activation of the cAMP-dependent PKA causes the phosphorylation of multiple serine residues within the R-domain.
- PKA is the primary kinase that phosphorylates the R-domain.
- *This allows* binding of ATP to NBD1.
- When ATP is hydrolyzed by NBD1, the channel opens and anions can flow, according to the electrochemical gradient, through the pore.
- When the R-domain is fully phosphorylated, the NBD2 can bind ATP.
- This event stabilizes the open state of the chloride channel and results in longer openings. In the next step, when ATP is hydrolyzed at NBD2 and ADP and 25 phosphate ions are released from both NBDs, the channel will close again.
- For this fine-tuning of channel activity, interaction between both NBDs

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Other functions of CFTR

- CFTR also acts as a regulator of other channels in the apical membrane.
- Before CFTR was identified, the defect in chloride transport found in CF epithelia was assigned to the malfunctioning of the outwardly rectifying chloride channel **(ORCC).**
- ***Studies have shown that*** CFTR activates the ORCC and this process is dependent on the presence of the NBD1 and R-domain of CFTR.
- A Ca**2+-sensitve Cl– secretory pathway is also present in the human airway,** and has been found to be intact in the epithelia of patients with CF.
- *Recent findings indicate the CFTR is able to inhibit endogenous* calcium-activated chloride currents.

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- The abnormal concentration of mucus in the lungs of patients with CF reflects a primary abnormality of airway epithelial ion–water transport.
- In CF, the airway epithelium is susceptible to fluid hyperabsorption because of a defect in the secretion of chloride and bicarbonate anions mediated by the *CFTR and by an intact sodium-absorptive path.*
- *This abnormal* epithelial fluid absorption raises osmotic pressures in the mucus layer that exceed those in the periciliary layer that deplete the airway surface of fluid, leading to hyperconcentrated (dehydrated) mucus, impaired mucus transport, and mucus adhesion to airway surfaces.

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- An increase in mucin (the principal component of airway mucus) secretion is suggested by the formation of endobronchial mucus plaques and plugs, which become the main sites of chronic airway infection, not airway epithelial cell surfaces.
- Air-flow obstruction and recurrent bacterial infections are common features of CF lung disease.
- Endobronchial infection induces an excessive and ineffective inflammatory response characterized by elevated airway levels of neutrophil elastase that contribute to excess mucus adhesiveness and cohesiveness (ie, sticky and stringy).
- A chronic cycle of infection and inflammation is initiated, resulting in damage to the structural integrity of the airways and leading to the

Other problems associated with CF

- Small growth (polyps) in the nose
- Increased roundness of finger and toe nails
- An enlarged liver and spleen
- Diabetes
- Infertility in men, because the vas deference (tube carrying sperms) may fail to form. Almost all males with CF are sterile because of congenital bilateral absence of vas deferens (CBAVD).
- Fertility problem in women ,due to thicker mucus making fertilization difficult.

Pancreatic disease

- Exocrine pancreatic insufficiency (PI) is present from birth in about 85–90% of CF patients.
- *Similarly to that observed in the* lung, abnormal mucus secretions cause obstruction of the pancreatic ducts, followed by dilatation of the secretory ducts and acini.
- The pancreatic enzymes continue to be produced, but cannot reach the gut, so the gland undergoes tissue destruction and fibrosis.
- This process begins *in utero and can continue over a period of many years, and the* degree of destruction usually correlates with the age of the patient.
- Clinically, pancreatic enzyme deficiency leads to malabsorption of protein and fat and fat-soluble vitamins, producing a distended abdomen and bulky, loose, greasy, foul-smelling stools.

