Cystic fibrosis

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Abstract | Cystic fibrosis is an autosomal recessive, monogenetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The gene defect was first described 25 years ago and much progress has been made since then in our understanding of how *CFTR* mutations cause disease and how this can be addressed therapeutically. CFTR is a transmembrane protein that transports ions across the surface of epithelial cells. CFTR dysfunction affects many organs; however, lung disease is responsible for the vast majority of morbidity and mortality in patients with cystic fibrosis. Prenatal diagnostics, newborn screening and new treatment algorithms are changing the incidence and the prevalence of the disease. Until recently, the standard of care in cystic fibrosis treatment focused on preventing and treating complications of the disease; now, novel treatment strategies directly targeting the ion channel abnormality are becoming available and it will be important to evaluate how these treatments affect disease progression and the quality of life of patients. In this Primer, we summarize the current knowledge, and provide an outlook on how cystic fibrosis clinical care and research will be affected by new knowledge and therapeutic options in the near future. For an illustrated summary of this Primer, visit: http://go.nature.com/4VrefN

Cystic fibrosis is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Close to 2,000 mutations in this gene have so far been described (see the Cystic Fibrosis Mutation Database), although fewer than 150 are known to be disease causing (see the Clinical and Functional Translation of CFTR (CFTR2) website). Although cystic fibrosis is a monogenetic disease, its phenotypic variability is substantial — as shown by the broad range of disease severity observed in patients with the same genotype[1]. The cystic fibrosis phenotype (BOX 1) is characterized by progressive lung disease, exocrine pancreatic insufficiency that results in gastrointestinal malabsorption, intestinal abnormalities that result in malnutrition, impaired growth, and a variety of other manifestations, including sinusitis and diabetes. CFTR primarily functions as a chloride channel that transports ions across the apical membrane of epithelial cells throughout the body, but has other functions, including bicarbonate secretion and inhibition of sodium transport, which are important for the pathophysiology of CFTR deficiency and dysfunction. Mutations in *CFTR* are grouped in classes that reflect their functional consequences; those leading to loss of CFTR expression on the cell surface or loss of its function are generally 'severe' mutations associated with a phenotype of both lung disease and pancreatic insufficiency. Mutations with residual CFTR function are often associated with preserved pancreatic function; some individuals show single organ manifestations, such a congenital bilateral absence of the vas deferens in the male reproductive tract[2]. *CFTR* mutations have also been described in patients with cystic fibrosis-like organ manifestations — including pancreatitis, sinusitis or 'idiopathic' bronchiectasis (widening of the airways) — and the threshold of CFTR function needed to prevent disease varies between different organs[3,4].

The discovery of the cystic fibrosis gene defect in 1989 has resulted in a better understanding of disease pathophysiology, but only in the past few years has this information led to targeted therapies that address the underlying cellular defect[5–7]. Important advances in addressing all aspects of the disease have been made over the past two decades and the prognosis of patients with cystic fibrosis is constantly improving. The development of clinical trial networks has enabled rapid testing of putative treatments through training of research staff, establishment of standard operating procedures and reference laboratories. Strong partnerships have also been developed between government agencies, academic centres, voluntary health organizations, individuals and families with cystic fibrosis. Although these advances can mainly be attributed to improvements in symptom-directed therapy of downstream complications, it is expected that upstream CFTR-directed therapy will further improve patients' longevity and quality of life. In this Primer, we summarize the current understanding about how *CFTR* mutations cause disease and

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we outline current diagnostic strategies and therapeutic interventions addressing disease manifestations. We also describe the epidemiology of cystic fibrosis and how cystic fibrosis and its treatment affect patients' quality of life. Lastly, we discuss how cystic fibrosis care and treatment is expected to change in future years.

Epidemiology

Changing diagnostic criteria and methods as well as improvements in clinical outcome have influenced the epidemiology of cystic fibrosis. Estimates of disease incidence are around 1 in 3,000 live births in persons of northern European descent[8,9], with Ireland having the highest incidence at 1 in 1,400 live births[10]. Incidence varies according to race and ethnicity; only 1 in 4,000 to 10,000 Latin Americans and 1 in 15,000 to 20,000 African Americans have cystic fibrosis, with even lower incidence rates in people of Asian background[9]. These estimates are based on information from western countries — epidemiological data are missing for large regions of the world, including the Middle East, Asia and Africa. Importantly, some small populations in eastern Europe have very high incidence rates, specifically Albania, where the incidence was noted to be 1 in 555 (REF. 11). This high incidence is also reflected in data noting very high incidence in Albanian immigrants to northern Italy[12]. The introduction of prenatal genetic screening in western countries seems to correlate with decreasing incidence in some countries[13]. Although the incidence is decreasing, data from registries suggest that the prevalence is increasing because of improvements in survival[14–16] (FIG. 1).

Box 1 | **Phenotypes of cystic fibrosis**

Classic cystic fibrosis
- Chronic sinusitis
- Pancreatic insufficiency
- Diabetes
- Obstructive lung disease from infancy with chronic bacterial infection
- Severe liver disease (5–10% of patients)
- Meconium ileus (15–20% of patients)
- Congenital bilateral absence of vas deference (males)
- High sweat chloride concentrations

Nonclassic cystic fibrosis
- Chronic sinusitis
- Pancreatic sufficiency
- Pancreatitis (5–20%)
- Obstructive lung disease (variable onset)
- Congenital bilateral absence of vas deference (males)
- Lower sweat chloride concentrations than classic cystic fibrosis

Recent analyses in the United States have shown that cystic fibrosis survival improved in the period from 2000 to 2010 at a rate of 1.8% per year (95% CI, 0.5–2.7%) and that the projected median survival of children born today is 56 years (95% CI, 54–58 years) if the mortality rate continues to decrease at this rate[15]. However, median age of death is still in the mid-twenties to early thirties[16]. These data mirror earlier findings from the United Kingdom[17]. Consistent with improved survival, the diagnosis of cystic fibrosis has moved to early in life with universal newborn screening in many countries. In 2010, more than half (58%) of the people with cystic fibrosis in the United States were diagnosed by newborn screening compared with only 8% of those diagnosed in 2000. Several countries are now reporting that >50% of their cystic fibrosis population is >18 years of age[18,19]. Not only has the patient population aged but also an increasing number of people with mild phenotypes of cystic fibrosis have been diagnosed on the basis of advances in genotyping of *CFTR* mutations, which contributes to, but does not fully account for, the increase in survival. In patients 40 years and older who were diagnosed after the age of 15 years, the median age of diagnosis has been reported to be 48.8 years (range, 24 to 72.8 years)[20]; these individuals are much more likely to have a nonclassic phenotype (see below)[21,22]. Survival is improving, even in patients with severe lung disease (defined as a forced expired volume in 1 second (FEV_1) <30% of predicted)[23], with median survival in those patients not receiving lung transplants increasing from 1.2 to 5.3 years in the period from 1991 to 2002.

Key questions in cystic fibrosis epidemiology remain unanswered. The first question relates to the exact effect of the almost universal use of newborn screening; newborn screening could enable children to maintain their lung health into adulthood. The next key question is how the advent of CFTR-directed therapies such as ivacaftor[24,25] will change the long-term morbidity and mortality. In the future, incidence might continue to fall, partly because of prenatal counselling and partly because of mixing of populations with differing incidence of *CFTR* mutations. However, prevalence could continue to increase as a consequence of the initiation of new therapies prior to end-organ injury.

