Cystic fibrosis

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Cystic fibrosis is the most common lethal genetic disease in white populations. The outlook for patients with the disease has improved steadily over many years, largely as a result of earlier diagnosis, more aggressive therapy, and provision of care in specialised centres. Researchers now have a more complete understanding of the molecular–biological defect that underlies cystic fibrosis, which is leading to new approaches to treatment. One of these treatments, hypertonic saline, is already in use, whereas others are in advanced stages of development. We review clinical care for cystic fibrosis and discuss recent advances in the understanding of its pathogenesis, implementation of screening of neonates, and development of therapies aimed at treating the basic defect.

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Introduction

The outlook for people diagnosed with cystic fibrosis—the most common lethal genetic disease in the white population—has improved substantially in the past 10–20 years. The US Cystic Fibrosis Foundation's projected life expectancy for patients has increased from 31 years to 37 years over the past decade,[1] and a UK model predicting that a child born with cystic fibrosis today will typically live to be 50 years of age or more seems to be realistic.[2]

Cystic fibrosis is caused by a mutation in a gene that encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is expressed in many epithelial cells and blood cells. Although CFTR functions mainly as a chloride channel, it has many other regulatory roles, including inhibition of sodium transport through the epithelial sodium channel, regulation of the outwardly rectifying chloride channel, regulation of ATP channels, regulation of intracellular vesicle transport, acidification of intracellular organelles, and inhibition of endogenous calcium-activated chloride channels.[3–7] CFTR is also involved in bicarbonate–chloride exchange. A deficiency in bicarbonate secretion leads to poor solubility and aggregation of luminal mucins.[8]

More than 1500 CFTR mutations have been identified, but only the functional importance of a small number is known. The table shows one classification system for the most common mutations based on their functional alterations. The absence of phenylalanine at position 508 (Phe508del, also known as F508del; see panel 1 for glossary of genetic terms), which is a class II mutation, accounts for about two-thirds of mutated alleles in northern European and North American populations. Although CFTR mutation frequency varies from population to population, worldwide no other single mutation accounts for more than approximately 5% of CFTR mutations.[10,11]

Pancreatic insufficiency is closely associated with class I–III mutations; however, variability in genetic background (ie, all other genes in the genome) and environment make genotype–phenotype associations weak, especially with regard to lung disease. Manifestations of

Search strategy and selection criteria

We searched Medline (up to September, 2008), Google Scholar for specific topics, and the Cochrane Library for English language reviews pertinent to cystic fibrosis. Additionally, we used systematic reviews prepared by Karen Robinson at Johns Hopkins University (Baltimore, MD, USA) for the US Cystic Fibrosis Foundation's Pulmonary Care Guidelines Committee for chronic therapy, airway clearance, and exacerbation sections of the Seminar. References from previously published reviews of cystic fibrosis were reviewed with inclusion of the most recent and relevant studies.

	Effect on CFTR	Functional CFTR present	Sample mutations
Class I	Lack of protein production	No	Stop codons (designation ending in X; eg, Trp1282X, Gly542X); splicing defects with no protein production (eg, 711+1G→T, 1717–1G→A)
Class II	Protein trafficking defect with ubiquitination and degradation in endoplasmic reticulum/golgi body	No/substantially reduced	Phe508del, Asn1303Lys, Gly85Glu, Leu1065Pro, Asp1507, Ser549Arg
Class III	Defective regulation; CFTR not activated by ATP or cyclic AMP	No (non-functioning CFTR present in apical membrane)	Gly551Asp, Ser492Phe, Val520Phe, Arg553Gly, Arg560Thr, Arg560Ser
Class IV	Reduced chloride transport through CFTR at the apical membrane	Yes	Ala455Glu, Arg117Cys, Asp1152His, Leu227Arg, Arg334Trp, Arg117His*
Class V	Splicing defect with reduced production of normal CFTR	Yes	3849+10kb C→T, 1811+1·6kb A→G, IVS8-5T, 2789+5G→A

Adapted from reference 9 by permission of Edward Arnold (Publishers) Ltd. CFTR=cystic fibrosis transmembrane conductance regulator. *Function of Arg117His is dependent upon the length of the polythymidine track on the same chromosome in intron 8 (IVS8): 5T, 7T, or 9T. There is more normal CFTR function with a longer poly-T track.

Table: **Classification of CFTR mutations**

Panel 1: Glossary of terminology used to identify common CFTR gene mutations

Ala455Glu: A455E
Ala559Thr: A559T
Ala561Glu: A561E
Arg1162X: R1162X
Arg117Cys: R117C
Arg117His: R117H
Arg334Trp: R334W
Arg347Pro: R347P
Arg553Gly: R553G
Arg533X: R533X
Arg560Thr: R560T
Arg1066Cys: R1066C
Arg560Ser: R560S
Asn1303Lys: N1303K
Asp1152His: D1152H
Asp1507: D1507
Gly85Glu: G85E
Gly542X: G542X
Gly551Asp: G551D
Ile507del: I507del
Leu227Arg: L227R
Leu346Pro: L346P
Leu1065Pro: L1065P
Phe508del: F508del
Ser492Phe: S492F
Ser549Arg: S549R
Ser549Asn: S549N
Trp846X: W846X
Trp1282X: W1282X
Val520Phe: V520F

CFTR=cystic fibrosis transmembrane conductance regulator. Left-hand terms use three-letter aminoacid codes; right-hand terms use one-letter aminoacid code.

cystic fibrosis can be very different between patients, even siblings, with the same CFTR genotype. Polymorphisms in non-CFTR genes might explain this discrepancy. Several studies have shown that polymorphisms in transforming growth factor β1 and mannose-binding lectin-2 genes are associated with more severe lung disease, with evidence of gene–gene interactions.[12–14] Similarly, two or more modifier genes seem to be the major determinants of intestinal obstruction in newborn babies with cystic fibrosis.[15] Identification of modifier-gene polymorphisms could lead to more accurate prediction of the course of illness in an individual patient; the gene products could become therapeutic targets.

Cystic fibrosis is most common in populations of northern European descent, among whom the disease occurs in approximately 1 in 3000 births.[11] Birth prevalence varies from country to country, and with ethnic background (figure 1). For example, the disease occurs in roughly 1 in 3000 white Americans, 1 in 4000–10 000 Latin Americans, and 1 in 15 000–20 000 African Americans.[11] Cystic fibrosis is uncommon in Africa and Asia, with a reported frequency of 1 in 350 000 in Japan.[21] In Europe the Phe508del mutation predominates in the northwest, and decreases in frequency towards the southeast; the most common mutation in Israel is Trp1282X.

There are several hypotheses regarding how CFTR dysfunction leads to the phenotypic disease known as cystic fibrosis. Four hypotheses are outlined below; it is possible that aspects of all four contribute to the pathogenesis of the disease.

The low-volume hypothesis postulates that the loss of inhibition of epithelial sodium channels, because of CFTR dysfunction, leads to excess sodium and water reabsorption, resulting in dehydration of airway surface materials.[22–24] Concomitant loss of chloride efflux prevents the epithelium from correcting the low airway surface water volume. The subsequent decrease in periciliary water volume results in a reduction in the lubricating layer between epithelium and mucus, with compression of cilia by mucus causing inhibition of normal ciliary and cough clearance of mucus. According to this hypothesis, mucus on the epithelium forms plaques with hypoxic niches that can harbour bacteria, particularly *Pseudomonas aeruginosa*.[24,25]

The high-salt hypothesis argues that in the absence of functional CFTR, excess sodium and chloride are retained in airway surface liquid.[26,27] The increased concentration of chloride in the periciliary layer disrupts the function of important innate antibiotic molecules (eg, human β-defensin 1), allowing bacteria that are cleared by normal airways to persist in lungs.[28]

Dysregulation of the host inflammatory response has been postulated as the putative basic defect in cystic fibrosis. Support for this hypothesis lies in the fact that abnormally high concentrations of inflammatory mediators are seen in cystic fibrosis cell cultures and uninfected ex-vivo tissue samples.[29–32] Furthermore, findings from lung lavage studies show that inflammation is present in children as young as 4 weeks of age who are apparently free of infection.[33] An increase in proinflammatory molecules such as interleukin 8, interleukin 6, tumour necrosis factor α, and arachidonic acid metabolites has been found in patients with cystic fibrosis.[34–36] Stimulation of the nuclear factor-κB pathway, platelet hyper-reactivity, and abnormalities in neutrophil apoptosis have also been reported.[37–39] At the same time, concentrations of native anti-inflammatory substances such as interleukin 10, lipoxin, and docosahexaenoic acid are reduced,[31,34,40] leading to an imbalance between proinflammatory and anti-inflammatory mediators that favours unabated inflammation.

Another hypothesis suggests that primary predisposition to infection is a mechanism by which CFTR dysfunction leads to cystic fibrosis. In normal hosts, *P aeruginosa* binds to functional CFTR and initiates an innate immune response, which is rapid and self-limiting. In patients with cystic fibrosis, an increase in asialo-GM1 in apical cell membranes allows increased binding of *P aeruginosa* and *Staphylococcus aureus* to airway epithelium, without initiation of the CFTR-mediated immune response.[41,42] The result is that in cystic fibrosis, the rapid, self-limiting response that eliminates *P aeruginosa* from the airways is lost at the same time as there is enhanced attachment of bacteria to the epithelial surface.

