The basic science of cystic fibrosis

Sarah-Jane Bowen

Jeremy Hull

Abstract

Cystic fibrosis (CF) is a monogenic autosomal recessive condition affecting approximately 1 in 3000 in the Caucasian population. It is a life-limiting condition with effects on multiple organs, predominantly the lungs, gastrointestinal tract, pancreas and liver. The cystic fibrosis transmembrane conductance regulator (CFTR) anion channel regulates chloride, bicarbonate and sodium transport across cell membranes, predominantly in epithelial tissues. In cystic fibrosis the CFTR protein is structurally or functionally abnormal due to mutations in the CFTR gene located on Chromosome 7. Approaching 2000 CFTR mutations have been identified to date though not all are disease-causing. There is phenotypic heterogeneity in CF due to variability in how mutations affect CFTR biosynthesis and the impact of genetic and environmental modifying factors. Advances in understanding of the pathophysiology of CF have led to improved outcomes though there remains a significant burden of disease. There have been promising results from the development of mutation-specific small molecule therapies and personalised care is felt to be crucial in further improving the outlook for those with CF.

Keywords CFTR; chloride channel; cystic fibrosis; genetics; lungs; mutations; paediatrics

Introduction

Cystic fibrosis (CF) is an autosomal recessive condition that affects around 70,000 people worldwide. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which lead to defective or insufficient amounts of functional CFTR protein. This causes abnormalities in chloride, bicarbonate and sodium transport across cell membranes with consequences on multiple organs. The predominant clinical manifestations of CF are lung disease, pancreatic insufficiency and gastrointestinal complications. Other potential consequences of CF include diabetes, liver and bone disease and infertility. CF lung disease is characterised by infection and inflammation with eventual bronchiectasis, and respiratory failure is the cause of death in over 90% of CF patients. The cornerstones of the management of CF are effective airway clearance, proactive treatment of infection and maintenance of good nutritional status. In the 1960s CF was universally fatal in childhood; current estimates suggest a median survival of 44 years.

Jeremy Hull ***BM Bch DM*** *is a Paediatric Respiratory Consultant at Oxford Children's Hospital, John Radcliffe Hospital, Headington, Oxford, UK. Conflict of interest: none declared.*

Sarah-Jane Bowen ***MBBS MRes*** *is a Paediatric Respiratory Registrar at Oxford Children's Hospital, John Radcliffe Hospital, Headington, Oxford, UK. Conflict of interest: none declared.*

CFTR gene discovery

The autosomal recessive inheritance pattern for CF was reported in 1946 and the gene was discovered in 1989. Based on existing hypotheses of pathogenesis at that time and the predicted structure of the protein, the gene was called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The CFTR gene is located on chromosome 7q31.2 spanning ~200 kb of genomic DNA and comprising 27 exons.

CFTR protein structure

The CFTR gene is transcribed into 6.13 kb of messenger ribonucleic acid (mRNA) encoding the 1480 amino-acid CFTR protein. CFTR belongs to the ATP-binding cassette (ABC) gene superfamily whose products include transmembrane proteins involved in transporting a wide variety of substrates across intra- and extra-cellular membranes. CFTR is the only known ABC transporter that acts as an anion channel. Normal CFTR structure and function is shown in Figure 1.

Biosynthesis of mature CFTR protein involves a delicate folding process in the endoplasmic reticulum (ER) membrane, the ER lumen and the cytosol. Correct folding of the CFTR protein is essential for a normally functioning and stable channel. If defective folding is identified by the chaperones involved in cellular quality control the abnormal protein is degraded by the ubiquitin-proteasome system. Only 30% of nascent CFTR proteins make it to the cell membrane where the levels of functional CFTR are meticulously regulated with recycling via endosomal pathways.

CFTR protein expression and function

CFTR is expressed in multiple tissues. The protein is predominantly found at the apical (luminal) membrane of polarised epithelial tissues including:

- Airway surface epithelium and submucosal glands
- Pancreatic duct epithelium
- Epithelium of crypt cells, submucosal glands and Brunner's glands in the gastrointestinal tract
- Epithelial lining of the gall bladder and intra-hepatic bile ducts
- Sweat gland epithelium
- Salivary gland epithelium
- Epithelium of the vas deferens and epididymis
- Cervical and uterine epithelium
- Kidney collecting duct epithelium

The epithelial tissues that express CFTR largely correspond to the sites affected in CF disease, with a few exceptions such as the kidney where other pathways appear to be more functionally significant. CFTR is also expressed, though generally to a lesser extent, in non-epithelial cells and tissues including:

- Ventricular cardiomyocytes and aortic smooth muscle cells
- Neurones in the brain, corneal and vascular endothelial cells
- Lymphocytes

CFTR predominantly functions as a cAMP-regulated chloride channel but it also mediates secretion of bicarbonate and smaller amounts of other anions. Although ATP is required to open the channel, once open there is passive movement of ions along