Mechanisms/pathophysiology

CFTR protein and genetic mutations

Cystic fibrosis is caused by gene mutations in *CFTR* on the long arm of chromosome 7 (REFS 5,26). This gene is a unique member of the ATP-binding cassette (ABC) or traffic ATPase family of genes[27,28], which carry a regulatory domain that is actively phosphorylated[29,30]. CFTR primarily functions as an apical anion channel of

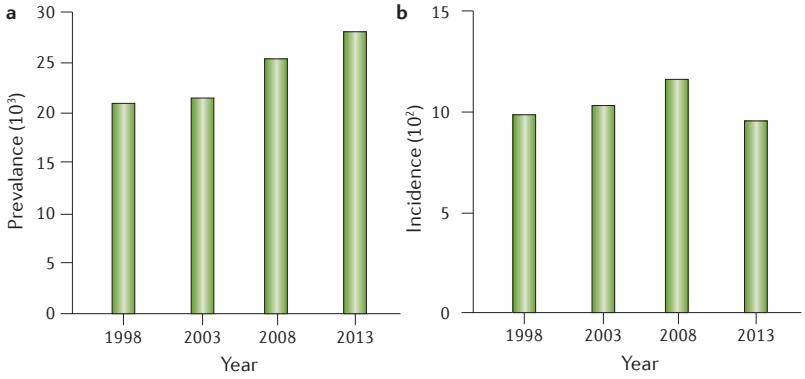

Figure 1 | **Cystic fibrosis in the United States. a** | The number of patients reported in the annual reports of the Cystic Fibrosis Foundation in 5-year intervals from 1998 to 2013 are shown. The figure notes a fairly substantial increase in the number of people living with cystic fibrosis in the US Cystic Fibrosis Foundation Patient Registry, which is probably attributable to improved survival of patients during this 15-year period and not merely newly diagnosed patients. **b** | The numbers of newly diagnosed patients in each of the given years over time are shown.

chloride and bicarbonate, rather than as an active pump. Similarly to other members of the ABC protein family, it includes two nucleotide-binding domains (NBDs) encoding sites capable of binding and hydrolysing ATP (Walker A and B motifs) and membrane-spanning domains that function as the ion channel pore through the plasma membrane (FIG. 2).

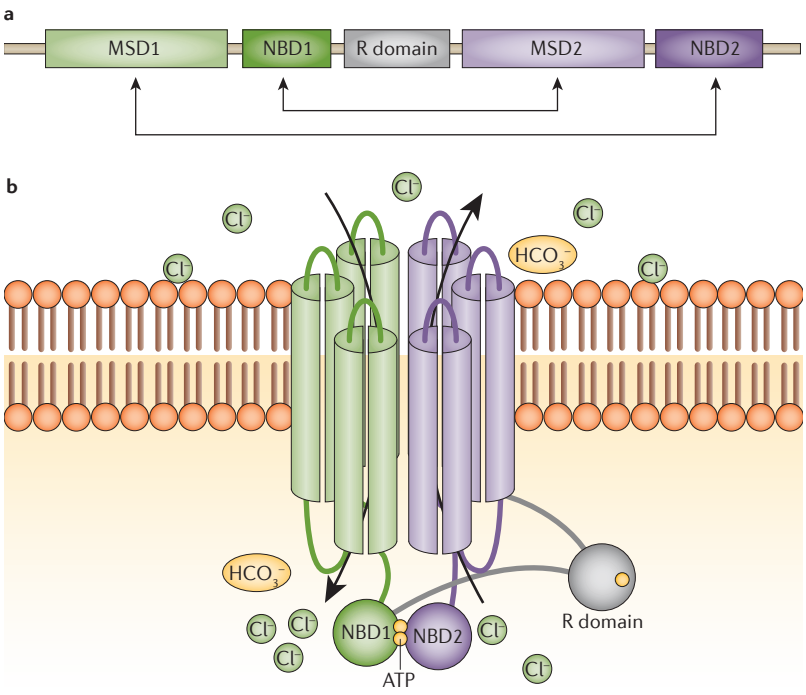

Figure 2 | **Structure of CFTR. a** | Linear structure of cystic fibrosis transmembrane conductance regulator (CFTR) is shown. **b** | The CFTR protein is comprised of two, six span membrane-bound regions each connected to a nuclear binding domain (NBD; NBD1 and NBD2). NBDs bind ATP, as well as a regulatory (R) domain that consists of many charged amino acids. The channel opens when its R domain is phosphorylated by protein kinase A and when ATP is bound at the NBDs. MSD, membrane spanning domain.

CFTR mutations can reduce channel number, function or both, and can vary in severity and occur through a variety of cellular mechanisms. The relative severity and completeness of each genetic defect has a major influence on the manifestations and the severity of disease (that is, the cystic fibrosis phenotype), although genetic modifiers and environmental factors also have a role (see the Clinical and Functional Translation of CFTR (CFTR2) website)[8,31]. The presence of at least one *CFTR* allele that is partially active can vastly improve clinical outcome; this is also evident from lower concentrations of sweat chloride or higher likelihood of pancreatic sufficiency compared with those that have no functional CFTR. When two mild or 'variable' mutations are present, or one mutation has sufficient function, atypical forms of cystic fibrosis can occur, such as congenital absence of the vas deferens[32], 'idiopathic' pancreatitis[33,34] or very late-onset respiratory disease without other characteristic features of cystic fibrosis[32–34]. A global catalogue of the phenotypes found in reported patients (patients who have been included in registries and report to the database) is available online (see the Clinical and Functional Translation of CFTR (CFTR2) website)[35] and can provide diagnostic and prognostic guidance to the management of individual patients.

The classification of *CFTR* alleles into molecular classes helps to simplify our understanding of the cellular defect (TABLE 1); however, it is now clear that many mutations have more than one feature[36]. This has led to a multinational effort to standardize the characterization of the mutations, particularly with regard to their response to therapeutics (that is, the 'theratype'), and to publicize the information through open data repositories[35]. The function of the CFTR protein can be reduced by disordered regulation and gating, which causes diminished ATP binding and hydrolysis (class III alleles) or defective chloride conductance (class IV alleles). Reduced channel number can be conferred by major disruptions of the *CFTR* gene (insertions, deletions and premature termination codons; each representative of a class I allele), aberrant splicing that reduces or eliminates full-length and stable mRNA transcripts (non-canonical and canonical splicing mutations, respectively; class V alleles), mutations that reduce surface stability (class VI alleles) or misfolding mutations that destabilize the CFTR protein and that subject it to premature degradation by endoplasmic reticulum-associated degradation, disrupting its normal localization to the plasma membrane (class II alleles). Class II mutations include the most common *CFTR* allele, deletion of the phenylalanine at position 508 (also known as F508del or c.1521_1523delCTT).

The severity of disease depends on whether a molecular defect is complete, in addition to other factors unrelated to the CFTR protein itself, such as modifier genes or environment. Mutations that affect chloride conductance are frequently only mild in severity; similarly, some class II and class VI alleles only partially disrupt protein stability, and non-canonical splice mutations are — by definition — partial, owing to the presence of normally spliced transcripts. Some alleles also show more than one

Table 1 | ***CFTR* mutations by molecular class, functional abnormality and primary therapy type**

Molecular classification	Functional abnormality	Molecular consequence	Primary therapy type	Example mutations
Class I	Decreased number	No functional protein produced	Translational readthrough*	G542X, 394delTT and 3905insT
Class II	Decreased number	Absent or diminished protein processing	Corrector* Potentiator*‡	F508del, N1303K and A455E
Class III	Decreased function§	Defective gating	Potentiator*	G551D, R117H and F508del‡
Class IV	Decreased function	Decreased conductance	Potentiator*	R347H, R334W and R117H‡
Class V	Decreased number	Abnormal splicing (canonical (complete) or non-canonical (partial))	Splice repair‖ Potentiator‖	621+1G>T, 3849+10kbC>T and R117H+5T
Class VI	Decreased number	Decreased cell surface stability	Cell surface stabilizer‖ Potentiator‖	S1455X, L1399X and F508del‡

*Therapy type that has shown activity in one or more clinical trials. ‡Secondary abnormality. §Open channel probability. ‖Potential therapy type.

abnormality, adding further complexity. Although the main defect that results from F508del is destabilized protein folding due to altered NBD1 stability, which subjects the protein to degradation in the proteasome, the protein also alters CFTR gating and cell surface residence time[37]. Indeed, F508del is at a key position within NBD1 such that it causes destabilization; furthermore, the deletion affects interdomain assembly and the communication of conformational changes to the transmembrane domains that are necessary for channel activation[38,39].

