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# Cystic Fibrosis in Human - A review

**R. K. Patel**
Genetic Group of Gujarat and Diagnostic Centre, Mehsana, India- 384002
*Corresponding author: rkpatel46@yahoo.com

**Abstract**

Cystic fibrosis is an inherited Autosomal recessive monogenic genetic disease in a various human population worldwide. The disease characterized by the accumulation of thick and sticky mucus that can damage many body organs starting with respiratory system associated with chronic digestive system most severely affected leading to death in 90% of patients. Various mutations in the *CFTR* gene located on human chromosome 7 with specific location 7q31.2 are the cause of disease. The *CFTR* gene synthesizes a protein called the cystic fibrosis transmembrane conductance regulator that controls the movement of salt and water in and out of human body's cells. Therefore, *CFTR* is an ion channel protein that transports chloride ions across the membranes of cells that line airways, glands, and the digestive tract. Chloride ions balance the water, making mucus thick or thin. The article discusses background, prevalence, etiology, complications, diagnosis, possible treatments and future development in clinical research of cystic fibrosis.

**Key words**: *CFTR* gene, Cystic fibrosis, mutation, nonsense mutation,

## Introduction

Though the disease was not named in the history of medical science, but people were aware for cystic fibrosis since 1857. It was popularized by German saying 'the child will soon die whose brow tastes salty when kissed' (1). The clinical entity of disease was first described by Dorothy Andersen in 1938 (2) and it was considered as a lethal disease of babyhood. Genetic cause and inheritance pattern of the disease were described in 1946. During 1950s the sweat test was developed as a result of discoveries made by Paul di Sant'Agnese during the heat wave in New York in 1953 (3). It was later standardized by Gibson and Cooke in 1955 (4). In 1955, Cystic Fibrosis Foundation was established in the US and chloride transport was identified as the basic physiologic defect of CF in 1983. In 1985, gene causing cystic fibrosis was narrowed down to chromosome number 7. Finally, Professor Lap-Chi Tsui and his colleagues identified the specific fault in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in 1989 (5).

Cystic fibrosis is an inherited Autosomal recessive monogenic genetic disease in humans. The disease, characterized by the accumulation of thick and sticky mucus that can damage many body organs starting with respiratory system associated with chronic digestive system most severely affected, leading to death in 90% of patients (6). Mucus is a substance that protects the linings of the respiratory system, digestive system, reproductive system, and other organs and tissues. Mucus is a viscous fluid containing inorganic salts, glycoproteins, antimicrobial enzymes, immunoglobulins and water and produced from cells found in mucous glands. In cystic fibrosis, the body produces mucus, which is abnormally thick and sticky. This abnormal

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mucus can block the windpipe of the respiratory system and can lead to severe breathing problems and bacterial infections in the lungs. Over time, mucus and infections result in permanent lung damage, including formation fibrosis and cysts in the lungs. Cystic fibrosis is a fatal disorder of childhood. Whereas, with improved treatments and better management many people with cystic fibrosis now live well into adulthood. The Cystic Fibrosis Foundation (CFF) is projecting a life expectancy of 37 years for CF patients currently (7). Whereas, a UK study predicts that a CF patient can expect to live more than 50 years of age (8). In countries with limited resources like India, the survival of children with CF is lagging behind considerably as compare to the developed countries.

***Genetic inheritance:*** The CF is monogenic autosomal recessive genetic disease that means when a mother carrier of CF mates with normal father will produce 50% carrier and 50% normal progeny (Figure 1). Similarly, when both father and mother are carriers, they will produce 25% normal, 50% carrier and 25% affected for CF (Figure 2).