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Pancreatic disease

- A further important consequence of the severe pancreatic damage is deficiency of pancreatic bicarbonate, resulting in a diminished capacity to buffer influxes of gastric acid into the duodenum.
- *This* reduces the efficacy of endogenous and exogenous pancreatic enzymes and favours the precipitation of bile salts, which thereafter are less effective in fat solubilisation, leading to impaired lipolysis.
- The endocrine function of the pancreas can also be impaired in CF disease.
- Insulin–dependent diabetes becomes prevalent with increasing age, and beyond the age of 10–15 years there is an almost linear decrease in the percentage of patients with a normal oral glucose tolerance test .
- About 9–12% of all CF patients require insulin therapy (Cystic Fibrosis Foundation, 2003; European Epidemiologic Registry of CF, 2000).

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Gastrointestinal disease

- Diseases of the gastrointestinal tract are usually less prominent in CF patients.
- However, about 10 to 15% of CF newborns suffer from obstruction of the small intestine, called meconium ileus (MI), due to reduced water secretion and sludging of intestinal contents.
- *The* accumulation of undigested proteins (e.g albumin), when mixed with intestinal mucus, produces an impervious and hyperviscid meconium substance.
- Dehydratation of the intestinal contents can already be detected *in utero* by ultrasonography as hyperechogenic fetal bowel.
- Later in life, recurrent episodes of bowel obstruction called distal intestinal obstruction syndrome or the equivalent of MI are also characteristic for CF, and are encountered by about 20% of CF patients.

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Hepatobiliary disease

- Severe liver disease is the second leading cause of mortality in CF.
- However, clinical evidence of severe liver disease with hepatic cirrhosis and portal hypertension occurs in only 2–7% of all CF*, but focal biliary cirrhosis is present at autopsy* in about 25% of patients.
- The secretion of chloride ions and thereafter water into the biliary duct is necessary to keep bile acids and proteins soluble in the bile fluid.
- Abnormal inspissated mucus secretions in the bile ductules causes obstruction, dilatation, inflammation and focal biliary cirrhosis that over time progress to multilobular cirrhosis.
- The early pathogenesis of CF liver disease is incompletely understood, but is known to involve biliary dysfunction, hepatic stellate cell activation and a disruption of the homeostasis of the extracellular matrix, leading to

Changes in sweat glands

- The most consistent functional alteration in CF has been elevated concentrations of chloride ion and sodium ion in sweat, which are the basis for the principal diagnostic test for the disease.
- *The number of sweat glands* is normal and there are no structural abnormalities in the eccrine sweat glands in CF.
- *Normal sweat chloride values in neonates range from 6* to 32 mmol/l (mean 16.9 mmol/l).
- The sweat gland is composed of two regions: the secretory coil and the resorptive duct, and the functions of both components are defective in CF patients.
- The coil secretes an ultrafiltrate in response to cholinergic and β-adrenergic agonists that is almost isotonic with plasma.
- The β-adrenergic response of the secretory coil is consistently absent in

Changes in sweat glands

- Normally, as the ultrafiltrate passes the water-impermeable duct, chloride ion and sodium ion are absorbed.
- CFTR is the only channel in the sweat duct capable of reabsorption of chloride ion and if defective, electrolyte concentrations are up to 5 times higher.
- In a sweat test, a chloride ion concentration of >60 mmol/l is found in approximately 98% of patients with CF.
- In patients with chloride concentration within the normal or borderline range and also bearing specific CFTR mutations e.g. 3849+10kbC→T, delayed diagnosis
- has been reported.
- The major clinical manifestation of abnormal sweat gland function is excessive salt loss, which in young children can sometimes lead to hypochloremic, hyponatremic alkalosis and dehydratation. Because of the sweat electrolyte defect, young CF patients are more prone to heat prostration .

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Pathogenesis of CF Lung Disease

- Efficient mucociliary clearance is essential for respiratory health.
- The mucociliary clearance apparatus consists of 2 hydrogels: a mucus layer and a periciliary layer.
- Effective mucociliary transport is dependent on proper hydration of the airway surface liquid.
- Mucociliary clearance may be impaired by abnormalities of ciliary motion or by changes in the composition of mucus that make it less responsive to ciliary propulsion.
- In healthy persons, airway epithelia can secrete or absorb ions and water, preserving normal airway surface hydration.