In addition to *CFTR* mutations, disease manifestations and progression are influenced by non-*CFTR* gene modifiers and environmental factors[31,40–43]. For example, multiple genes that encode apical plasma membrane proteins found near CFTR lead to an increased risk of meconium ileus (a condition whereby the bowel is obstructed by viscous secretions) in the newborn[42,44,45]. The meconium ileus risk alleles in solute carrier family 26 member 9 (*SLC26A9*; which mediates processes near CFTR), *SLC9A3* and *SLC6A14* are pleiotropic and influence other cystic fibrosis comorbidities in early life, including earlier development of lung damage and acquisition of *Pseudomonas aeruginosa* infection at a younger age[46]. A large and ongoing genome-wide association study and linkage study of 3,600 patients with cystic fibrosis reported a strong association between lung disease severity and loci on chromosome 11 and chromosome 20 (REF. 47).

In several analyses from an international twin and sibling study, genetic modifiers independent of CFTR are estimated to account for up to 50% of the variation in lung function in patients with cystic fibrosis, with the remainder attributed to environmental exposures and stochastic effects[48,49]. Genetic variations in innate immune molecules (for example, mannose-binding lectin 2 and chloride channel accessory 4)[50] and cytokines (for example, transforming growth factor β1 (TGFβ1) and tumour necrosis factor (TNF)) have been examined in many studies for their influence on clinical phenotype[41,51,52]. The results have not been consistent in all populations studied, which probably reflects differences in the populations studied (in terms of population size and the distribution of *CFTR* mutation status). These studies have identified potential modifiers that can be further assessed in the large cohorts that have been established for genome-wide association studies. For example, the risk for the development of cystic fibrosis-related diabetes has recently been shown to be influenced by modifier genes that include variants at *SLC26A9* and at four susceptibility loci for type 2 diabetes mellitus[43].

Environmental factors that have been shown to influence disease progression include exposure to environmental factors or toxins (for example, second-hand smoke, pollutants, climate and exposure to microorganisms), access to specialized care, adherence to treatment and variation in clinical practice[53–56]. Importantly, tissue-specific differences are evident in the susceptibility to *CFTR* mutations, non-*CFTR* gene modifiers and environmental factors. For example, specific *CFTR* gene mutations strongly affect pancreatic function (phenotype)[57], but environmental and other non-genetic factors have a greater influence on the pulmonary phenotype. The combination of large cohorts with the reduced costs and the enhanced processing of vast genetic data in the coming years are likely to provide understanding of the complex relationships between *CFTR*, non-*CFTR* gene modifiers and environmental factors on disease manifestations in cystic fibrosis.

Airway pathophysiology

Cystic fibrosis affects the function of epithelial tissues in which CFTR is highly expressed; in particular, glandular epithelia. The disease primarily manifests in the lungs, pancreas, gastrointestinal tract, vas deferens and sweat glands, although airway disease is the main cause of morbidity and mortality. In the lungs, cystic fibrosis results in mucus accumulation that compromises the airway lumen and that contributes to obstructive pulmonary disease (FIG. 3). Submucosal gland hyperplasia and thickened mucus secretions are also prominent.

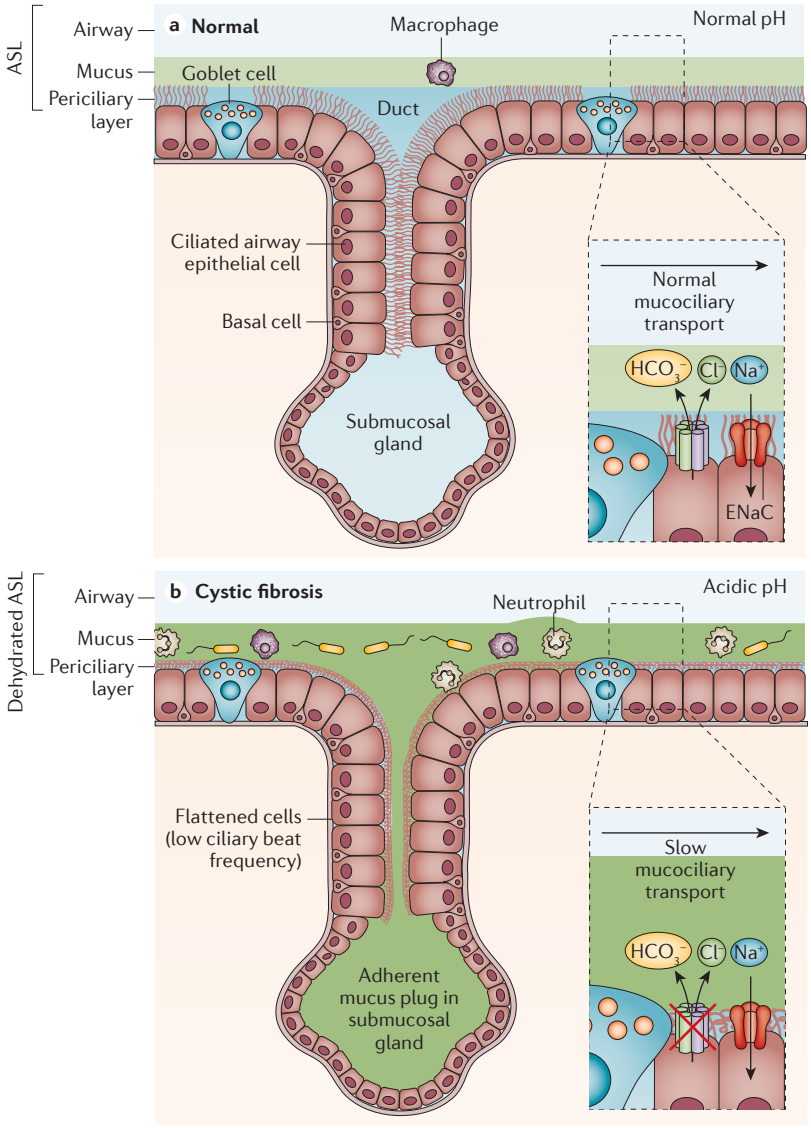

Figure 3 | **The mucociliary transport defect in cystic fibrosis. a** | In the healthy state, adequate airway surface homeostasis enables effective transport of mucus extruding from the airway surface goblet cells and submucosal glands. Appropriate bicarbonate and pH regulation enable normal mucus to form, which facilitates the formation of a two-layer gel that optimizes mucociliary clearance and airway defence. **b** | Depletion of the airway surface liquid occurs through the absence of cystic fibrosis transmembrane conductance regulator (CFTR)-mediated fluid secretion accompanied by tonic fluid absorption via the epithelial sodium channel (ENaC; inset). CFTR-dependent liquid desiccation decreases airway surface liquid (ASL) depth, including the periciliary layer, ultimately contributing to mucus stasis. Decreased bicarbonate transport contributes to an acidic pH. Abnormally adherent mucus also emerges from the glands in cystic fibrosis and can become fixed to the gland orifice or to the originating goblet cells. These events contribute to a proinflammatory airway environment that further accelerates pathogenesis.