***Symptoms:*** The prominent sign of the CF in affected babies as they have salty skin that can be realized when mother or any person kisses baby. The disease appears in many organs but mainly upper and lower respiratory tracks, pancreas, digestive system, and reproductive tracts (9). For most patients, lung disease is the serious problem that sometimes causes death of a person. Respiratory system complications include bronchiectasis, pneumonia, nasal polyps, hemoptysis, pneumothorax and eventually respiratory failure (10). An affected person suffers from persistent cough with phlegm. Wheezing or dyspnea (shortness of breath) on exertion is commonly observed in patients of CF.

Digestive system complications elucidate nutritional deficiency. Affected babies have a blockage intestine that occurs shortly after birth. Nearly 20% of people with cystic fibrosis develop diabetes by age of 30. The pancreas is the vital gland responsible for the digestion of carbohydrate, protein and lipid through the secretion of various digestive enzymes into the duodenum (11). The concentrated mucus secretion causes obstruction of the ducts inhibiting secretion of digestive enzymes. CF condition is associated with abdomen pain, diarrhoea, heartburn, severe constipation, common gastrointestinal problems, etc. Other complications cause poor digestion and absorption due to small intestine bacterial overgrowth, enteric circular muscle dysfunction, abnormal intestinal mucus, and intestinal inflammation. As a result, frequent greasy and bulky stools with difficult bowel movements are also observed in the CF patients. Because of poor digestion and absorption, patients exhibit poor growth and less weight gain sometimes in spite of a good appetite and food intake.

The majority of adult males with CF (99%) are characterized by congenital bilateral absence of vas deferens (CBAVD) or blocked vas deferens, which carry sperm. CBAVD is encountered in 1- 2% of infertile males without CF (12). Females with CF are found to be less fertile than normal healthy women. In females with CF, delayed puberty and amenorrhoea are common due to malnutrition. CF in females is associated with congenital absence of the uterus and vagina (13). Epididymal obstruction (14), bilateral ejaculation duct obstruction with seminal vesicle abnormality (15) was also reported in CF patients.

Other complications may include Osteoporosis, Electrolyte imbalances and dehydration manifesting as increased heart rate, fatigue, weakness and low blood pressure, etc.

***Occurrence:*** There are approximately 70,000 worldwide cases and probably 1000 new cases are added every year. CF is very common in the white population of northern European ancestry having 1 in 2000–3000 births as reported by the Cystic Fibrosis Foundation (16, 17), and less in Asian- Americans having 1:30,000 newborns (18). As reported by the National Institute of Health,

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USA, (19), the Cystic fibrosis (CF) is the most common life-shortening disease among the white population of the United States, affects more than 30,000 people in the United States and 80,000 people worldwide. The incidence varies considerably among different ethnic groups and country. The birth prevalence is estimated to be approximately 1 in 2500 children born in the United Kingdom (20). It is less common in African Americans (1 in 15000), American black population (1:17,000) and the Native American population (1:80,000) (18, 21). The total births in India during year 2012 were estimated to be 27.271 million (22). The number of children born each year with CF may be approximately 10908 presuming incidence to be about 1 in 2500 live births (5). However, most of these children are dying may be due to poor health condition, malnutrition, ignorance for diagnosis and non-availability of diagnostic tests.

***Genetic cause:*** Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on human chromosome 7 with specific location 7q31.2. Normally, the *CFTR* gene provides instructions for making a protein called the cystic fibrosis transmembrane conductance regulator. This protein controls the movement of salt and water in and out of human body's cells. Therefore, CFTR is an ion channel protein that transports chloride ions across the membranes of cells that line airways, glands, and the digestive tract. Chloride ions balance the water, making mucus thick or thin (Figure 3). When Chloride ions do not pass through due to absence of CFTR membrane protein, the mucus viscosity becomes thick, which cannot be moved by the cilia of epithelium cells. The thick mucus attracts invading pathogens which becomes a layer over mucus and develops infection.