Diagnosis

The diagnosis of cystic fibrosis should be considered in any child or adult who presents with the signs or symptoms listed in panel 2. Diagnostic algorithms for classic and non-classic cystic fibrosis have been published by the European Union Cystic Fibrosis Diagnostic Working Group and the US Cystic Fibrosis Foundation.[43,44] These guidelines concur that the diagnosis of cystic fibrosis consists of finding specific clinical (phenotypic) characteristics in combination with biochemical or genetic markers of CFTR dysfunction. In general, a diagnosis of cystic fibrosis can be made in a patient with clinical features of the disease if the concentration of chloride in sweat is greater than 60 mmol/L or if it is in the intermediate range (30–59 mmol/L for infants less than 6 months of age, 40–59 mmol/L for older individuals), and two disease-causing CFTR mutations are identified.[44]

The sweat test remains the most readily available and clinically useful way of making the diagnosis of cystic fibrosis, provided it is done according to strict guidelines, with pilocarpine iontophoresis and a quantitative determination of chloride concentration.[45] Sweat chloride concentration increases with age in people without cystic fibrosis; however, a concentration greater than 60 mmol/L is still diagnostic of the disease.[46] Panel 3 lists some of the causes of false-negative and false-positive sweat test results.

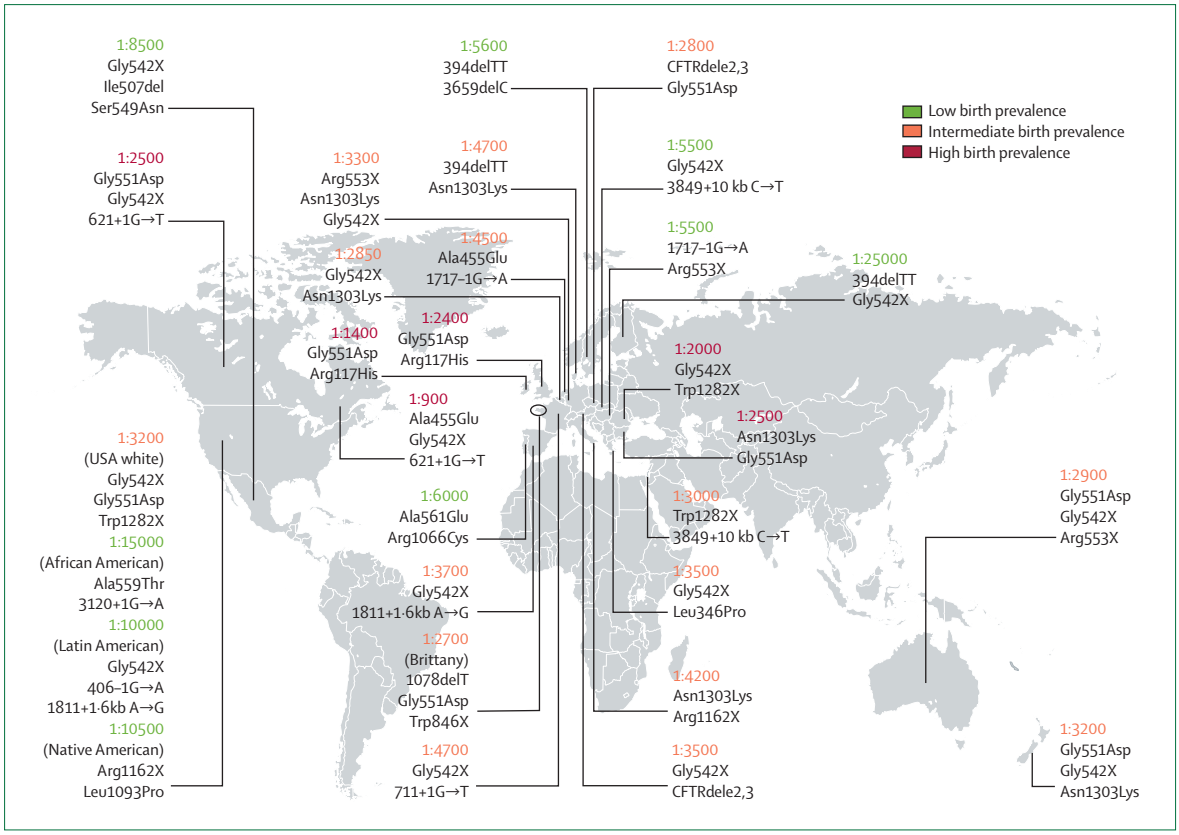

***Figure 1*: Approximate cystic fibrosis birth prevalence and common mutations for selected countries**
Birth prevalence is reported as number of live births per case of cystic fibrosis. Common/important mutations in each region are listed below the prevalence. The birth prevalence can vary greatly between ethnic groups in a country. Data from references 10, 11, and 16–20.

Several methods of CFTR mutation detection are commercially available. In general, use of a discrete group of mutation probes is faster and less costly than expanded mutation analysis, and incorporation of the 40 most frequent disease-associated mutations will detect over 90% of affected individuals in most populations. A concern with limited analysis is that the diagnosis will be missed if the patient is affected by a mutation that is not screened. Full sequence analysis will detect most CFTR mutations; however, it might reveal polymorphisms and novel mutations of unknown importance.

A helpful method in assessing individuals who might have cystic fibrosis, but who do not meet classic diagnostic criteria, is measurement of nasal transepithelial potential difference (NPD).[43,44] Unfortunately, measurement of NPD is labour intensive, technically difficult, and not available at all cystic fibrosis centres. NPD might be useful in the research setting as a marker of the ability of pharmaceutical agents to alter chloride channel function.

The number of people recognised as having milder problems possibly associated with CFTR dysfunction is growing. These problems include male infertility, recurrent pancreatitis, chronic sinusitis, and primary sclerosing cholangitis.[47,48] Sweat testing is rarely helpful, since these patients will frequently have borderline chloride concentrations; however, a sweat chloride concentration more than 60 mmol/L will confirm the diagnosis of classic cystic fibrosis. CFTR mutation analysis, if done, should be interpreted by someone with good knowledge of cystic fibrosis genetics, since variants that are not diagnostic might be found. For practical and psychological reasons, it is best to use the term "CFTR-related diseases" for this group of disorders, rather than saying that the patient has cystic fibrosis.

Newborn screening

Newborn screening is done by the measurement of immunoreactive trypsinogen (IRT) in blood spots taken from newborn infants. A very high IRT concentration suggests pancreatic injury consistent with (but not specific for) cystic fibrosis. This marker is increased even in infants with class IV or V mutations that are associated with pancreatic sufficiency. Infants who have a high IRT concentration on initial testing undergo further assessment via a repeat IRT 1–3 weeks later (IRT/IRT), or by analysis of the initial blood spot for a specified group of CFTR mutations (IRT/DNA).[49,50] The advantages of IRT/IRT screening are that it avoids the problems associated with detecting mutations of uncertain clinical

Panel 2: Signs and symptoms of cystic fibrosis

General (any age)
- Family history of cystic fibrosis
- Salty-tasting skin
- Clubbing of fingers and toes
- Cough with sputum production
- Mucoid *Pseudomonas aeruginosa* isolated from airway secretions
- Hypochloraemic metabolic alkalosis

Neonatal
- Meconium ileus
- Protracted jaundice
- Abdominal or scrotal calcifications
- Intestinal atresia

Infancy
- Persistent infiltrates on chest radiographs
- Failure to thrive
- Anasarca or hypoproteinaemia
- Chronic diarrhoea
- Abdominal distention
- Cholestasis
- *Staphylococcus aureus* pneumonia
- Idiopathic intracranial hypertension (vitamin A deficiency)
- Haemolytic anaemia (vitamin E deficiency causes anaemia by increasing fragility and reducing lifespan of red blood cells)

Childhood
- Chronic pansinusitis or nasal polyposis
- Steatorrhoea
- Rectal prolapse
- Distal intestinal obstruction syndrome or intussusception
- Idiopathic recurrent or chronic pancreatitis
- Liver disease

Adolescence and adulthood
- Allergic bronchopulmonary aspergillosis
- Chronic pansinusitis or nasal polyposis
- Bronchiectasis
- Haemoptysis
- Idiopathic recurrent pancreatitis
- Portal hypertension
- Delayed puberty
- Azoospermia secondary to congenital bilateral absence of the vas deferens

significance, and does not require immediate discussion of carrier status or non-paternity. The IRT/IRT algorithm needs a second blood spot to be taken 1–3 weeks after the first one, which could be a major logistical problem in some populations, whereas the IRT/DNA method allows complete testing on one blood specimen. Mutations included in the DNA analysis should reflect the frequency of specific cystic fibrosis mutations in the local population.[50] A positive screening result by either method only indicates that a child is at increased risk for cystic fibrosis; a sweat test must still be done to confirm the diagnosis.

In regions that undertake newborn screening, it has become highly unusual to see infants present with cystic fibrosis at an advanced age, or with the classic symptoms of respiratory disease and emaciation. Several studies have shown that newborn screening for cystic fibrosis leads to improved nutritional outcomes.[51,52] Other studies have shown that weight for age in infancy correlates positively with lung function at 6 years of age.[53,54] There are data supporting the hypothesis that improved growth in infancy as a result of newborn screening leads to improved pulmonary function later in life.[55] Two UK Cystic Fibrosis Trust registry-based studies have shown the clinical benefit and cost-effectiveness of newborn screening;[56,57] the US Centers for Disease Control and Prevention supports use of cystic fibrosis newborn screening throughout the USA.[58]

Clinical manifestations

Cystic fibrosis-related symptoms appear throughout life, with great overlap and variability of symptoms and timing from patient to patient. Figure 2 shows the approximate age of onset of some of the major clinical complications of the disease.

Gastrointestinal symptoms

Around 15% of infants with cystic fibrosis are born with meconium ileus, an obstructive condition secondary to inspissated material in the small and large bowels. 85–90% of infants with cystic fibrosis develop pancreatic insufficiency, which can be present at birth or evolve over the first year of life. Typical signs of pancreatic insufficiency are greasy stools, flatulence, abdominal bloating, and poor weight gain. Pancreatic insufficiency leads to steatorrhoea, fat-soluble-vitamin deficiency, and malnutrition. At the time cystic fibrosis was first recognised in 1938, the life expectancy of patients was only months; death was caused by malnutrition.[59] With the introduction of pancreatic enzyme replacement therapy, malnutrition became manageable; however, adequate caloric intake and correction of fat-soluble-vitamin deficiencies remain crucial components of disease control.[60] Thickened intestinal secretions, malabsorption, and decreased gut motility can lead to distal intestinal obstruction or chronic constipation in older patients.[61] Poor absorption of fat soluble vitamins (A, D, E, and K) can lead to acrodermatitis, anaemia, neuropathy, night blindness, osteoporosis, and bleeding disorders.