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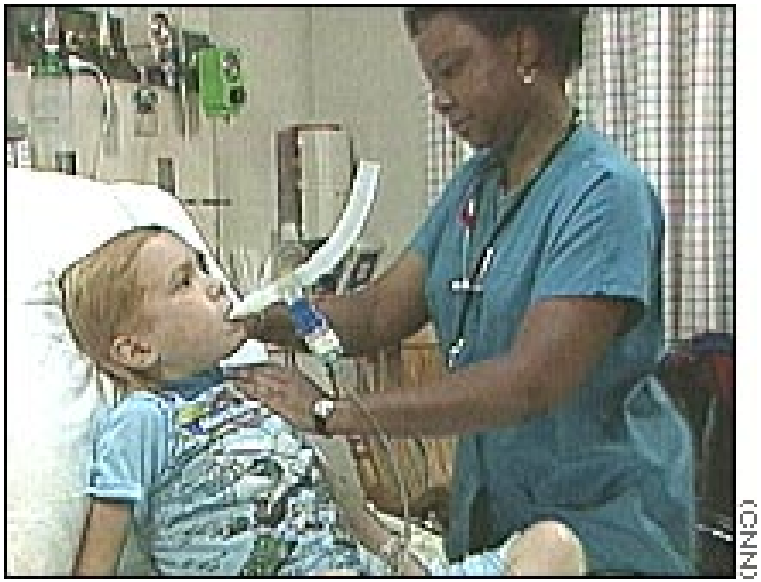
- Each mutation has different effects
- Severity of disease is also affected by mutations in other genes

Methods of Detection/Diagnosis

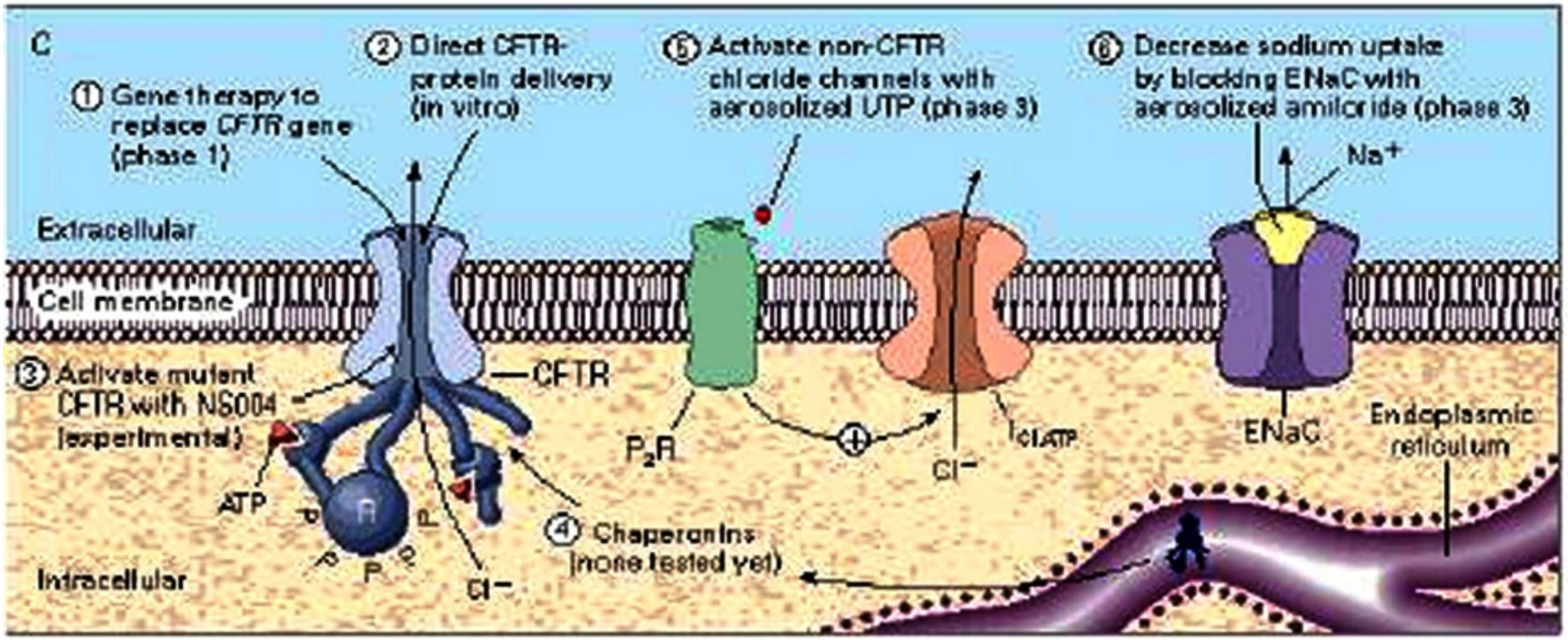
- A test to measure the amount of salt
- Tag-It Cystic Fibrosis Kit
- Amniocentesis (for prenatal testing)