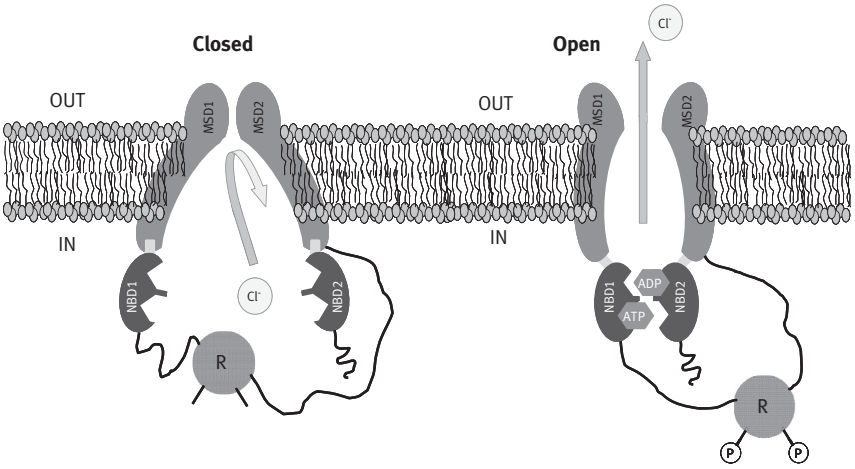

Figure 1 CFTR Structure and Regulation of CFTR Opening (Adapted from Hwang and Sheppard 2009*). Binding of ATP places the channel in an 'open-ready' state and facilitates movement of chloride across the epithelial cell membrane. Subsequent ATP hydrolysis causes return of the CFTR protein to the 'closed' conformation. *Abbreviations: MSD, membrane-spanning domain; NBD, nucleotide-binding domain; R, regulatory domain; P, phosphorylation*. *Hwang TC, Sheppard DN. Gating of the CFTR Cl^- channel by ATP-driven nucleotide-binding domain dimerization. J Physiol. 2009 May 15; 587 (Pt 10): 2151–61.

electrochemical gradients. In the airway epithelium the CFTR protein also has an inhibitory effect on nearby epithelial sodium channels (ENaC).

CFTR mutations

CF is a monogenic disease with an autosomal recessive inheritance pattern. It is most common in populations of northern European descent with an incidence approximating 1 in 3000 and a carrier frequency of 1 in 25. In contrast CF occurs in 1/4000–10,000 Latin Americans, 1/15,000–20,000 African Americans and 1/350,000 Japanese.

Approaching 2000 CFTR gene mutations have been reported to date though the majority are rare and not all are CF-causing. In some populations a small number of CFTR mutations predominate while other areas display greater mutational diversity. The most common mutation in Europe is Phe508del (previously referred to as ΔF508), which is an in-frame (3 base pair) deletion of the phenylalanine amino acid at position 508 of the protein. Across Europe and North America this mutation accounts for around two-thirds of known alleles. Twenty-three other mutations are described as 'relatively common' though none of these accounts for more than around 5% of mutations.

Mutations can be classified according to their known or postulated effect on the CFTR protein, as shown in Table 1. There are caveats to this system however in that many mutations are as yet unclassified and some confer features of more than one class.

Class I mutations tend to confer severe disease as the non-functional CFTR protein produced is degraded within the cell. Class II 'trafficking' mutations where, at best, only negligible amounts of CFTR protein reach the apical membrane are the commonest, specifically Phe508del. In Class III 'gating' mutations normal CFTR protein reaches the apical membrane but defective regulation means that the channel cannot be activated and, therefore, fails to open. The most common Class III mutation is Gly551Asp (formerly known as G551D), which is found in around 5% of those with CF. Class IV mutations lead to reduced chloride ion conductance and the phenotype is usually milder. In Class V mutations functional CFTR reaches the apical membrane but in reduced amounts also causing less severe disease. Class VI mutations cause unstable CFTR protein with reduced longevity at the apical membrane, which confers a poor prognosis.

Advances in understanding of CFTR biosynthesis and how different mutations disrupt this in CF have prompted a shift in therapeutic approaches in CF. Rather than seeking to ameliorate the downstream effects of absent or abnormal CFTR, the emphasis is now on targeting and correcting the underlying defect. Small molecule drugs with the potential to regulate the basic pathways affected in CF, i.e. cellular processing of CFTR and chloride ion transport via the CFTR channel, have been identified using high-throughput cellular assays. Small molecule agents have been classified as CFTR 'correctors', which aim to partially correct defective CFTR folding or trafficking, or CFTR 'potentiators', which increase CFTR ion channel activity. It is now recognised that some agents possess both properties.

The most promising results in this field have come from the development of Ivacaftor; a CFTR potentiator that has been shown to increase chloride secretion and airway surface liquid *in-vitro* in the Class III gating mutation Gly551Asp. Clinical trials have demonstrated that this orally-bioavailable drug reduces sweat chloride, improves lung function, reduces exacerbation rates, increases weight gain and positively affects quality of life. It is now being used in adults and children over 6 years of age with Gly551Asp and some other gating mutations.

Efforts to develop a drug targeted at the commonest CFTR mutation Phe508del have been hampered by the apparent need to not only correct abnormal CFTR processing but also to enhance CFTR function at the cell membrane. Recently published trials of the combination of a corrector called Lumacaftor and the potentiator Ivacaftor have proved inconclusive as to the impact on sweat chloride and clinical outcome measures.

Another agent that has been developed is Ataluren; a CFTR corrector that can allow read-through of the premature termination codon often seen in Class I nonsense mutations. However the positive results seen in Phase 1 and 2 trials were not observed