Patients with cystic fibrosis are at risk for focal biliary cirrhosis caused by obstruction of intrahepatic bile ducts, but clinically apparent cirrhosis occurs in only about 5% of patients, and usually presents by 15 years of age.[61] Bleeding from oesophageal varices is life-threatening for patients who have portal hypertension; intensive intervention by gastroenterologists and surgeons is needed to control it.

Pulmonary disease

The lungs of children with cystic fibrosis are normal in appearance at birth, but quickly become infected and inflamed, with polymorphonuclear cells present in bronchoalveolar lavage fluid obtained from even healthy-looking infants.[33] Chronic airway infection, progressing to bronchiectasis, gas trapping, hypoxaemia, and hypercarbia is the hallmark of cystic fibrosis lung disease; pulmonary insufficiency is responsible for at least 80% of cystic fibrosis-related deaths.[1]

Typically, infants with cystic fibrosis are rapidly colonised by *Haemophilus influenzae* or *S aureus*, or both. Within a short time, *P aeruginosa* becomes the predominant organism found in the airways.[62] One group of investigators showed that 39 (98%) of a cohort of 40 cystic fibrosis infants had serological or culture evidence of *P aeruginosa* infection by 3 years of age.[63] Persistent infection leads to generation and secretion of chemotactic cytokines, which recruit large numbers of polymorphonuclear cells into the airways. *P aeruginosa* amplifies the cycle of infection and inflammation by releasing toxins and elastases that cleave crucial surface markers on polymorphonuclear cells. These cells then release their own proteases and elastases that exacerbate injury to any viable polymorphonuclear cells in the region.[64] Thereafter, bacterial exotoxins and products of the damaged neutrophils spur further polymorphonuclear cell recruitment, more inflammation, and increased tissue damage. Release of DNA from senescent polymorphonuclear cells leads to increased sputum viscosity.[65]

The airways of cystic fibrosis patients are conducive to the growth of *P aeruginosa* for several reasons: permissive microenvironments within the hypoxic niches of adherent mucous plaques, increased bacterial binding to the epithelium, and decreased bacterial clearance via innate immune mechanisms.[23,25,41,42] Initially, *P aeruginosa* grows as a non-mucoid strain that can be cleared by the host, or eradicated with aggressive antibiotic treatment.[66,67] Over time, *P aeruginosa* colonies synthesise an alginate coat and form biofilms.[42] These biofilms, once established, are difficult if not impossible to clear with standard antibiotic treatment. There is a pronounced survival benefit for those patients who remain free of pseudomonas infection.[68,69] For this reason, heightened surveillance for *P aeruginosa* has become commonplace, with strategies to eradicate early infection by use of inhaled antibiotics with or without oral quinolones under investigation.[66,70,71]

Cystic fibrosis airways can be infected with other pathogens, such as *Burkholderia cepacia* (a complex of at least nine different species), *Stenotrophomonas maltophilia*, meticillin-resistant *S aureus* (MRSA), and atypical mycobacteria.[72] Many *Burkholderia* species have innate antibiotic resistance, are transmissible from person to person, and are highly virulent. Infection with *B cepacia* complex can cause a rapid decline in pulmonary function, and increased mortality in patients with cystic fibrosis.[73] Occasionally, infection with the complex can cause an invasive, fatal bacteraemia—the so-called "cepacia syndrome". *Burkholderia cenocepacia*, one of the species in the complex, is highly transmissible: infection with it is associated with a striking deterioration in

Panel 3: Causes of false-positive or false-negative sweat test results

False-positive result

- Atopic dermatitis (eczema)
- Malnutrition
- Congenital adrenal hyperplasia
- Mauriac syndrome
- Fucosidosis
- Ectodermal dysplasia
- Klinefelter's syndrome
- Nephrogenic diabetes insipidus
- Adrenal insufficiency
- Hypothyroidism
- Autonomic dysfunction
- Environmental deprivation
- Munchausen syndrome by proxy

False-negative result

- Dilution of sample
- Malnutrition
- Peripheral oedema
- Low sweat rate (quantity not sufficient)
- Hypoproteinaemia
- Dehydration
- CFTR mutations with preserved sweat duct function (eg, 3849+10kb C→T; Arg117His–7T)

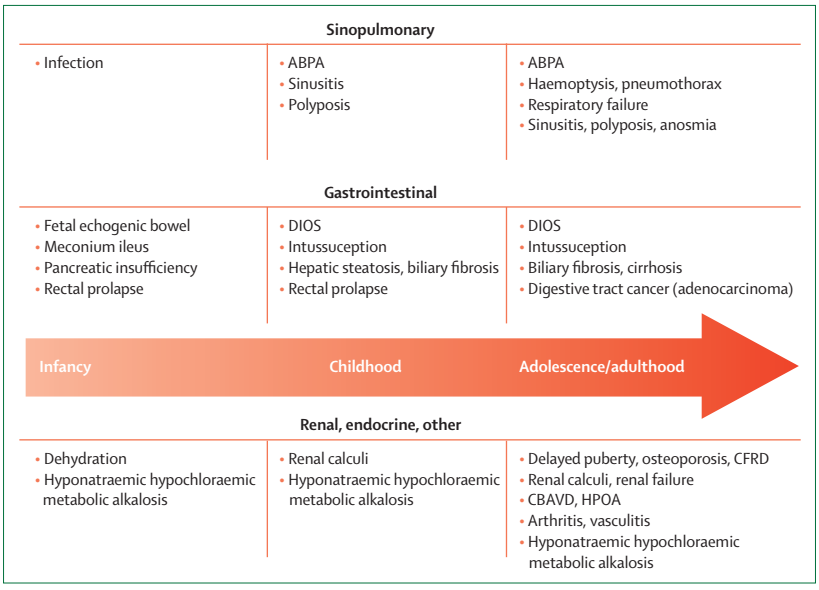

***Figure 2*: Approximate age of onset of clinical manifestations of cystic fibrosis**
ABPA=allergic bronchopulmonary aspergillosis. CBAVD=congenital bilateral absence of the vas deferens. CFRD=cystic fibrosis-related diabetes mellitus. DIOS=distal intestinal obstruction syndrome. HPOA=hypertrophic pulmonary osteoarthritis.

health, perhaps because of the organism's ability to elicit a more robust inflammatory response from host cells than other *B cepacia* species.[74,75] Other *B cepacia* complex species can also cause acute deterioration, highlighting the need for effective infection control at all cystic fibrosis centres.[76]

Approximately 15–20% of patients with cystic fibrosis carry MRSA in their airways; this colonisation is associated with poorer lung function.[77] *S maltophilia* has been found in increasing numbers in patients with cystic fibrosis, but so far has not been shown to be associated with more rapid decline in pulmonary function or wellbeing.[78] Atypical mycobacteria are sometimes found in airway secretions from patients with cystic fibrosis; it remains unclear whether this finding represents true infection in all cases, or is only saprophagous colonisation in some patients. *Mycobacterium avium* complex (72%) and *Mycobacterium abscessus* (16%) were the most common atypical mycobacteria found in a survey of US cystic fibrosis centres.[79] A cross-sectional study from Israel showed an association between non-tuberculous mycobacterial infection and more severe underlying disease, a result not found by the US study.[80] The clinical significance of recovering atypical mycobacteria in sputa from cystic fibrosis patients is not fully understood, but patients with persistently positive sputum smears or cultures should be monitored closely for development of worsening disease.

Another organism that can cause colonisation without invasive infection is *Aspergillus fumigatus*. An intense allergic response to this fungus—known as allergic bronchopulmonary aspergillosis (ABPA)—is seen in 1–15% of patients with cystic fibrosis, with a frequency that varies geographically.[81,82] Clinical manifestations of ABPA are wheezing, pulmonary infiltrates, and central bronchiectasis. The minimum diagnostic criteria for ABPA are acute or subacute clinical deterioration, total serum IgE concentration more than 500 IU/mL (1200 ng/mL), immediate cutaneous reactivity to aspergillus, and one of the following: (1) precipitins to *A fumigatus* or IgG antibody to *A fumigatus*, or (2) abnormalities on chest radiograph or CT scan that have not cleared with standard antibiotic treatment.[81]

Endocrine disorders

Pancreatic dysfunction is caused by obstruction of intrapancreatic ducts with thickened secretions. With time, the pancreas undergoes autolysis with replacement of the body of the pancreas with fat. When a certain proportion of islet cells are no longer functional, the patient will develop insulin insufficiency and carbohydrate intolerance, possibly coexisting with insulin resistance.[83,84] Cystic fibrosis-related diabetes mellitus (CFRD) is not the same as typical type I or type II diabetes mellitus. Several factors unique to cystic fibrosis affect glucose metabolism, including raised energy expenditure, acute and chronic infection, glucagon deficiency, liver dysfunction, decreased intestinal transit time, and increased work of breathing.[83] As cystic fibrosis patients get older, clinically apparent CFRD is more likely to develop; up to 30% of patients aged over 25 years are reported to have the condition.[1] However, in one study, nearly 40% of adolescent cystic fibrosis patients not previously diagnosed with CFRD had abnormal findings on oral glucose tolerance testing.[84] Notably, female patients with diabetes have poorer survival than male patients.[85] Because of the association between CFRD and more severe pulmonary disease, more frequent pulmonary exacerbations, and poorer nutritional status, any patient irrespective of age who has unexplained weight loss or a decrease in pulmonary function should be assessed for CFRD. Periodic screening with a random glucose concentration should be done in all cystic fibrosis patients; a yearly oral glucose tolerance test is thought to be the best screening method for those 10 years of age and older.[86]