Airway disease is thought to begin in the small airways[7]. Development of bronchiectasis leads to irreversible changes that encourage continued infection and that accelerate disease pathogenesis.

Despite decades of research, the understanding of the origins of airway pathogenesis remains incomplete and continues to evolve. Several key manifestations include delayed mucociliary clearance through airway surface liquid depletion, abnormalities of the physical properties and adhesion of mucus, and a predisposition to infection owing to abnormal mucosal defences[58,59]. Dysregulated inflammation intrinsic to the *CFTR* defect is also apparent. These processes initiate and perpetuate a cycle of destruction that ultimately results in irreversible lung injury, bronchiectasis and respiratory failure[58,59].

Loss of apical CFTR leads to reduced chloride and bicarbonate secretion[60]. Given that the release of water and electrolytes onto the airway surface is mostly driven by CFTR-dependent fluid secretion through both the glands and the surface epithelia, CFTR deficiency leads to diminished airway surface hydration, which can impair mucociliary transport in itself[60]. CFTR has also been shown to regulate the activity of the epithelial sodium channel (ENaC; also known as the amiloride-sensitive sodium channel), which is also activated by cleavage events conferred by free proteases such as prostasin and neutrophil elastase that are enriched in the inflamed airway[61–63]. CFTR decrement also confers unopposed ENaC-dependent sodium and water absorption, which exacerbates airway surface liquid depletion[64]. Moreover, the periciliary layer, which is a mucin gel layer between the cell surface and the mucus layer (FIG. 3), is sensitive to the osmolar forces of the overlying mucus; as the overlying mucins become concentrated in the absence of adequate fluid transport, the periciliary layer collapses and failure of mucociliary transport ensues[65]. However, this view is not without controversy. Specifically, ENaC hyperactivity has been questioned owing to the absence of sodium hyperabsorption from airway mucosa in newborn pigs with cystic fibrosis (together with evidence of normal airway surface liquid depth)[66], the lack of heightened amiloride-sensitive currents in rat models[67] and the finding that elevated amiloride-sensitive currents can be increased by reduced CFTR-dependent transepithelial resistance in primary human airway epithelial monolayers derived from patients with cystic fibrosis[68]. Even in the presence of adequate airway hydration, mucus abnormalities can adversely affect mucociliary transport[69], and mucus adhesion can occur at the gland outlet even under submerged conditions, which suggests multifactorial causes to delayed mucociliary clearance[70].

Mucus and glandular epithelium abnormalities

Hyperviscous respiratory secretions obstruct small and medium airways, leading to marked failure of mucociliary clearance that can be macroscopically verified by radioligand imaging[71]. A primary biochemical defect in mucus composition has been explored but is not well established as a fundamental cause of the disease, although enzymatically driven events that lead to the release of intestinal mucins have been identified[72]. Mucus includes a complex range of extracellular proteins, which are found in high concentrations in the airway lumen[73].

In extrapulmonary organs (for example, the pancreas and the liver), marked ductular obstruction is observed in the absence of polymicrobial infection, which enables direct studies of the relationship between CFTR and

mucus formation to be carried out. An emerging idea implicates defective bicarbonate transport as a mediator of hyperviscosity and mucosal adhesion in cystic fibrosis[74]. In this model, exocrine mucus — which is highly negative in charge — is produced by acinar and other epithelial cells. This mucus binds calcium ions, which condenses the mucus and shields the negative repulsive force between sulphates and other anionic groups on constituent mucins[74]. Bicarbonate secretion via CFTR chelates calcium and permits mucinous expansion and a viscoelastic state that is compatible with physiological clearance. Failure of bicarbonate release is hypothesized to result in defective mucin expansion, leading to hyperviscous secretion with abnormally adherent properties; that is, the mucus is tightly bound to the epithelial surface and is difficult to mobilize. Evidence of excessive mucus viscosity and adhesion that depends on bicarbonate secretion has been observed in the intestines of mouse models of cystic fibrosis[75,76] and excised airways of model pigs[69]. Notably, CFTR is highly expressed in the glandular epithelium, where it activates fluid and electrolyte secretion[68,70]; CFTR-dependent anion transport is also crucial for the release of elastic mucus from the gland duct, even under submerged (hydrated) conditions, which indicates its importance to normal mucus maturation and transport[70]. As only large animal models of cystic fibrosis (for example, pig, ferret and, to a lesser extent, rat) have prominent glandular expression, this might explain why mouse models are minimally affected by lung disease[67,77,78].

Defects in airway defence

Although CFTR mainly functions as an anion transporter, it also regulates numerous processes, including fundamental aspects of airway defence and inflammatory cell function. CFTR is situated within membrane complexes in close proximity to several integral membrane proteins, including other ion channels. In addition to ENaC, CFTR can directly or indirectly regulate anion secretion through other chloride channels, such as transmembrane member 16A (TMEM16A; also known as anoctamin 1), or can contribute to airway pH regulation through chloride exchangers, including anion exchange protein 2 (REFS 79,80). The absence of bicarbonate secretion leads to an acidic pH airway surface liquid in cystic fibrosis, which has been reported as a possible cause of defective bacterial killing by the highly pH-sensitive innate defensins[81]. CFTR also has a direct effect on neutrophil killing, as it affects degranulation by interfering with granule trafficking[82]. Dysfunctional macrophages might also be biased towards a proinflammatory response[83]. Proteomic and transcriptomic analyses show hundreds of cellular gene products that directly bind to or that are regulated by CFTR. Accordingly, additional effects of CFTR are likely to emerge, partly fostered by studies using agents that specifically activate the protein[84].

Inflammation in lung disease. Whether infection is required to cause airway inflammation in cystic fibrosis remains under debate. Post-mortem studies have reported that babies with cystic fibrosis who died from meconium ileus had normal (or near to normal) airway epithelium and no signs of inflammation or infection[85]. However, animal models have suggested that abnormalities of mucus can be proinflammatory in the absence of infection[86], and endoplasmatic reticulum-associated protein degradation induced by misprocessed F508del CFTR might also provide a stimulus for a heightened inflammatory response[87]. Despite this controversy, what is clear is that infection exaggerates the inflammatory milieu. Excessive neutrophil-dominated inflammation is observed in the absence of infection[88], and neutrophil elastase activity (a marker of airway inflammation) in bronchoalveolar lavage fluid in infants (3 months of age) was associated with early bronchiectasis at 12 months and 3 years of age in children with cystic fibrosis[89].

Factors released in chronic neutrophilic inflammation in cystic fibrosis can markedly reduce airway surface liquid height[90]. As well as aggravating airway dehydration, the imbalance between neutrophil elastase, other proteolytic enzymes (derived from inflammatory cells or bacteria) and anti-proteases results in exaggerated tissue damage[91,92]. Oxidative stress and persisting airway inflammation might be associated with local airway deficiency in glutathione (which normally protects from reactive oxygen species)[93,94]. Over time, a vicious cycle of reduced mucus clearance, neutrophil-dominated inflammation and bacterial infection damages the airways[95].

Lung infection. Airway pathogens that are most commonly detected include *P. aeruginosa*, *Staphylococcus aureus*, and Aspergillus species. *P. aeruginosa* infection has been associated with increased mortality, frequent exacerbations, rapid decline in lung function and heightened inflammation in patients with cystic fibrosis, which has fuelled programmes of eradication over the past two decades[96–98]. Viral infection is also a common cause of exacerbations in people with cystic fibrosis[99,100] — although these patients are not more susceptible to viral infection, the effect is greater[101]. Fungi, including Aspergillus species, are also increasingly recognized as pathogens in cystic fibrosis and are associated with an increased rate of pulmonary exacerbations[102].