CYSTIC FIBROSIS :Treatment

- Chest physiotherapy
- Mucolytics (DNAase)
- Bronchodilators and Decongestants
- Antibiotics (inhaled and systemic)
- Pancreatic enzyme replacement
- Nutritional support (Turmeric)
- Gane therapy



Cystic Fibrosis: possible therapies



Diagnosis

- **Diagnostic Imaging**
- **Pulmonary Function Testing**

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Diagnosis of CF

- About one in five babies with CF are diagnosed at birth ,when their gut becomes blocked by extra thick meconium (the black fat-like bowel contents that all babies pass soon after their birth).This condition may need surgery.
- Just over half of the people with CF are diagnosed as babies because they are not growing or putting on weight as they should .This is because the pancrease is not producing enzymes which pass into gut as food leaves the stomach.Without theses enzymes the fat in the food cannot be properly digested .In children who are affected ,the fat passes straight through the gut .The children does not benefit from get the energy from fat.

Sweat chloride test

- A sweat chloride level of 60 mmol/L confirms a clinical diagnosis of CF.
- A sweat chloride level in the normal range (29 mmol/L) indicates that CF is improbable; however, sweat chloride values in the normal range have been reported in the presence of certain *CFTR utations,* thus genetic analysis may be indicated in some clinical situations even if the sweat test is in the normal range.
- For individuals with intermediate sweat chloride level of 30–59 mmol/L, genetic analysis is required.
- Further testing for *CFTR function, such as nasal potential* difference, and intestinal current measurement may also be indicated and performed in a specialized diagnostic CF care center.

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Diagnostic Imaging

- Progressive lung disease is the life-limiting factor in CF.
- Capturing the severity of CF lung disease and monitoring it over time are important in guiding clinical care and in measuring outcomes in clinical trials.
- Imaging offers information on the regional distribution of CF lung disease, so longitudinal imaging is recommended for disease tracking in clinical practice. Chest radiography, CT, and magnetic resonance imaging (MRI) are now available as common imaging techniques.

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Pulmonary Function Testing

- Pulmonary function tests are routinely performed in the clinical management of patients with CF, and they are being increasingly applied in infants and preschool children.
- They are useful to determine the severity of lung disease, to gauge response to therapy, and to monitor disease progression.

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Suggested Reading

1. Human Molecular Genetics – Tom Stratchen & Andrew P. Read. Pub: John Wiley & Sons.
2. An introduction to Genetic Analysis – Griffith, Miller, Suzuki, Lewontin, Gelbard. Pub: W.H. Freeman & Co.
3. Genomes 2 – T.A. Brown, Pub: Wiley-Liss. John W. & Sons.
4. Emery's Elements of Medical Genetics – R.F. Mueller, I.D. Young, Pub: Churchill
5. An Introduction to Human Molecular Genetics – J.J. Pasternak, Pub: Fitzgerald Science