Osteoporosis secondary to vitamin D deficiency, chronic systemic inflammation, and intermittent corticosteroid use is increasingly being recognised as a complication of cystic fibrosis. Osteopenia starts in childhood, but generally manifests in adulthood. Bone resorption exceeds bone formation even in well nourished, clinically stable patients.[87]

Reproduction

The vas deferens is very sensitive to CFTR dysfunction. Virtually all men with classic cystic fibrosis have azoospermia and are infertile because of congenital bilateral absence of the vas deferens, which can also be seen in men with only one CFTR mutation and no other manifestations of cystic fibrosis.[47,88] Women with cystic fibrosis are fertile. Although there is some controversy about the effects of pregnancy in cystic fibrosis, the consensus is that a woman who has adequate nutritional and pulmonary reserve can successfully complete the term of pregnancy.[89]

Treatment

Chronic pulmonary treatment

An aggressive approach to cystic fibrosis care is supported by two epidemiological studies showing that cystic fibrosis centres with high median pulmonary function test results see patients more frequently, obtain more frequent respiratory-tract cultures, and use more oral and intravenous antibiotics than do centres with lower median lung function results.[54,90] In an evidence-based review, the US Cystic Fibrosis Foundation used the US Preventive Services Task Force recommendation grades to assess chronic pulmonary therapies.[91] The highest recommendations were given to inhaled dornase alfa (recombinant human deoxyribonuclease; given daily), and inhaled tobramycin (300 mg twice daily, given in 28-day on-off cycles for use in patients with moderate to

severe disease with *P aeruginosa* in their airways).[91] These two agents were also recommended for use in patients with mild lung disease, although the evidence for benefit is not as robust. The Cystic Fibrosis Foundation guidelines also support the use of inhaled hypertonic saline, chronic azithromycin, ibuprofen, and inhaled β agonists in specific patient populations.[91]

Despite the inflammatory nature of the disease, neither oral nor inhaled corticosteroids were recommended for routine use in patients with cystic fibrosis, because of an unacceptable adverse event profile of oral corticosteroids, and absence of proof of efficacy for the inhaled medication.[91–93] Intravenous colistin has been found to be beneficial in the acute setting when combined with another antipseudomonal antibiotic.[94] Inhaled colistin has been used widely in Europe as a chronic, suppressive treatment, but only two trials met the Cystic Fibrosis Foundation Pulmonary Guidelines Committee's eligibility criteria for inclusion in their report. Neither of these studies showed a benefit from inhaled colistin.[95,96]

Hypertonic saline, macrolide antibiotics, and ibuprofen deserve special note. These are inexpensive, readily available drugs, which are easy to administer and have very few side-effects, and thus could be successfully used in worldwide cystic fibrosis care. Inhaled hypertonic saline represents an exciting change to the notion of cystic fibrosis care that treats only symptoms to one that corrects the underlying defect.[97] By acting as a hyperosmolar agent, hypertonic saline presumably draws water into the airways even when CFTR dysfunction is present. Rehydration of the periciliary layer then allows improved mucociliary clearance. Elkins and colleagues[98] found that patients with cystic fibrosis who received hypertonic saline (4 mL of 7% hypertonic saline twice a day via nebulisation for 48 weeks) had a larger increase in forced expiratory volume in 1 s (FEV_1) and fewer pulmonary exacerbations than patients who received 0·9% saline. Similarly, in a study of 24 patients who received 7% hypertonic saline four times a day for 14 days with or without amiloride, Donaldson and co-workers[99] showed that hypertonic saline improved mucociliary clearance and FEV_1 more when given alone than when given with amiloride. In-vitro data further suggested that sustained hydration of airway surfaces was the factor that caused the improved mucociliary clearance.[99] If hypertonic saline can correct the basic hydration defect in airways of cystic fibrosis patients, as the results from these studies suggest, it would be most effective used early in life, before pulmonary disease becomes established. In a pilot study, inhalation of 7% hypertonic saline was well tolerated in a cohort of 13 infants between 6 months and 3 years of age.[100] Larger studies of hypertonic saline in infants are underway.

Macrolide antibiotics have been used for many years to treat patients with diffuse panbronchiolitis, a disease that shares many features with cystic fibrosis.[101] Four studies have addressed the chronic use of macrolides in cystic fibrosis.[102–105] The largest of these showed that improvement in FEV_1 and reduction in pulmonary exacerbations were higher after treatment with thrice weekly azithromycin than after placebo in *P aeruginosa*-positive patients.[105] The precise mechanisms of action of macrolide antibiotics remain unclear, but azithromycin reduces virulence factor production, decreases biofilm production, and has bactericidal effects on *P aeruginosa* when it is growing in its stationary (biofilm) phase.[106] Furthermore, macrolides can affect cytokine production by many cell types and alter polymorphonuclear cell function, making them effective as both antibiotic and anti-inflammatory agents.[107]

High-dose oral ibuprofen has been studied in two large, long-term, placebo-controlled trials.[108,109] In a single-centre study, Konstan and colleagues[108] showed a decrease in the rate of loss of lung function over 4 years after ibuprofen treatment compared with placebo, with the largest benefit seen in younger patients (5–13 years). A multicentre trial in Canada enrolled patients 6–18 years of age with mild lung disease.[109] In this study there was no significant effect of ibuprofen treatment on the primary endpoint, FEV_1, compared with placebo, although the ibuprofen-treated group spent fewer days in the hospital than patients in the placebo group (1·8 days *vs* 4·1 days per year). No significant adverse events were reported in either of these studies; however, a retrospective report from another institution showed that many patients treated with high-dose ibuprofen chose to discontinue treatment, often because of gastrointestinal side-effects.[110] Ibuprofen treatment, if used, seems to be most beneficial when started before the development of severe inflammation and pathological changes in the lung.[108]

Frequent use of oral antibiotics to reduce symptoms such as cough and sputum production is warranted in the treatment of symptomatic cystic fibrosis patients. Whether prophylactic antibiotics should be used in the asymptomatic infant who is colonised or infected with *S aureus*, however, is less clear. In a randomised, placebo-controlled trial, Stutman and colleagues[111] showed that use of prophylactic cefalexin for up to 6 years decreased rates of positive *S aureus* airway cultures, but at the cost of increased rates of *P aeruginosa* infection. UK studies of the use of prophylactic flucloxacillin in younger children, for a shorter duration, showed reduction of clinical symptoms without an increased rate of pseudomonas infections.[112,113] In view of the severe consequences of *P aeruginosa* infection in patients with cystic fibrosis, the US Cystic Fibrosis Foundation recommends against the use of prophylactic antistaphylococcal agents,[91] but universal agreement about this issue has not been reached.[114]

Pulmonary exacerbations

Treating flares of cystic fibrosis lung disease aggressively, especially with intravenous antibiotics, improves pulmonary outcomes, and presumably extends life

Panel 4: Signs and symptoms of an acute pulmonary exacerbation in cystic fibrosis

This mnemonic is based on Dorothy Anderson's original description of cystic fibrosis as "cystic fibrosis of the pancreas".[59] Adapted with permission from reference 121 (copyright Elsevier, 1994).

C=Cough: increase or change in character
F=Fever: low-grade rise in body temperature
P=Pulmonary function tests (decrease in FEV_1)
A=Appetite: decrease in appetite
N=Nutrition: weight loss
C=Complete blood count: increase in white blood cell count
R=Radiograph: new findings on chest radiograph
E=Examination: new crackles or wheezes
A=Activity: reduction in activity level
S=Sputum: increase in quantity or change in quality

FEV_1=forced expiratory volume in 1 s.

expectancy.[90,115,116] Unfortunately, what constitutes a pulmonary exacerbation of cystic fibrosis is not clearly defined, as highlighted in a series of reviews on pulmonary exacerbation epidemiology, prevention, and treatment.[117–120] Increased cough, change in sputum colour or quantity, decreased appetite or weight, and change in respiratory rate and examination (ie, presence of new crackles or wheezes on auscultation of the chest) are particularly important features (panel 4). The importance of a unified approach to the definition of exacerbations and then treating them appropriately has been shown by Kraynack and colleagues[122] who reported that the median FEV_1 of the patients at their centre rose substantially in a very short time after uniform, aggressive standards were adopted.

Once identified, treatment for a pulmonary exacerbation of cystic fibrosis generally includes antibiotics (oral, inhaled, or intravenous), increased use of airway clearance techniques, and improved nutrition. Intravenous antibiotic treatment has been shown to reduce sputum *Pseudomonas* spp density, and improve pulmonary function.[116] Combination antibiotic treatment with agents that have different modes of action is preferred to single agent treatment to avoid emergence of resistant strains, with treatment lasting about 14 days.[123] Since most patients with exacerbations will have *P aeruginosa* in their airways, the usual in-hospital treatment is a combination of a β lactam, which interferes with cell wall biosynthesis, and an aminoglycoside, which binds bacterial ribosome subunits and inhibits protein production;[123] however, addition or substitution of other antibiotics specific for *S aureus*, *H influenzae*, or MRSA might be necessary. Home-based treatment with intravenous antibiotics is feasible,[124] but might not be as effective as hospital-based treatment.[125,126] Use of combined oral and inhaled antibiotics without hospital admission might be sufficient for milder exacerbation and allows the patient's daily life to continue unimpeded.