With increasing age, infections with other bacteria, including *Achromobacter xylosoxidans*, *Stenotrophomas maltophilia* and *Burkholderia cepacia* complex, become increasingly common[16]. The prevalence of each of these pathogens varies between cystic fibrosis populations, although they generally occur in <15% of patients and rates increase with disease severity. Methicillin-resistant *S. aureus* (MRSA) is an increasing threat that occurs in up to 30% of patients presenting at some US centres[16,103], although the rates are lower in Europe. In the late 1980s, epidemic strains of *Burkholderia cenocepacia* were associated with clear evidence of cross-infection, rapid clinical deterioration in many, and poor outcomes following lung transplantation[104]. Both MRSA and *B. cenocepacia* infection in patients with cystic fibrosis have been reported to have adverse effects on prognosis[103,105]. Although *P. aeruginosa* was previously thought to be acquired from the environment, sharing of strains (patients with

Box 2 | **Conventional diagnostic criteria for cystic fibrosis**

Individuals must have at least one clinical feature:

- Meconium ileus
- Diarrhoea and failure to thrive
- Recurrent respiratory infections
- Nasal polyps
- Rectal prolapse
- Male infertility
- Electrolyte depletion

or a diagnosis of cystic fibrosis in a sibling and/or a positive newborn screening test, plus laboratory evidence of an abnormality in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene or protein:

- Chloride channel dysfunction (positive sweat test or abnormal transepithelial potential difference)
- Known disease-causing mutations on chromosome 7 in *trans*.

the same strains) has been shown to be common over the past 15 years, with some strains being associated with adverse clinical outcomes[106–108]. In some settings, strong evidence supports person-to-person spread of shared strains, although in other clinical settings common strains in patients with cystic fibrosis are genetically indistinguishable from common environmental strains[106,108–110]. Prevention of cross-infection has become a key part of the management of all patients and implementation of intensive infection control measures has reduced rates of shared strain infection in treatment centres[111,112]. However, such control measures remain challenging in resource-limited settings, especially with the rapid growth in the numbers of adults with cystic fibrosis.

Non-tuberculous mycobacteria cause infection in 3–20% of patients with cystic fibrosis[113], and prevalence rates have increased over the past 10 years[114]. Rapidly growing species (for example, *Mycobacterium abscessus*) are difficult to treat because prolonged courses of multiple antibiotics are required, which are often associated with toxicity and are a contraindication for lung transplantation in many transplant centres[65]. Of recent concern are the reports suggesting the potential for person-to-person transmission of *M. abscessus*[115].

Over the past several years, the airway has been revealed to not be sterile in health; the airway in cystic fibrosis is now considered to harbour a polymicrobial milieu. Both non-culture-based and culture-based methods have shown a wide range of atypical bacterial species, including *Prevotella*, *Fusobacterium* and *Veillonella* spp. among others[116,117]. The clinical significance of the microbiome in disease progression remains to be established, but several features are emerging, including a heterogeneous microbiome composition in patient populations and lower bacterial diversity with increasing age and poorer lung function[118].

The lung in cystic fibrosis is a microaerophilic environment and *P. aeruginosa* survives — and in fact thrives — within low oxygen tension biofilms[119]. Chronic *P. aeruginosa* infection is also associated with ongoing microevolution, which alters virulence, regulatory networks and acquisition of antimicrobial resistance mechanisms[120,121]. These factors probably contribute to the persistence of *P. aeruginosa*. The interaction of the host with pathogens is crucial for the development and the progression of pulmonary disease in cystic fibrosis, which leads to perpetual neutrophil recruitment to the lungs (where neutrophils are the dominant inflammatory cells)[121]. A key component of this dysregulation is the development of neutrophil extracellular traps (NETs), which are stimulated by bacterial pathogens (such as *P. aeruginosa* and *S. aureus*)[122]. NETs have detrimental effects in the cystic fibrosis airway by enhancing the viscosity of airway secretions, dampening pathogen clearance and potentially enhancing biofilm development and persistence[122]. The macrophage is likely to be an important scavenger in the lungs in patients with cystic fibrosis, but the mechanisms of action are debated. Some studies have suggested that T cells accumulate within the subepithelial airway but are limited in the lumen[123]. Whether specific lymphocyte subsets have an important role in lung disease remains controversial. Evidence would suggest that T helper 17 (T_H17) cells are important drivers of neutrophilic inflammation and an abnormal regulatory T cell response has been described to be linked to CFTR deficiency[124,125]. The effect of polymicrobial infection is likely to add to the complexity of regulation of immune responses in the cystic fibrosis airway.

Diagnosis, screening and prevention

Principles of diagnosis

The conventional diagnostic criteria for cystic fibrosis are given in BOX 2. Diagnosis is increasingly made by newborn screening (see below). However, physicians should be aware of symptoms and signs of disease in older children and adults, particularly bronchiectasis, recurrent respiratory tract infection, nasal polyps, male infertility and portal hypertension; late-presenting patients are usually — but not invariably — pancreatic sufficient. Screening can miss some mild cases if the child is born in a region without routine screening, the child missed screening despite being born in a screening region or a laboratory error occurred.

More than 95% of patients presenting symptomatically can be diagnosed with a sweat test. The test measures chloride concentration after pilocarpine (a muscarinic receptor agonist) application, which stimulates sweat production, but in a small minority of patients the diagnosis is unclear even after extensive testing[126]. The upper limit of normal sweat chloride concentration remains under contention; in Europe, the upper limit of normal for an indeterminate result is 30 mmol l^{-1} regardless of age. Given that sweat chloride concentration can increase with age, 40 mmol l^{-1} is considered the upper limit after 6 months of age only in the United States. Above 60 mmol l^{-1} is definitely abnormal for all ages and, provided the clinical presentation is compatible, is diagnostic[127]. However, rare but unequivocal cases of cystic fibrosis have been reported with sweat chloride of <30 mmol l^{-1}, which probably occurred owing to heterogeneity in end-organ CFTR expression or the influence of other related genes such as the gene that encodes ENaC[128].

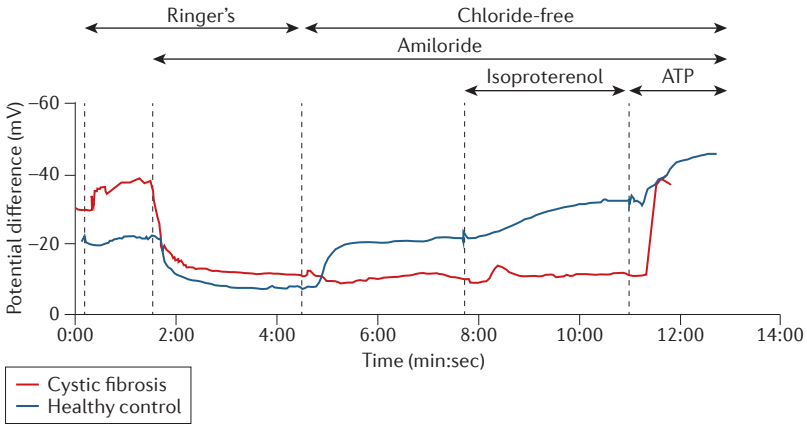

Figure 4 | **Diagnosing cystic fibrosis: nasal potential difference measurement.** Representative nasal potential difference tracings from a healthy control (blue) and a patient with cystic fibrosis (red) are shown. The nasal mucosa is sequentially perfused with Ringer's solution (an isotonic solution relative to the bodily fluids), Ringer's solution with amiloride to block the epithelial sodium channel (ENaC), chloride-free solution with amiloride, chloride-free solution with amiloride and isoproterenol to activate the cystic fibrosis transmembrane conductance regulator (CFTR), and finally the addition of ATP to activate non-CFTR-dependent anion transport. The change in potential difference upon addition of amiloride is used to estimate sodium transport, which is elevated in cystic fibrosis. The change in potential difference with chloride-free isoproterenol is used to measure CFTR-dependent anion transport, which is reduced in cystic fibrosis. Patients with mild phenotypes of cystic fibrosis generally show intermediate results.