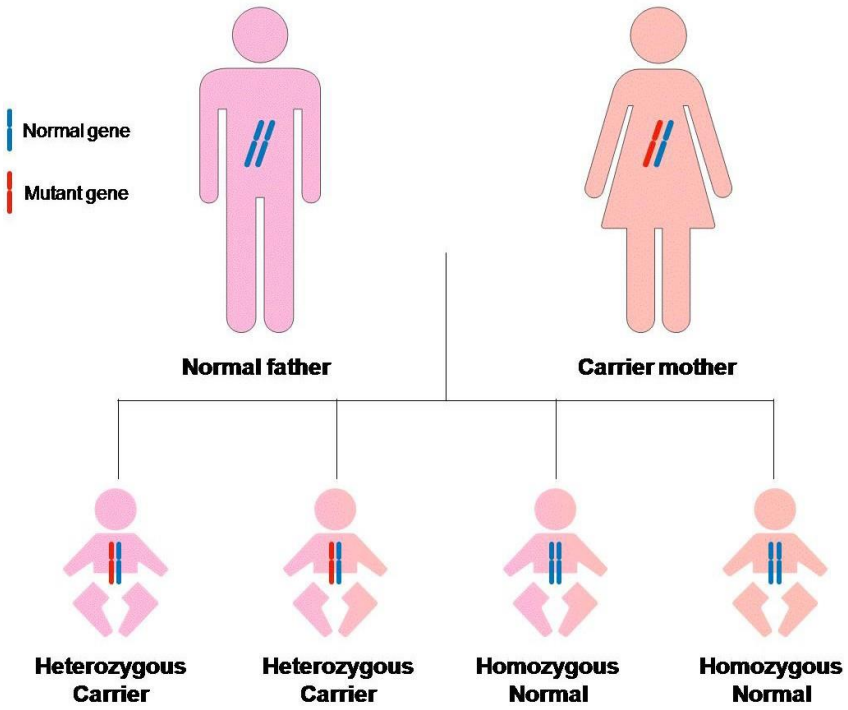


**Fig. 1.** Heterozygous carrier and homozygous normal

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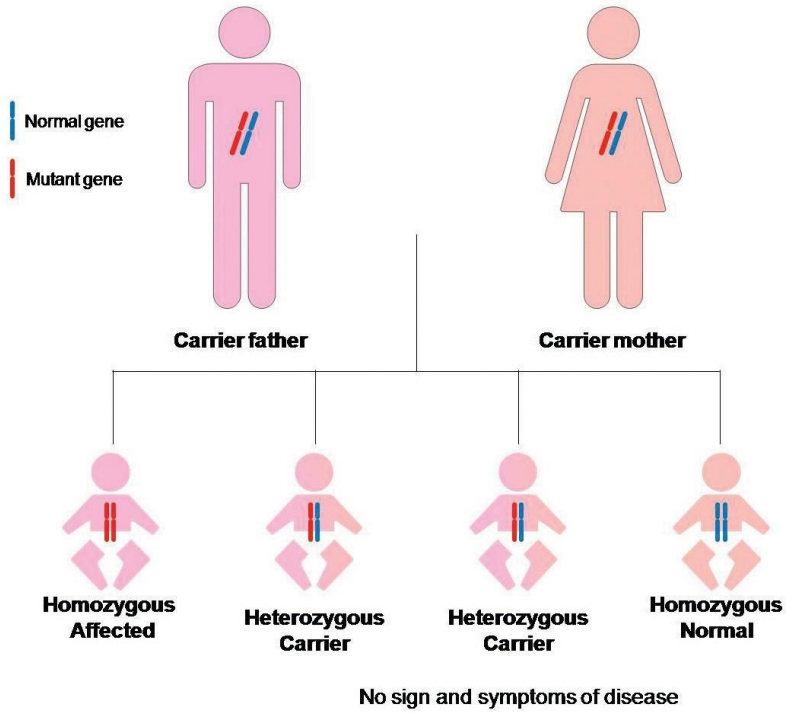