Airway clearance techniques

There are many techniques used by patients with cystic fibrosis to augment clearance of tenacious airway secretions. These methods include percussion and postural drainage, positive expiratory pressure (PEP) devices, high pressure PEP devices, active cycle of breathing techniques, airway-oscillating devices, high-frequency chest wall oscillation devices, and autogenic drainage (ie, chest physiotherapy in which the patient does a series of respiratory huffs and coughs designed to move mucus from distal to proximal airways so it can be coughed out). Despite a paucity of well designed, controlled studies, nearly all cystic fibrosis caregivers believe in the benefit of airway clearance techniques as a part of the therapeutic regimen.[127] Bradley and colleagues,[128] summarising several Cochrane systematic reviews, and McCool and Rosen,[129] in guidelines written for the American College of Chest Physicians, describe evidence to support the inclusion of chest physical treatment in the care of patients with cystic fibrosis, but could not find evidence that one form of airway clearance was better than another. Thus, convenience, ease of administration, and patient satisfaction are the main driving forces for the choice of airway clearance method. Notably, percussion and postural drainage done early in life should avoid the head-down position, which could increase the risk of gastroesophageal reflux and aspiration.[130]

Exercise has many beneficial effects on cardiovascular fitness and sense of wellbeing. Several studies show that aerobic and anaerobic exercise improve quality of life in patients with cystic fibrosis and might stabilise lung function to some degree, but there is no evidence that exercise alone should be used as an alternative to airway clearance.[131]

Lung transplantation

Lung transplantation is the final therapeutic option for patients with endstage lung disease. Transplantation has the potential to extend and substantially improve quality of life in properly selected patients. How best to select patients, especially children, for this high-risk procedure is the subject of vigorous debate.[132–134] In Europe, it is unusual for children with cystic fibrosis to be considered for transplantation unless they have a projected life expectancy of less than 2 years despite maximum medical therapy. This cautious approach improves the risk–benefit ratio.[134] 5-year survival post transplant for children is less than 50%, with slightly better outcomes in adults (50% of recipients are alive 6 years post transplant).[135,136] For adults, referral for transplantation generally occurs when a patient's FEV_1 plateaus at less than 30% of that predicted. However, age, sex, lung infection and colonisation, and rate of decline of FEV_1 all affect the decision. The presence

or absence of certain species of *B cepacia* can affect outcome and should be taken into account when assessing lung transplant candidates.[137] Use of nocturnal non-invasive ventilation can improve chest symptoms, sleep-associated hypoventilation, and quality of life in patients with awake hypercapnia who are awaiting transplantation.[138]

Nutrition

The benefits of maintaining good nutrition in regard to long-term survival and lung health cannot be overstated. In a classic study from 1988, Corey and co-workers[139] reported a clear-cut survival advantage for well nourished cystic fibrosis patients compared with less well nourished patients. Peterson and colleagues[140] reported improved FEV_1 trajectory in children who gained weight at an appropriate and uninterrupted rate.

Supplementation with pancreatic enzymes should be used in patients who present with pancreatic insufficiency either on clinical grounds (steatorrhoea, failure to thrive), or as shown by low human faecal elastase-1 concentration.[60,141] Fat-soluble-vitamin supplementation is mandatory in all patients with pancreatic insufficiency. Infants with cystic fibrosis can be safely breastfed, and this form of feeding might confer lifelong benefits.[142] Patients' height and weight should be measured and their body-mass index (BMI) calculated at every cystic fibrosis clinic visit; those showing a decrease in BMI (or BMI percentage in children) or stunting should receive nutritional counselling. In view of the strong correlation between nutritional status and pulmonary function, attention to nutritional wellbeing should be regarded as one of the cornerstones of good lung health in cystic fibrosis; supplements (given orally or via gastrostomy tube) should be strongly considered in any patient with less than optimum growth.

New horizons

The pronounced improvement over the past two decades in life expectancy for patients with cystic fibrosis is largely the result of centralisation of care at cystic fibrosis centres and aggressive treatment of symptoms. Large patient registries have been used to examine treatment outcomes, and to implement quality improvement programmes.[54,57,90,143,144] Recent advances in the understanding of cystic fibrosis pathophysiology have not yet had time to result in substantial improvements in clinical care. The great hope for the future is that therapies that treat the basic defect will normalise life expectancy for those born with CFTR mutations.

Animal models

A frustrating aspect of cystic fibrosis research has been the lack of a good animal model of the disease. The widely used mouse models do not have pronounced lung disease, making them poor surrogates for the study of pulmonary treatments. Recently, both cystic fibrosis heterozygote ferrets and pigs have been developed,[145,146] as has a litter of CFTR-deficient piglets that shows phenotypic similarities to human infants with the disease.[147] It is hoped that these models will lead to improved understanding and treatment of cystic fibrosis.

Mutation-specific therapies

Class I nonsense mutations are single base substitutions that lead to premature termination of mRNA transcripts and result in a loss of production of full length CFTR. About 10% of patients with cystic fibrosis carry in-frame nonsense mutations, with Trp1282X and Gly542X being the most common.[10] Welch and co-workers[148] have reported a molecule—PTC124—that has been shown to allow read-through of premature stop codons without disruption of normal termination signals. Administration of the compound to a mouse model expressing the CFTR-Gly542X mutation suppressed the mutation and restored CFTR protein and function.[149] These promising results led to a phase II human trial, in which an improvement in NPD measurements was seen in many, but not all, of the patients who received the test material.[150]

Class II mutations result in faulty processing of nascent CFTR protein. In the most common example, Phe508del, mRNA is translated into a protein that has a folding defect. This defect is recognised by the quality control mechanisms in the endoplasmic reticulum where the misfolded protein forms a stable conformer with a chaperone, is ubiquinated, and marked for degradation before it can leave the endoplasmic reticulum.[151,152] CFTR proteins with class III and IV mutations reach the apical cell membrane, but do not function properly.[153] Hypothetical agents that correct the localisation of Phe508del from the endoplasmic reticulum to the cell membrane have been called "correctors"; drugs that increase function of CFTR that is correctly located at the cell membrane are termed "potentiators".

If a misfolded protein does reach the cell surface, it might retain a degree of its normal function. This is true for the Phe508del-CFTR protein. Discovery of a drug that could overcome the endoplasmic reticulum quality control mechanism and allow mutant protein to leave intracellular organelles and proceed to the cell membrane would have important implications not only for cystic fibrosis, but also for many other diseases that are caused by altered protein folding.[151] Several chemicals and small molecules that either act as chaperones or that could allow passage of altered CFTR to the cell membrane are under investigation.[154–158] Chaperones are small intracellular molecules that regulate protein trafficking and help nascent proteins to achieve their native structure. With normal folding, chaperones engage and disassociate from proteins as the folding process proceeds. If an aminoacid deletion or substitution interferes with normal protein folding, chaperones fail to disengage and instead mark the nascent protein for degradation. Modifiers of chaperone function, including phenylbutyrate, have

shown promise in vitro, but no derivatives have reached clinical trials. A nutritional supplement, curcumin, generated a great deal of interest when it was first proposed as a corrector of CFTR misfolding.[156] Unfortunately, other investigators have not been able to replicate the initial findings.

Potentiators of CFTR channel activity could benefit patients who are affected by mutations in which CFTR reaches the apical cell membrane but does not respond appropriately to cAMP-mediated phosphorylation (class III), or does not have normal chloride conductance (class IV). Examples of potentiators include genistein, VX-770 (Vertex Pharmaceuticals, Cambridge, MA, USA), alkylxanthines (eg, CPX [SciClone Pharmaceuticals, Foster City, CA, USA]), and phosphodiesterase-5 inhibitors.[155,158–162] In a phase IIa clinical trial, patients with at least one copy of the Gly551Asp mutation showed greater improvement in chloride channel function, as indicated by decreased sweat chloride concentration and improved NPD results, after treatment with VX-770 for 28 days than did patients given placebo.[161] These findings with VX-770 and PTC124 pave the way for targeted therapy of the molecular defect in the near future.

A cocktail of a corrector and a potentiator might be the ultimate treatment for most patients with cystic fibrosis, since Phe508del-CFTR protein that is delivered to the apical cell membrane via a corrector will still have functional abnormalities because of loss of the phenylalanine residue in the first nucleotide binding domain.[158,159] However, even before a corrector is available, Phe508del-homozygous patients might benefit from potentiators, since some of their defective protein reaches the apical membrane despite the endoplasmic reticulum's quality control mechanisms.

Ways of correcting the biochemical aberrations of cystic fibrosis without affecting CFTR function directly include upregulation of non-CFTR-associated chloride channels (eg, with Moli1901)[162] or reduction of epithelial sodium reabsorption through epithelial sodium channels (eg, with denufosol).[163]

Gene therapy

It should be possible to treat an autosomal recessive disease such as cystic fibrosis with insertion of one copy of normally functioning DNA into the affected cells, independent of the class of mutation the recipient had before gene therapy. Although easy in concept (and in vitro), in practice gene therapy has proven to be quite difficult. Initial gene therapy trials with adenovirus vectors proved to be impractical because of immunogenicity and low efficiency of viral vectors to insert DNA into epithelial cells.[164–166] Recently, attention has turned to adeno-associated viruses (AAV) and liposomes as potential vectors. Flotte and colleagues[167] have shown a physiological correction of chloride movement in nasal epithelial cells from recombinant AAV-serotype-2 CFTR gene therapy recipients, even in those with low CFTR mRNA expression. Unfortunately, a phase IIb trial of repeated doses of aerosolised AAV CFTR treatment did not result in significant improvement in spirometric values.[168] Additionally, concerns remain about toxicity, and immunological responses to repeated administration of this vector.[169]

The UK Cystic Fibrosis Gene Therapy Consortium has worked to develop non-viral vectors for gene transfer.[170] So far, their best results in animal models have been with a cationic lipid vector. In their 2006 review, they anticipated that repeat doses of non-viral vectors and use of new plasmids—and new methods of delivering these vectors—would be developed in the next few years.[170] It is hoped that next-generation vectors will lead to effective gene transfer and sustained cure of the pulmonary disease associated with cystic fibrosis. For now, however, the prospect of gene therapy remains a hope more than a reality.

Conclusions

Cystic fibrosis is a multifaceted disease that requires close attention to pulmonary and nutritional variables. Patients should be seen in centres that have experience of caring for individuals with the disease and that can offer expertise in a broad range of areas. Physicians alone cannot provide adequate care; a team consisting of nurses, nutritionists, respiratory therapists, social workers, and others is necessary to achieve the best outcomes. The goal in 2009 is to preserve lung function by maximising current treatment regimens, so that patients can benefit fully from future therapies that could correct the basic defect and turn cystic fibrosis into a manageable disease.