Genetic testing is an important part of diagnostics because mutation-specific therapy is an increasing reality and can help to resolve unclear cases. Given the marked variation in the prevalence of *CFTR* mutations between different ethnic groups, test panels that account for this variation should be used unless whole-exome sequencing is carried out. In addition, the unknown significance of most of the rare *CFTR* mutations does not support the use of extended genotyping in patients with equivocal diagnostic tests[126].

Measurement of the transepithelial potential difference is an adjunct diagnostic test but is only available in a few specialist centres. The *in vivo* technique usually measures the potential difference across the nose or the respiratory epithelium via a catheter referenced to a peripheral electrode[129–131]. Rectal potential difference can also be measured *in vivo*; alternatively, *in vitro* measurement of CFTR activity on excised rectal biopsy tissue can be carried out via open-circuit or closed-circuit currents[132,133]. Other *in vitro* methods for assessing CFTR function, such as intestinal organoid swelling or the function of nasal epithelial cells grown in culture, are also of emerging interest[134]. The nasal potential difference test in patients with cystic fibrosis has several cardinal features, including elevated potential difference at baseline, a heightened response to amiloride and a diminished response to chloride-free isoproterenol compared with healthy controls (FIG. 4). A modified sweat test that assesses β-adrenergic sweat secretion has recently been developed and might have advantages in individuals with mild disease. However, its diagnostic use is not yet well established[135]. Other supportive tests can be considered to clarify the diagnosis, including stool human faecal elastase measurement for pancreatic insufficiency and, in postpubertal men, semen analysis to test for azoospermia[32].

Diagnosis is hindered in patients with positive diagnostic tests but without symptoms and in patients with a cystic fibrosis phenotype but negative or equivocal diagnostic tests[136]. The case for the latter patients is relatively straightforward; irrespective of the underlying diagnosis, any organ disease should be treated on its merits and the patient should be carefully monitored. Seemingly symptom-free patients with positive diagnostic tests should be carefully followed to detect the development of complications[137] whilst not overburdening the patients with treatment.

Newborn screening

Newborn screening for cystic fibrosis has been controversial because of its cost, the creation of anxiety around the procedure in seemingly healthy infants and the lack of established pulmonary treatments for infants. The cost of treatment has been shown to be reduced in patients who have been diagnosed through newborn screening compared with those who have had later diagnoses[138]. A randomized controlled trial has clearly shown the efficacy of screening and has provided evidence of nutritional — but not respiratory — benefits[139]. However, a factor in the unexpectedly poor respiratory outcomes in that study might have been failure to apply modern infection control precautions in one of the participating centres. Much evidence shows benefit when comparing outcomes before and after the introduction of screening[140–142]. For example, The London Cystic Fibrosis Collaboration showed that infants diagnosed later (in the first 2 years of life) had airway obstruction at presentation, even if they had had no respiratory symptoms or signs, and this never recovered despite specialist treatment[143,144]. By comparison, the outcomes in babies diagnosed by screening were much more favourable (see below). Another benefit of newborn screening is that parents have the opportunity to make informed choices about antenatal diagnosis in future pregnancies.

A large number of screening protocols are available, including measurements of serum immunoreactive trypsin and *CFTR* mutation analysis[145–147]. Screening diagnosis must always be confirmed with a sweat test, not least to ensure that there has not been an error in the screening laboratory. The possible outcomes are: a definite diagnosis of classic cystic fibrosis depending on the protocol, cystic fibrosis definitely excluded or an indeterminate outcome. Indeterminate outcomes are the most challenging situations; these children fall into two groups, namely those harbouring *CFTR* mutations who will develop late-onset, mild-variant disease and those with true diagnostic uncertainty[148]. Thus, screening can lead to the child being given a diagnosis of cystic fibrosis that might not have been possible until middle age. Such a situation raises the question of whether an early diagnosis affects the quality of life of the individual and their family. If accurate information is carefully given, and the intensity of treatment and monitoring is appropriately applied according to the severity of the clinical state,

quality of life need not be negatively affected. However, there is disagreement even between experts about some variants, for example, R117H with the 7T intron 8 variant in *trans*, with some believing this to be a benign variant, whereas others have identified late-presenting patients with this mutation[149–152].

True diagnostic uncertainty is another outcome of screening. The US Cystic Fibrosis Foundation has coined the phrase 'CFTR-related metabolic syndrome' to describe infants with sweat chloride values of <60 mmol l^{-1} (which could be too high an upper limit of normal in infancy, given that virtually all healthy babies have a sweat chloride of <30 mmol l^{-1}) and two *CFTR* mutations, one of which has not been shown to be disease causing[153]. An alternative term — cystic fibrosis screen positive with inconclusive diagnosis (CFSPID) — has been proposed by the European Cystic Fibrosis Working Group[154]. However, some of these infants develop cystic fibrosis-like symptoms and current recommendations are for careful follow-up monitoring[155]. Nonetheless, despite these issues, the case in favour of newborn screening is overwhelming.

Detection of early lung disease

Infants who are diagnosed through screening are often asymptomatic with few if any clinical signs of lung disease. When investigating these infants, it is important not to miss important warning signs or to cause anxiety by overinterpreting trivial abnormalities. Current techniques to identify lung disease are lung function measurements, imaging (mainly high-resolution CT, although MRI is being increasingly used[156]) and bronchalveolar lavage.

In terms of lung function tests, spirometry measures lung volume and air flow but is insensitive to distal airway disease. In preschool and school-age children, multi-breath washout (the lung clearance index (LCI)) is the most sensitive test in clinical practice and outperforms spirometry and lung volume assessment by body plethysmography[157–159]. However, in the first 2 years of life, LCI will fail to detect a substantial number of children who have abnormal infant spirometry, so the two tests should be combined in this age group[160]. Two large collaborative projects have evaluated lung function in infants, with differing results: although both groups reported that lung function was abnormal at diagnosis[161,162], the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF) reported rapid and marked deterioration[163], whereas the London Cystic Fibrosis Collaboration (LCFC) showed improvement over the first year of life, with stabilization in the second year[164,165]. These differences could be explained by differences in treatment strategies or in the characteristics of the population studied.

LCI has also been used as a clinical trial end point; one study has shown that an elevation in LCI predicts pulmonary exacerbations[166–168]. The role of high-resolution CT is controversial owing to the radiation exposure and the potentially increased baseline risk of some epithelial cancers in patients with cystic fibrosis[169]. Indeed, its role in infants diagnosed through screening is debated; the AREST-CF group has shown a large number of lung abnormalities[89], whereas the LCFC group found that CT changes at 1 year of age were so mild that they could not be reproducibly scored, and accordingly abandoned the technique[170]. In school-age children, if LCI is abnormal then the CT will also probably be abnormal and the number of scans could, therefore, be reduced[171,172]. If high-resolution CT is carried out, dilated airways should not be assumed to be 'bronchiectatic' (that is, irreversibly dilated) as air-trapping might simply reflect transient mucus plugging rather than any structural disease. No study has shown that regular CT scans improve prognosis, and scanning should be reserved for selected cases.