**Fig. 2.** Affected, carrier and normal

Because of the bacterial infection, massive neutrophil infiltration takes place that releases elastase (proteases enzyme). The protease enzymes counteract with the lung antiproteases resulting in tissue destruction (23). Additionally, degranulating neutrophils release large quantities of nucleic acids and cytosol matrix proteins contributing to the mucus hyper-viscosity (24) that causes a narrow passage of the airways, glands, digestive system, etc. When it happens in exocrine gland like pancreas it develops fibrosis and cyst. *CFTR* is highly expressed in the pancreas, particularly in the small intercalated ducts that connect the acini (25). Deficiency of functional CFTR in CF thus leads to decreased ductal cell secretions of chloride ions ($Cl^-$), water and bicarbonate ($HCO3^-$), which also reduces the pH (26, 27).

More than 2,000 mutations in the *CFTR* gene have been identified in people with cystic fibrosis. Most of these mutations change amino acids in the CFTR protein or delete a small amount of DNA from the *CFTR* gene. The most common mutation, called delta F508, is a deletion of one amino acid (phenylalanine in the tenth exon) at position 508 in the CFTR protein causing frame shifting. The delta F508 (ΔF508) is also renamed as p.F508del, constitutes about 70% of the total cases worldwide. As a result of ΔF508 mutation in the *CFTR* gene, it produces abnormal channel of CFTR protein, which breaks down shortly after it is synthesized, so it never reaches the cell membrane to transport chloride ions. The mucus becomes thick because of less water and no ions transportation in and out of the cell membrane. The normal allelic variant of this gene is about 250,000 base pairs long with 27 exons. The product of this gene, CFTR protein, is made of 1480 amino acids and is a member of the ATP Binding Cassette (ABC) transporter super family (28).

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Since AF508 was reported, a number of other, different mutations have been described in the CFTR locus. Granell et al. (29) reported 84-bp deletion in exon 13 of the CFTR gene in a six month old female baby with signs of CF. the 84bp deletion was detected by DNA amplification and direct sequencing of 500 bp of the 5' end of exon 13 and, as a consequence, 28 amino acid residues should be lost in the regulatory domain of the CFTR protein.

The DNA samples from 16 Hutterite CF families were analysed. The Hutterite most inbred population in North America. A new CF homozygous mutation, M1l01K was identified in exon 17b by Polymerase Chain Reaction- single -strand conformation polymorphism (PCR-SSCP) analysis of a 263bp segment of amplified DNA. Sequencing analysis identified the mutation as a T-to-A transaction at position 3434, leading to a predicted change of methionine (codon 1101, ATG) to lysine (codon AAG). The mutation was associated with maldigestion in three siblings (30). Another mutation, M1101R, which is a transversion of T to G at the same nucleotide position and should also result in a basic amino acid substitution, were identified by other investigators in a Turkish CF patient who was ΔF508/M1101R and had pancreatic insufficiency (W. Lissens et al - personal communication).

Severe pancreatic disease, but the mild pulmonary disease with nonsense mutations in each CF gene was observed in one of African American patients (31). The genetic investigation revealed that a patient with two nonsense mutations (stop codon); R553X and W1316X in the CF gene had exhibited an intense degree of reduced quantity of CFTR mRNA in respiratory epithelial cells. The nonsense mutation R553X (C→T at nucleotide 1789) occurs in exon 11 of the CFTR whereas, W1316X (A→G at nucleotide 4079)1 occurs exon 21 gene and produces a truncated protein missing the regulatory domain, leading to a reduction or absence of cytoplasmic CFTR mRNA (32). However, when mRNA is severely decreased or undetectable, the respective protein product is absent. Occasionally, nonsense mutations are associated with normal mRNA levels, but truncated proteins (33). Hamosh and his group reported a compound heterozygote for this R553X