Contributors

BPO'S and SDF both participated in the writing of this Seminar. Both authors saw and approved the final version.

Conflicts of interest

BPO'S declares that he has no conflict of interest. SDF is a co-inventor on a patent application through Beth Israel Deaconess Medical for the use of docosahexaenoic acid for the treatment of conditions related to cystic fibrosis gene mutations. BPO'S and SDF do not hold equity in any company producing a cystic fibrosis-related project. BPO'S and SDF have both served on committees for the US Cystic Fibrosis Foundation.

References

1 Anon. 2006 annual data report to the center directors. Bethesda, MD: Cystic Fibrosis Foundation Patient Registry, 2007.

2 Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947–2003. *Eur Respir J* 2007; **29:** 522–26.

3 Reisin IL, Prat AG, Abraham EH, et al. The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J Biol Chem* 1994; **269:** 20584–91.

4 Schwiebert EM, Egan ME, Hwang TH, et al. CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. *Cell* 1995; **81:** 1063–73.

5 Stutts MJ, Canessa CM, Olsen JC, et al. CFTR as a cAMP-dependent regulator of sodium channels. *Science* 1995; **269:** 847–50.

6 Vankeerberghen A, Cuppens H, Cassiman J-J. The cystic fibrosis transmembrane conductance regulator: an intriguing protein with pleiotropic functions. *J Cyst Fibros* 2002; **1:** 13–29.

7 Mehta A. CFTR: more than just a chloride channel. *Pediatr Pulmonol* 2005; **39:** 292–98.

8 Quinton PM. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. *Lancet* 2008; **372:** 415–17.

9 Nissim-Rafinia M, Kerem B, Kerem E. Molecular biology of cystic fibrosis: CFTR processing and functions, and classes of mutations. In: Hodson M, Geddes DM, Bush A, eds. Cystic fibrosis, 3rd edn. London: Edward Arnold, 2007; 54–55.

10 Lao O, Andres AM, Mateu E, Bertranpetit J, Calafell F. Spatial patterns of cystic fibrosis mutation spectra in European populations. *Eur J Hum Genet* 2003; **11:** 385.

11 Walters S, Mehta A. Epidemiology of cystic fibrosis. In: Hodson M, Geddes DM, Bush A, eds. Cystic fibrosis, 3rd edn. London: Edward Arnold Ltd, 2007: 21–45.

12 Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med* 2005; **353:** 1443–53.

13 Dorfman R, Sandford A, Taylor C, et al. Complex two-gene modulation of lung disease severity in children with cystic fibrosis. *J Clin Invest* 2008; **118:** 1040–49.

14 Collaco JM, Vanscoy L, Bremer L, et al. Interactions between secondhand smoke and genes that affect cystic fibrosis lung disease. *JAMA* 2008; **299:** 417–24.

15 Blackman SM, Deering-Brose R, McWilliams R, et al. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. *Gastroenterology* 2006; **131:** 1030–39.

16 Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. *Hum Mutat* 1997; **10:** 135–54.

17 Bobadilla JL, Macek M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. *Hum Mutat* 2002; **19:** 575–606.

18 Castellani C, Cuppens H, Macek M, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 2008; **7:** 179–96.

19 Farrell PM. The prevalence of cystic fibrosis in the European Union. *J Cyst Fibros* 2008; **7:** 450–53.

20 Orozco L, Velazquez R, Zielenski J, et al. Spectrum of CFTR mutations in Mexican cystic fibrosis patients: identification of five novel mutations (W1098C, 846delT, P750L, 4160insGGGG and 297-1 G-A). *Hum Genet* 2000; **106:** 360–65.

21 Yamashiro Y, Shimizu T, Oguchi S, Shioya T, Nagata S, Ohtsuka Y. The estimated incidence of cystic fibrosis in Japan. *J Pediatr Gastroenterol Nutr* 1997; **24:** 544–47.

22 Matsui H, Grubb BR, Tarran R, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell* 1998; **95:** 1005–15.

23 Matsui H, Wagner VE, Hill DB, et al. A physical linkage between cystic fibrosis airway surface dehydration and *Pseudomonas aeruginosa* biofilms. *Proc Natl Acad Sci USA* 2006; **103:** 18131–36.

24 Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. *Annu Rev Med* 2007; **58:** 157–70.

25 Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway pseudomonas infections of cystic fibrosis patients. *J Clin Invest* 2002; **109:** 317–25.

26 Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996; **85:** 229–36.

27 Zabner J, Smith JJ, Karp PH, Widdicombe JH, Welsh MJ. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro. *Mol Cell* 1998; **2:** 397–403.

28 Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* 1997; **88:** 553–60.

29 Carlstedt-Duke J, Bronnegard M, Strandvik B. Pathological regulation of arachidonic acid release in cystic fibrosis: the putative basic defect. *Proc Natl Acad Sci USA* 1986; **83:** 9202–06.

30 Tirouvanziam R, de Bentzmann S, Hubeau C, et al. Inflammation and infection in naive human cystic fibrosis airway grafts. *Am J Respir Cell Mol Biol* 2000; **23:** 121–27.

31 Karp CL, Flick LM, Park KW, et al. Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat Immunol* 2004; **5:** 388–92.

32 Machen TE. Innate immune response in CF airway epithelia: hyperinflammatory? *Am J Physiol Cell Physiol* 2006; **291:** C218–30.

33 Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 1995; **151:** 1075–82.

34 Freedman SD, Blanco PG, Zaman MM, et al. Association of cystic fibrosis with abnormalities in fatty acid metabolism. *N Engl J Med* 2004; **350:** 560–69.

35 Zaman MM, Gelrud A, Junaidi O, et al. Interleukin 8 secretion from monocytes of subjects heterozygous for the deltaF508 cystic fibrosis transmembrane conductance regulator gene mutation is altered. *Clin Diagn Lab Immunol* 2004; **11:** 819–24.

36 Colombo C, Costantini D, Rocchi A, et al. Cytokine levels in sputum of cystic fibrosis patients before and after antibiotic therapy. *Pediatr Pulmonol* 2005; **40:** 15–21.

37 Carrabino S, Carpani D, Livraghi A, et al. Dysregulated interleukin-8 secretion and NF-kappaB activity in human cystic fibrosis nasal epithelial cells. *J Cyst Fibros* 2006; **5:** 113–19.

38 O'Sullivan BP, Michelson AD. The inflammatory role of platelets in cystic fibrosis. *Am J Respir Crit Care Med* 2006; **173:** 483–90.

39 Rottner M, Kunzelmann C, Mergey M, Freyssinet J-M, Martinez MC. Exaggerated apoptosis and NF-kappaB activation in pancreatic and tracheal cystic fibrosis cells. *FASEB J* 2007; **21:** 2939–48.

40 Bonfield TL, Konstan MW, Berger M. Altered respiratory epithelial cell cytokine production in cystic fibrosis. *J Allergy Clin Immunol* 1999; **104:** 72–78.

41 Imundo L, Barasch J, Prince A, Al-Awqati Q. Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface. *Proc Natl Acad Sci USA* 1995; **92:** 3019–23.

42 Campodónico VL, Gadjeva M, Paradis-Bleau C, Uluer A, Pier GB. Airway epithelial control of *Pseudomonas aeruginosa* infection in cystic fibrosis. *Trends Mol Med* 2008; **14:** 120–33.

43 De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. *Thorax* 2006; **61:** 627–35.

44 Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008; **153:** S4–14.

45 LeGrys VA, Yankaskas JR, Quittell LM, Marshall BC, Mogayzel PJ. Diagnostic sweat testing: the Cystic Fibrosis Foundation guidelines. *J Pediatr* 2007; **151:** 85–89.

46 Mishra A, Greaves RF, Carlin J, et al. The diagnosis of cystic fibrosis by sweat test: age-specific reference intervals. *J Pediatr* 2008; **153:** 758–63.

47 Boyle MP. Nonclassic cystic fibrosis and CFTR-related diseases. *Curr Opin Pulm Med* 2003; **9:** 498–503.

48 Pall H, Zielenski J, Jonas MM, et al. Primary sclerosing cholangitis in childhood is associated with abnormalities in cystic fibrosis-mediated chloride channel function. *J Pediatr* 2007; **151:** 255–59.

49 Therrell BL, Lloyd-Puryear MA, Mann MY. Understanding newborn screening system issues with emphasis on cystic fibrosis screening. *J Pediatr* 2005; **147** (suppl 1)**:** S6–10.

50 Comeau AM, Accurso FJ, White TB, et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report. *Pediatrics* 2007; **119:** e495–518.

51 Farrell PM, Kosorok MR, Laxova A, et al. Nutritional benefits of neonatal screening for cystic fibrosis. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *N Engl J Med* 1997; **337:** 963–69.

52 Farrell PM, Kosorok MR, Rock MJ, et al. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. *Pediatrics* 2001; **107:** 1–13.

53 Konstan MW, Butler SM, Wohl ME, et al. Growth and nutritional indexes in early life predict pulmonary function in cystic fibrosis. *J Pediatr* 2003; **142:** 624–30.

54 Padman R, McColley SA, Miller DP, et al. Infant care patterns at epidemiologic study of cystic fibrosis sites that achieve superior childhood lung function. *Pediatrics* 2007; **119:** E531–37.

55 McKay KO, Waters DL, Gaskin KJ. The influence of newborn screening for cystic fibrosis on pulmonary outcomes in New South Wales. *J Pediatr* 2005; **147** (suppl 1)**:** S47–50.

56 Sims EJ, McCormick J, Mehta G, Mehta A. Neonatal screening for cystic fibrosis is beneficial even in the context of modern treatment. *J Pediatr* 2005; **147** (suppl 1)**:** S42–46.

57 Sims EJ, Mugford M, Clark A, et al. Economic implications of newborn screening for cystic fibrosis: a cost of illness retrospective cohort study. *Lancet* 2007; **369:** 1187–95.