Identification of lower respiratory tract pathogens can be hampered by the patients' inability to spontaneously produce a sputum sample; for these patients, bronchoalveolar lavage can be considered. Early bronchoalveolar lavage surveillance programmes have shown that infection and inflammation in the absence of symptoms is common[88,98,173]. A 5-year, prospective randomized controlled trial showed no benefit from an aggressive programme of regular bronchoscopies; this technique should only be reserved for children who are symptomatic despite antibiotic treatment[174]. Early elevation of bronchoalveolar lavage neutrophil elastase activity has been shown to predict later structural changes on CT[89], but no evidence currently supports that detecting neutrophil elastase facilitates an intervention that improves prognosis.

Extrapulmonary manifestations

Cystic fibrosis is a multisystem disease that affects many organs in which CFTR is expressed. Chronic rhinosinusitis is extremely common and nasal polyposis a complication in up to 45% of patients[175,176]. Persistent infection in the upper airways and sinuses can be a source of lower respiratory tract infection[177]. The gastrointestinal tract is affected in numerous ways, including increased nutrient loss secondary to pancreatic insufficiency, reduced fat-soluble vitamin absorption, fat-soluble vitamin deficiency states, frequent gastro-oesophageal reflux and impaired bowel transit (complicated by distal intestinal obstruction syndrome, constipation and small intestinal bacterial overgrowth)[178–182]. The early recognition of nutritional deficits is vital, as poor growth and malnutrition adversely affect pulmonary function and patient survival[183,184]. Hepatic involvement is also common, with up to one in three patients affected including those with evidence of hepatic steatosis, cholelithasis, ductal stenosis and focal biliary cirrhosis[185,186]. Biliary cirrhosis usually becomes clinically evident in late childhood or early adolescence and leads to portal hypertension.

Cystic fibrosis-related diabetes is increasingly common with advancing age and is clinically distinct from type 1 and type 2 diabetes mellitus in the general population[187]. Importantly, its occurrence adversely affects survival in patients with cystic fibrosis[188]. Other endocrinological complications of cystic fibrosis include delayed menarche in malnourished adolescent females and reduced bone mineral density,

which can increase the risk of bone fractures and is multifactorial[189]. Congenital bilateral absence of the vas deferens occurs in 98% of males with cystic fibrosis and results in azoospermia; intracytoplasmatic sperm injection or related procedures are required for these men to father children. Renal complications can occur and include nephrocalcinosis, and salt and water depletion owing to excess fluid losses, which contribute to acute kidney injury and proteinuria. These renal complications can occur even in those without evidence of diabetes. Chronic kidney disease is more common in adult patients and risk factors for its development include age, diabetes, prior episodes of acute kidney injury and prior organ transplantation. As survival for patients with cystic fibrosis has increased, several complications have been recognized, and these include an increased risk of colorectal and other gastrointestinal malignancies, the potential for macrovascular disease to complicate longstanding inflammatory disease and venous insufficiency.

Management

Clinical outcomes in cystic fibrosis vary between countries and centres[56,190–193], and evidence for many areas of clinical decision making is lacking. Thus, the current focus is on benchmarking projects[56], the establishment of standards of care and management guidelines[194,195] and the development of multidisciplinary specialist care programmes that work in collaboration with the patient and their family[196]. National and international data registries of patients with cystic fibrosis were established in the 1960s[18,197,198]. These registries are central for clinical research and care[15,19,55,199,200]. Public reporting of data and benchmarking between centres have led to improvements of clinical outcomes in less well-achieving centres and high-achieving centres[56,201].

Over the past 20 years, therapies and the time and effort required by patients and their families have markedly increased. Not surprisingly, adherence to prescribed therapies is quite low[202], with lowest rates of adherence observed for airway clearance and nebulized medications. One US study indicated that, overall, adherence to pulmonary therapies is ≤50%, with decreases in adherence related to increasing age from childhood to adulthood[203,204]. Similarly, use of complex and life-long therapies has led to the emergence of treatment-related toxicities, β-lactam allergies, aminoglycoside nephrotoxicity and vascular complications from long-term intravenous access devices[205].

Early recognition and treatment of lung disease[206] and exacerbations[207,208], *P. aeruginosa* eradication and prevention of chronic infection, optimization of nutritional status, adequate mobilization of airway secretions, physical fitness and psychosocial management require regular consideration[208]. Indeed, with the adult cystic fibrosis population growing rapidly, comprehensive transitional care programmes should be available at all centres with regular communication between paediatric and adult teams[208,209]. The key components of pulmonary care of all people with cystic fibrosis are knowledge, participation and monitoring of airway clearance and the appropriate use of maintenance therapies such as mucolytic therapy, hydrators and antibiotics (FIG. 5).

Pulmonary disease

Airway clearance and exercise. Daily airway clearance is considered a standard of care, although the strength of evidence to support long-term benefit is limited[210]. Modes of airway clearance include percussion, device assistance (for example, positive expiratory pressure and vest and handheld vibratory devices) and breathing modalities (for example, autogenic drainage). Although few comparative trials have been carried out, one has shown positive expiratory pressure to be associated with a lower rate of pulmonary exacerbations — an outcome measure that is associated with lung function decline — compared with an oscillating vest device[211]. Aerobic exercise results in improved exercise tolerance, and increased physical activity has been linked to reduced lung function decline[212,213].

Mucolytic and hydrator therapies. Dornase alfa, which is a recombinant human deoxyribonuclease, breaks down DNA derived from degrading neutrophils that accumulate in the airways of patients with cystic fibrosis, thereby reducing viscosity of airway secretions and leading to improved lung function and reduced exacerbations[214,215]. The first large-scale study to show benefit of nebulized dornase alfa was reported >20 years ago[214]. Benefits have subsequently been shown in patients with advanced lung disease (FEV_1 <40% of predicted) and in younger patients with mild disease[216,217]. This therapy is considered the standard of care in patients ≥5 years of age[208,218].

Hypertonic saline functions as a hydrating agent that increases mucociliary clearance and has been shown to improve lung function and to reduce exacerbations in a randomized controlled trial[219]. A reduction in exacerbations was not observed in a study of children aged 4 months to 5 years, but substudies have shown positive effects on lung function[220,221]. Additional studies to assess the efficacy of hypertonic saline in younger children are currently ongoing. Inhaled dry powder mannitol is another osmotic agent that has shown lung function improvements in two trials; effects have been more consistent in adults than in children[222,223].

Inhaled antibiotics. In patients with chronic *P. aeruginosa* infection, nebulized tobramycin (an aminoglycoside antibiotic) improved lung function, reduced exacerbations and increased weight[224,225]. A dry powder preparation of tobramycin that decreases delivery time has more recently been shown to have equal efficacy and to improve patient satisfaction[226]. Inhaled aztreonam (a β-lactam antibiotic) has also been shown to be efficacious when compared with both placebo and inhaled tobramycin[227,228]. Other antibiotics such as colistin are used in some countries and several new preparations of inhaled antibiotics are currently being studied[229]. Few studies have so far examined the role of inhaled antibiotics for other bacterial infections that are common in patients with cystic fibrosis (for example, *B. cepacia* complex, MRSA and *S. Maltophilia*)[230].