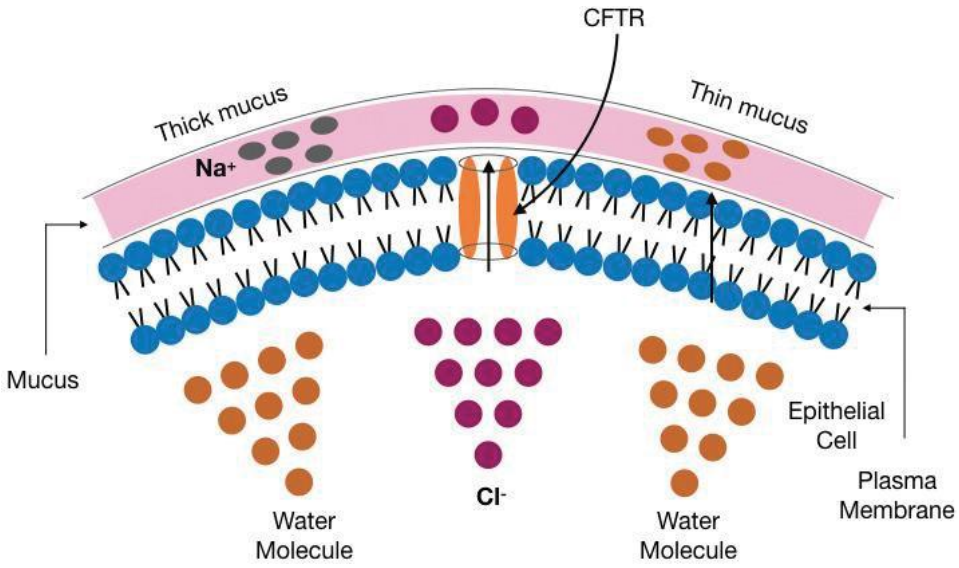


**Fig. 3.** Ion exchange through CFTR protein resulting thin and thick mucus

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mutation and the S549N missense mutation in another patient.

In the UK, a new mutation G551D (G'!A at neucleotide 1784) in exon 11 was reported along with Delta F508 and R553X mutations in 111 children and teenagers with cystic fibrosis (34). A nonsense R1162X mutation (C→T at nucleotide 3616) in exon 19 in nine CF patients characterized by mild pulmonary disease phenotypes was reported (35). A new mutation G542X (G→T at nucleotide 1756) in exon 11 of the CFTR gene that was responsible for a stop mutation in codon 542 was found in Canadian patients (36). The same mutation was observed in a Belgian patient. The G542X mutation accounted for 7.3% of the CF chromosomes in Belgium, being probably the second most frequent mutation (37). A novel mutation *1429 del7bp* was also reported in a one year old Hispanic female in combination with the well-established G542X mutation in exon 11 (38). Currently, there are 2089 mutations listed in the CFTR mutation database includes missense, Frameshift, Splicing, nonsense, deletions, etc. and most of them are very rare.

***Diagnosis of CF:*** The primary diagnostic test for cystic fibrosis is the measurement of sweat electrolyte levels (39). Patients with the disease reveals the greater chloride value (>60 mmol/L) than normal and baby skin tastes salty when kisses. The UK now has a screening programme for all newborns for cystic fibrosis using the Guthrie blood spot test (40) and positive samples are then tested for the CFRT gene mutation. A blood test of a baby to estimate the levels of a chemical produced by the pancreas called immunoreactive trypsinogen (IRT) is also a very useful test for diagnosis of CF.

However, CF results in thick and sticky mucus obstructing the pathways leading to serious lung infections especially pseudomonas. Lung infection may be diagnosed by simple chest X-rays indicate inflated lungs, fibrosis in lung and scaring. A sinus X-ray shows the signs of sinusitis. Similarly lung function test, sputum culture can also help in the diagnosis. If CF is confirmed, pulmonary radiographs are used to monitor disease progression. Radiographs also are commonly used as a diagnostic tool for patients with symptoms reflective of CF that have not been previously diagnosed (17).