58 Grosse SD, Boyle CA, Botkin JR, et al. Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs. *MMWR Recomm Rep* 2004; **53:** 1–36.

59 Anderson DH. Cystic fibrosis of the pancreas and its relation to celiac disease. *Am J Dis Child* 1938; **56:** 344.

60 Borowitz D, Baker R, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2002; **35:** 246–59.

61 Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut* 2007; **56:** 1153–63.

62 Rosenfeld M, Ramsey BW, Gibson RL. Pseudomonas acquisition in young patients with cystic fibrosis: pathophysiology, diagnosis, and management. *Curr Opin Pulmonol Med* 2003; **9:** 492–97.

63 Burns J, Gibson R, McNamara S, et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J Infect Dis* 2001; **183:** 444–52.

64 Hartl D, Latzin P, Hordijk P, et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. *Nat Med* 2007; **13:** 1423–30.

65 Chernick WW, Barbero GJ. Composition of tracheobronchial secretions in cystic fibrosis of the pancreas and bronchiectasis. *Pediatrics* 1959; **24:** 739–45.

66 Høiby N, Frederiksen B, Pressler T. Eradication of early *Pseudomonas aeruginosa* infection. *J Cyst Fibros* 2005; **4:** 49–54.

67 Taccetti G, Campana S, Festini F, Mascherini M, Doring G. Early eradication therapy against *Pseudomonas aeruginosa* in cystic fibrosis patients. *Eur Respir J* 2005; **26:** 458–61.

68 Konstan MW, Morgan WJ, Butler SM, et al. Risk factors for rate of decline in forced expiratory volume in one second in children and adolescents with cystic fibrosis. *J Pediatr* 2007; **151:** 134–39.

69 Kosorok MR, Zeng L, West SE, et al. Acceleration of lung disease in children with cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* 2001; **32:** 277–87.

70 Treggiari MM, Rosenfeld M, Retsch-Bogart G, Gibson R, Ramsey B. Approach to eradication of initial *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Pediatr Pulmonol* 2007; **42:** 751–56.

71 Ratjen E, Munck A, Campello V. Safety of inhaled tobramycin nebuliser solution for treatment of early *Pseudomonas aeruginosa* infection: first results from the ELITE study (abstract). *J Cyst Fibros* 2006; **5:** S22.

72 Steinkamp G, Wiedemann B, Rietschel E, et al. Prospective evaluation of emerging bacteria in cystic fibrosis. *J Cyst Fibros* 2005; **4:** 41–48.

73 De Boeck K, Malfroot A, Van Schil L, et al. Epidemiology of *Burkholderia cepacia* complex colonisation in cystic fibrosis patients. *Eur Respir J* 2004; **23:** 851–56.

74 De Soyza A, Ellis CD, Khan CM, Corris PA, de Hormaeche RD. *Burkholderia cenocepacia* lipopolysaccharide, lipid A, and proinflammatory activity. *Am J Respir Crit Care Med* 2004; **170:** 70–77.

75 Jones AM, Dodd ME, Govan JR, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. *Thorax* 2004; **59:** 948–51.

76 Kalish LA, Waltz DA, Dovey M, et al. Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. *Am J Respir Crit Care Med* 2006; **173:** 421–25.

77 Dasenbrook EC, Merlo CA, Diener-West M, Lechtzin N, Boyle MP. Persistent methicillin-resistant *Staphylococcus aureus* and rate of FEV1 decline in cystic fibrosis. *Am J Respir Crit Care Med* 2008; **178:** 814–21.

78 Goss CH, Mayer-Hamblett N, Aitken ML, Rubenfeld GD, Ramsey BW. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. *Thorax* 2004; **59:** 955–59.

79 Olivier KN, Weber DJ, Wallace RJ, et al. Nontuberculous mycobacteria: I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003; **167:** 828–34.

80 Levy I, Grisaru-Soen G, Lerner-Geva L, et al. Multi-center cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. *Emerg Infect Dis* 2008; **14:** 378–84.

81 Stevens D, Moss R, Kurup V, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; **37:** S225–64.

82 Mastella G, Rainisio M, Harms H, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000; **16:** 464–71.

83 Marshall BC, Butler SM, Stoddard M, Moran AM, Liou TG, Morgan WJ. Epidemiology of cystic fibrosis-related diabetes. *J Pediatr* 2005; **146:** 681–87.

84 Elder DA, Wooldridge JL, Dolan LM, D'Alessio DA. Glucose tolerance, insulin secretion, and insulin sensitivity in children and adolescents with cystic fibrosis and no prior history of diabetes. *J Pediatr* 2007; **151:** 653–58.

85 Milla CE, Billings JM, Moran AM. Diabetes is associated with dramatically decreased survival in female but not male subjects with cystic fibrosis. *Diabetes Care* 2005; **28:** 2141–44.

86 O'Riordan SM, Robinson PD, Donaghue KC, Moran AM. Management of cystic fibrosis-related diabetes. *Pediatric Diabetes* 2008; **9:** 338–44.

87 Aris RM, Merkel PA, Bachrach LK, et al. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab* 2005; **90:** 1888–96.

88 Yankaskas JR, Marshall BC, Sufian B, Simon RH, Rodman D. Cystic fibrosis adult care: consensus conference report. *Chest* 2004; **125:** 1S–39.

89 McMullen AH, Pasta DJ, Frederick PD, et al. Impact of pregnancy on women with cystic fibrosis. *Chest* 2006; **129:** 706–11.

90 Johnson C, Butler SM, Konstan MW, Morgan W, Wohl ME. Factors influencing outcomes in cystic fibrosis: a center-based analysis. *Chest* 2003; **123:** 20–27.

91 Flume PA, O'Sullivan BP, Robinson KA, et al. Cystic fibrosis pulmonary guidelines: chronic medications for maintenance of lung health. *Am J Respir Crit Care Med* 2007; **176:** 957–69.

92 Eigen H, Rosenstein BJ, FitzSimmons S, Schidlow DV. A multicenter study of alternate-day prednisone therapy in patients with cystic fibrosis. *J Pediatr* 1995; **126:** 515–23.

93 Balfour-Lynn IM, Lees B, Hall P, et al. Multicenter randomized controlled trial of withdrawal of inhaled corticosteroids in cystic fibrosis. *Am J Respir Crit Care Med* 2006; **173:** 1356–62.

94 Conway S, Pond M, Watson A, Etherington C, Robey H, Goldman M. Intravenous colistin sulphomethate in acute respiratory exacerbations in adult patients with cystic fibrosis. *Thorax* 1997; **52:** 987–93.

95 Jensen T, Pedersen SS, Garne S, Heilmann C, Hoiby N, Koch C. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. *J Antimicrob Chemother* 1987; **19:** 831–38.

96 Hodson ME, Gallagher CG, Govan JR. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. *Eur Respir J* 2002; **20:** 658–64.

97 Ratjen F. Restoring airway surface liquid in cystic fibrosis. *N Engl J Med* 2006; **354:** 291–93.

98 Elkins MR, Robinson M, Rose BR, et al. A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis. *N Engl J Med* 2006; **354:** 229–40.

99 Donaldson SH, Bennett WD, Zeman KL, Knowles MR, Tarran R, Boucher RC. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med* 2006; **354:** 241–50.

100 Subbarao P, Balkovec S, Solomon M, Ratjen F. Pilot study of safety and tolerability of inhaled hypertonic saline in infants with cystic fibrosis. *Pediatr Pulmonol* 2007; **42:** 471–76.

101 Koyama H, Geddes D. Erythromycin and diffuse panbronchiolitis. *Thorax* 1997; **52:** 915–18.

102 Ordoñez CL, Stulbarg M, Grundland H, Liu JT, Boushey HA. Effect of clarithromycin on airway obstruction and inflammatory markers in induced sputum in cystic fibrosis: A pilot study. *Pediatr Pulmonol* 2001; **32:** 29–37.

103 Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet* 2002; **360:** 978–84.

104 Wolter J, Seeney S, Bell S, Bowler S, Masel P, McCormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax* 2002; **57:** 212–16.

105 Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA* 2003; **290:** 1749–56.

106 Kohler T, Dumas J-L, Van Delden C. Ribosome protection prevents azithromycin-mediated quorum-sensing modulation and stationary-phase killing of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2007; **51:** 4243–48.

107 Schultz MJ. Macrolide activities beyond their antimicrobial effects: macrolides in diffuse panbronchiolitis and cystic fibrosis. *J Antimicrob Chemother* 2004; **54:** 21–28.

108 Konstan MW, Byard PJ, Hoppel CL, Davis PB. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; **332:** 848–54.

109 Lands LC, Milner R, Cantin AM, Manson D, Corey M. High-dose ibuprofen in cystic fibrosis: Canadian safety and effectiveness trial. *J Pediatr* 2007; **151:** 249–54.

110 Fennell PB, Quante J, Wilson K, Boyle M, Strunk R, Ferkol T. Use of high-dose ibuprofen in a pediatric cystic fibrosis center. *J Cystic Fibros* 2007; **6:** 153–58.

111 Stutman HR, Lieberman JM, Nussbaum E, Marks MI. Antibiotic prophylaxis in infants and young children with cystic fibrosis: a randomized controlled trial. *J Pediatr* 2002; **140:** 299–305.

112 Chatfield S, Owen G, Ryley H, et al. Neonatal screening for cystic fibrosis in Wales and the west midlands: clinical assessment after five years of screening. *Arch Dis Child* 1991; **66:** 29–33.

113 Weaver L, Green M, Nicholson K, et al. Prognosis in cystic fibrosis treated with continuous flucloxacillin from the neonatal period. *Arch Dis Child* 1994; **70:** 84–89.

114 Smyth A. Prophylactic antibiotics in cystic fibrosis: a conviction without evidence? *Pediatr Pulmonol* 2005; **40:** 471–76.

115 Szaff M, Hoiby N, Flensborg EW. Frequent antibiotic therapy improves survival of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. *Acta Paediatr Scand* 1983; **72:** 651–57.

116 Regelmann WE, Elliott GR, Warwick WJ, Clawson CC. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. *Am Rev Respir Dis* 1990; **141:** 914–21.

117 Block JK, Vandemheen KL, Tullis E, et al. Predictors of pulmonary exacerbations in patients with cystic fibrosis infected with multi-resistant bacteria. *Thorax* 2006; **61:** 969–74.

118 Goss CH, Burns JL. Exacerbations in cystic fibrosis 1: epidemiology and pathogenesis. *Thorax* 2007; **62:** 360–67.

119 Bell SC, Robinson PJ. Exacerbations in cystic fibrosis: 2: prevention. *Thorax* 2007; **62:** 723–32.

120 Smyth A, Elborn JS. Exacerbations in cystic fibrosis: 3: management. *Thorax* 2008; **63:** 180–84.

121 Schidlow DV. Cystic fibrosis. In: A practical guide to pediatric respiratory diseases. Schidlow DV, Smith DS, eds. Philadelphia: Hanley and Belfus Inc, 1994.

122 Kraynack NC, Singh C, Sheers T, Bryson E, McBride J. Standardization of a pulmonary exacerbation designed to uniformly identify pulmonary exacerbations in cystic fibrosis is associated with continued improvement in pulmonary function. *Pediatr Pulmonol* 2007; **42** (suppl 30)**:** 372.

123 Doring G, Conway S, Heijerman H, et al. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur Respir J* 2000; **16:** 749–67.

124 Wolter J, Bowler S, Nolan P, McCormack J. Home intravenous therapy in cystic fibrosis: a prospective randomized trial examining clinical, quality of life and cost aspects. *Eur Respir J* 1997; **10:** 896–900.

125 Thornton J, Elliott R, Tully MP, Dodd M, Webb AK. Long term clinical outcome of home and hospital intravenous antibiotic treatment in adults with cystic fibrosis. *Thorax* 2004; **59:** 242–46.

126 Nazer D, Abdulhamid I, Thomas R, Pendleton S. Home versus hospital intravenous antibiotic therapy for acute pulmonary exacerbations in children with cystic fibrosis. *Pediatr Pulmonol* 2006; **41:** 744–49.

127 van der Schans C, Prasad A, Main E. Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis. *Cochrane Database Syst Rev* 2000; **2:** CD001401.

128 Bradley JM, Moran FM, Stuart Elborn J. Evidence for physical therapies (airway clearance and physical training) in cystic fibrosis: an overview of five Cochrane systematic reviews. *Respir Med* 2006; **100:** 191–201.

129 McCool FD, Rosen MJ. Nonpharmacologic airway clearance therapies: ACCP evidence-based clinical practice guidelines. *Chest* 2006; **129** (suppl 1)**:** 250S–59.

130 Button BM, Heine RG, Catto-Smith AG, et al. Chest physiotherapy in infants with cystic fibrosis: to tip or not? A five-year study. *Pediatr Pulmonol* 2003; **35:** 208–13.

131 Bradley J, Moran F. Physical training for cystic fibrosis. *Cochrane Database Syst Rev* 2008; **1:** CD002768.

132 Liou TG, Adler FR, Cox DR, Cahill BC. Lung transplantation and survival in children with cystic fibrosis. *N Engl J Med* 2007; **357:** 2143–52.

133 Egan TM. Solid benefit of lung transplantation for some children with cystic fibrosis. *Pediatr Transplant* 2008; **12:** 125–28.

134 Aurora P, Spencer H, Moreno-Galdo A. Lung transplantation in children with cystic fibrosis: a view from Europe. *Am J Respir Crit Care Med* 2008; **177:** 935–36.

135 Goldberg HJ, Deykin A. Advances in lung transplantation for patients who have cystic fibrosis. *Clin Chest Med* 2007; **28:** 445–57.

136 Trulock EP, Christie JD, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult lung and heart-lung transplantation report—2007. *J Heart Lung Transplant* 2007; **26:** 782–95.

137 Murray S, Charbeneau J, Marshall BC, LiPuma JJ. Impact of burkholderia infection on lung transplantation in cystic fibrosis. *Am J Respir Crit Care Med* 2008; **178:** 363–71.

138 Young AC, Wilson JW, Kotsimbos TC, Naughton MT. Randomised placebo controlled trial of non-invasive ventilation for hypercapnia in cystic fibrosis. *Thorax* 2008; **63:** 72–77.

139 Corey M, McLaughlin FJ, Williams M, Levison H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. *J Clin Epidemiol* 1988; **41:** 583–91.

140 Peterson ML, Jacobs DR, Milla CE. Longitudinal changes in growth parameters are correlated with changes in pulmonary function in children with cystic fibrosis. *Pediatrics* 2003; **112:** 588–92.

141 Borowitz D, Lin R, Baker SS. Comparison of monoclonal and polyclonal ELISAs for fecal elastase in patients with cystic fibrosis and pancreatic insufficiency. *J Pediatr Gastroenterol Nutr* 2007; **44:** 219–23.

142 Parker EM, O'Sullivan BP, Shea JC, Regan MM, Freedman SD. Survey of breast-feeding practices and outcomes in the cystic fibrosis population. *Pediatr Pulmonol* 2004; **37:** 362–67.

143 Stern M, Wiedemann B, Wenzlaff P. From registry to quality management: the German cystic fibrosis quality assessment project 1995–2006. *Eur Respir J* 2008; **31:** 29–35.

144 Schechter MS. Patient registry analyses: seize the data, but caveat lector. *J Pediatr* 2008; **153:** 733–35.

145 Rogers CS, Hao Y, Rokhlina T, et al. Production of CFTR-null and CFTR-dF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. *J Clin Invest* 2008; **118:** 1571–77.

146 Sun X, Yan Z, Yi Y, et al. Adeno-associated virus-targeted disruption of the CFTR gene in cloned ferrets. *J Clin Invest* 2008; **118:** 1578–83.

147 Rogers CS, Stoltz DA, Meyerholz DK, et al. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* 2008; **321:** 1837–41.

148 Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007; **447:** 87–91.

149 Du M, Liu X, Welch EM, Hirawat S, Peltz SW, Bedwell DM. PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc Natl Acad Sci USA* 2008; **105:** 2064–69.

150 Kerem E, Hirawat S, Armoni S, et al. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet* 2008; **372:** 719–27.

151 Amaral M. CFTR and chaperones. *J Mol Neurosci* 2004; **23:** 41–48.

152 Macario AJ, de Macario EC. Sick chaperones, cellular stress, and disease. *N Engl J Med* 2005; **353:** 1489–501.

153 Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; **352:** 1992–2001.

154 Rubenstein RC, Zeitlin PL. Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of Delta F508-CFTR. *Am J Physiol Cell Physiol* 2000; **278:** C259–67.

155 Illek B, Zhang L, Lewis NC, Moss RB, Dong JY, Fischer H. Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. *Am J Physiol* 1999; **277:** C833–39.

156 Egan ME, Pearson M, Weiner SA, et al. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004; **304:** 600–02.

157 Wang X, Venable J, LaPointe P, et al. Hsp90 co-chaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. *Cell* 2006; **127:** 803–15.

158 Amaral MD, Kunzelmann K. Molecular targeting of CFTR as a therapeutic approach to cystic fibrosis. *Trends Pharmacol Sci* 2007; **28:** 334–41.

159 Zeitlin PL. Emerging drug treatments for cystic fibrosis. *Expert Opin Emerg Drugs* 2007; **12:** 329–36.

160 Lubamba B, Lecourt H, Lebacq J, et al. Preclinical evidence that sildenafil and vardenafil activate chloride transport in cystic fibrosis. *Am J Respir Crit Care Med* 2008; **177:** 506–15.

161 Vertex. Vertex announces positive 28-day results for VX-770, an oral investigational agent that targets a defective protein responsible for cystic fibrosis. http://investors.vrtx.com/releasedetail.cfm?ReleaseID=341350 2008 (accessed Jan 2, 2009).

162 Grasemann H, Stehling F, Brunar H, et al. Inhalation of Moli1901 in patients with cystic fibrosis. *Chest* 2007; **131:** 1461–66.

163 Deterding RR, LaVange LM, Engels JM, et al. Phase 2 randomized safety and efficacy trial of nebulized denufosol tetrasodium in cystic fibrosis. *Am J Respir Crit Care Med* 2007; **176:** 362–69.

164 Crystal RG, McElvaney NG, Rosenfeld MA, et al. Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis. *Nat Genet* 1994; **8:** 42–51.

165 Joseph PM, O'Sullivan BP, Lapey A, et al. Aerosol and lobar administration of a recombinant adenovirus to individuals with cystic fibrosis. I. Methods, safety, and clinical implications. *Hum Gene Ther* 2001; **12:** 1369–82.

166 Pickles RJ. Physical and biological barriers to viral vector-mediated delivery of genes to the airway epithelium. *Proc Am Thorac Soc* 2004; **1:** 302–08.

167 Flotte TR, Schwiebert EM, Zeitlin PL, Carter BJ, Guggino WB. Correlation between DNA transfer and cystic fibrosis airway epithelial cell correction after recombinant adeno-associated virus serotype 2 gene therapy. *Hum Gene Ther* 2005; **16:** 921–28.

168 Moss RB, Milla C, Colombo J, et al. Repeated aerosolized AAV-CFTR for treatment of cystic fibrosis: a randomized placebo-controlled phase 2B trial. *Hum Gene Ther* 2007; **18:** 726–32.

169 Tosi M, van Heeckeren A, Ferkol T, Askew D, Harding C, Kaplan J. Effect of pseudomonas-induced chronic lung inflammation on specific cytotoxic T-cell responses to adenoviral vectors in mice. *Gene Ther* 2004; **11:** 1427–33.

170 Griesenbach U, Geddes DM, Alton EW. Gene therapy progress and prospects: cystic fibrosis. *Gene Ther* 2006; **13:** 1061–67.