Genetic test is the appropriate to detect mutations in the *CFTR* gene. As mutations are large in number in the CFTR gene, it is advisable for PCR and DNA sequencing, and full mutation scan of the gene. The screening test for people without a family history of CF will also be done on the most common gene mutations, and so cannot be said to be 100% accurate. The most common mutation is delta F508 (ΔF508) in 10$^{th}$ exon exists in 70% cases that can be carried out by simply PCR-sequencing. When this mutation is not the cause of CF, one has to find out other mutations in *CFRT* gene preferably by the Ambry Test. The test is a full mutation scan of the CFTR gene by temporal temperature gradient electrophoresis analysis (TTGE) followed by dye terminator DNA sequencing of suspect regions. The Ambry Test or exome sequencing covers all *CFTR* exons and at least 20 bases 5' and 3' into each intervening sequence, and select deep intronic mutations (38).

***Treatment and management:*** Presently, there is no cure for cystic fibrosis but treatments are available to manage the symptoms, prevent complications, and make the condition easier for the patients to live with.

To prevent and control lung infections of CF patients, wide range of antibiotics; azithromycin, tobramycin, aztreonam and levofloxacin, ciprofloxacin, cephalexin, amoxicillin and doxycycline, depending on the severity are administrated. These medicines also include inhaled forms to control respiratory inflammation (41, 42). In order to reduce viscous, thick and sticky mucus from the lungs and to dilate the airways, bronchodilators like beta-agonists are advised to inhale with humidify oxygen therapy. Inhalation of Dornase Alfa (synthetic protein or pulmozyme) can also be useful that breaks down

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excess DNA in the pulmonary secretions of people with cystic fibrosis and reduce the risk of respiratory tract infections (43,44). A lung transplant may be required if the lungs are damaged.

For gastrointestinal track blockage in CF patients, oral rehydration therapy (ORT) to replace fluid to prevent and treat dehydration is usually practiced. Osmotic laxatives (stool softener) are useful and hyperosmolar contrast enemas are given in case of distal intestinal obstruction syndrome (DIOS). Electrolyte intestinal lavage solution (washing solution) is also very effective agent in the treatment of chronic constipation in the CF patients (45). Medicines are given to patients so that they can absorb food better for digestion and special diet and food supplements are provided to prevent malnutrition. Pancreatic insufficiency may be overcome in CF patients by the pancreatic enzyme replacement therapy (PERT) (46). PERT is the use of medications that contain enzymes which are produced by a normal pancreas. These medications contain proteases to digest protein, amylases to digest carbohydrates and lipases to digest fat. Digestion of protein, carbohydrates and fats helps prevent malabsorption. Providing appropriate nutrition and preventing dehydration, a high calorie fat diet, A, D, E, K, vitamins and minerals are supplemented in CF patients. Additionally, sodium chloride is also supplemented to the patients depending on age and environmental conditions (47).

***Development approach in treatment:*** The current and future therapeutic targets are mainly focused on correcting structural and functional abnormalities of the CFTR protein. These therapies include messenger RNA therapy, DNA or gene therapy, and gene editing. All of these therapies can be possible in the early stages of development so that normal and functional CFTR protein may be synthesized. CFTR modulators like *I vacaftor* is a drug approved by FDA used to treat cystic fibrosis in people with certain mutations in the CFTR gene (primarily the G551D mutation), which account for 4-5% cases of cystic fibrosis (48). However, it is not found to be effective in the most common mutation F508del. Another CFTR modulator, lumacaftor has shown favourable results in F508del mutation common in 72% patients (49). Most promising current drug Ataluren has shown to improve chloride transport in CF patients with nonsense mutation (50). Limitations are also observed in using CFTR modulators which includes some time non-significant effects, other daily symptomatic treatment, side effect, cost effective treatment and no guarantee of cure. However, DNA screening of heterozygosity or suspected parents and prenatal molecular screening can reduce the bourdon of CF patients in the population.

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