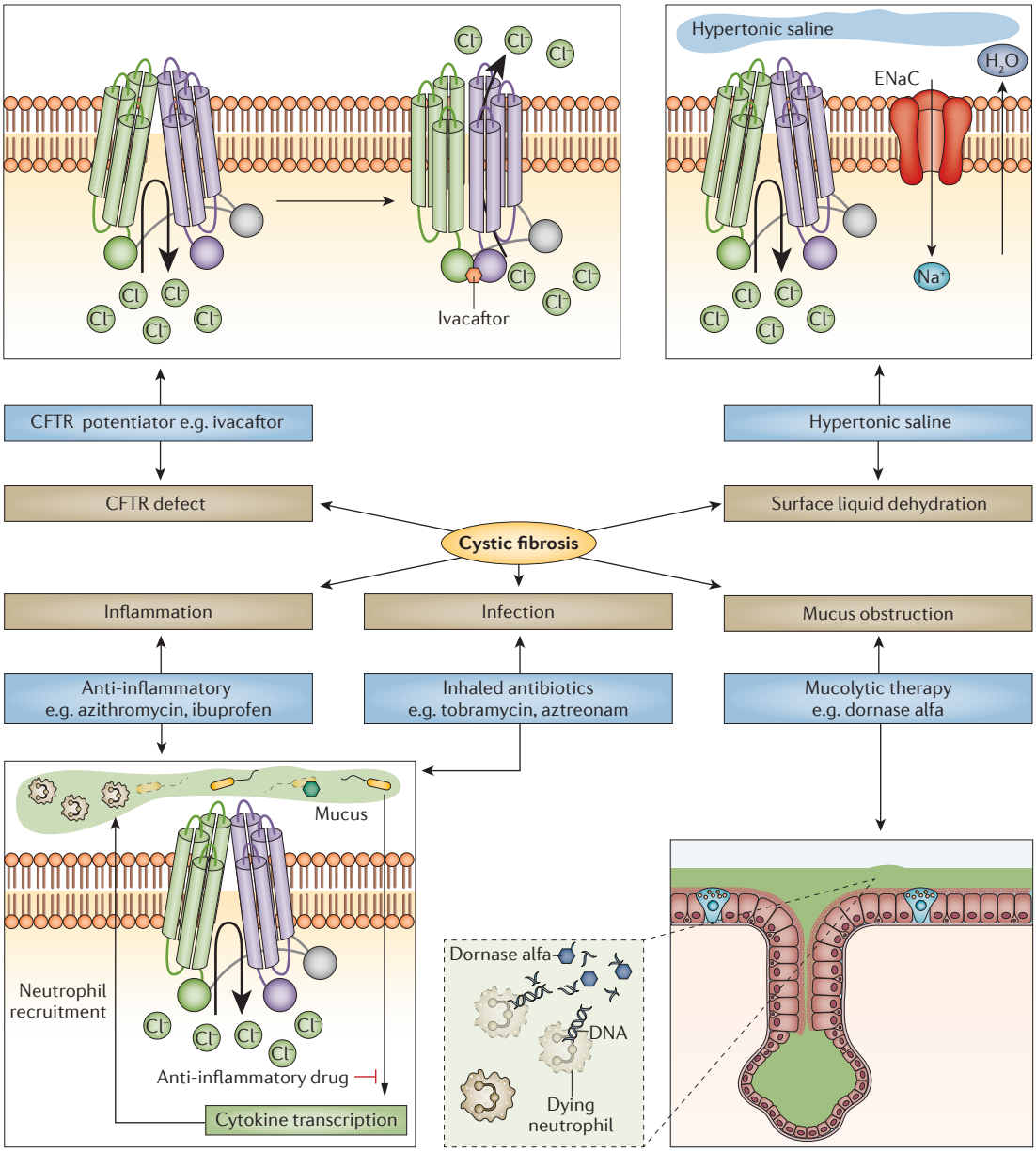

Figure 5 | **Currently available therapies to treat patients with cystic fibrosis.** Cystic fibrosis transmembrane conductance regulator (CFTR) therapies are based on the cellular mechanism, generally address a specific aspect of the molecular defect and do not therefore alleviate the effects of all classes of CFTR mutations. The CFTR potentiator ivacaftor directly activates CFTR and is currently licensed in most countries for patients with class III (gating) mutations. Hypertonic saline increases airway surface liquid, which is reduced in patients with cystic fibrosis as a consequence of defective chloride and increased sodium absorption. Dornase alfa cleaves extracellular DNA, thereby reducing the viscosity of airway secretions. Inhaled tobramycin and aztreonam are used as chronic maintenance therapy in patients with chronic *Pseudomonas aeruginosa* infection. Azithromycin has multiple potential modes of action but mainly ameliorates airway inflammation, as is the case for high-dose ibuprofen, which reduces neutrophil influx into the airways.

Macrolides. Several randomized controlled trials have shown improved lung function, quality of life, weight and reduced time to next exacerbations in *P. aeruginosa*-infected patients treated with azithromycin[231,232]. The mechanism of action of macrolides in cystic fibrosis might be anti-inflammatory rather than antibacterial. In patients without *P. aeruginosa*, when treated with azithromycin, lung function improvement was not observed but there was evidence of a reduced pulmonary exacerbation rate[233,234]. An increasing prevalence of nontuberculous mycobacteria in a single-centre study has raised concerns of whether long-term azithromycin treatment is a contributory factor. This finding was not supported by a subsequent national data registry analysis[113,115], but the effect of macrolides on nontuberculous mycobacterial infection remains controversial as complete case ascertainment is unlikely in registry analysis.

Anti-inflammatory therapies. Several anti-inflammatory therapies have been studied in patients with cystic fibrosis, including oral corticosteroids, inhaled corticosteroids, non-steroidal anti-inflammatory drugs (such as ibuprofen) and leukotriene inhibitors. Although ibuprofen reduced the rate of decline in lung function, especially in younger patients and adolescents, its uptake has been limited because of the need to monitor blood levels to optimize benefit[235,236]. Oral steroids are associated with adverse side effects and inhaled steroids have limited effect in patients who do not have asthma in addition to cystic fibrosis[237–240]. A leukotriene B4 inhibitor (known as BIIL 284) was associated with increased exacerbations and led to an early termination of one trial, raising concerns that anti-inflammatory therapies involve a delicate balance in reducing inflammation without adversely affecting the patient's response to infection[241].

Lung transplantation. Lung transplantation is the established treatment for patients with end-stage pulmonary disease. The outcomes for patients undergoing transplantation for cystic fibrosis have rapidly improved and median survival now approaches or even exceeds 10 years in many treatment centres. Timely referral and close communication between the cystic fibrosis and transplant centres is required to provide sufficient time for assessment of suitability (of the donor and of the recipient), to determine whether indications are met and to establish that there are no foreseeable contraindications. Donor allocation programmes vary globally but aim to prioritize those waiting for transplants who have the most limited pre-transplant survival[242]. Adjunctive therapies, including noninvasive ventilation, can function as a bridge to transplantation[243]. The most common cause of graft failure following lung transplantation is bronchiolitis obliterans, which is thought to be a form of chronic allograft rejection[242].

Extrapulmonary disease

As median survival from cystic fibrosis has approached or even exceeded 40 years, complications have emerged. For example, gastrointestinal malignancy, hyperlipidaemia, metabolic and endocrine complications and multiresistant infections have emerged and are providing new challenges[205]. Involvement of an interdisciplinary team and access to specialist support, including experts in microbiology, general surgery, thoracic surgery, gastroenterology and hepatology, otolaryngology, obstetrics and gynaecology, clinical genetics, endocrinology, palliative care and transplantation services are increasingly important for the adult with cystic fibrosis[208].

Treatment of the basic defect

In terms of treating the basic genetic defect, therapies targeting CFTR dysfunction work by inserting a normal copy of *CFTR* into patients cells (gene therapy), improving the expression of CFTR on the cell surface, increasing the 'opening probability' of existing channels (CFTR pharmacotherapy) or by targeting other ion channels to compensate for its dysfunction.

Gene therapy. Somatic gene therapy has mainly been studied as topical therapy administered to the airways to minimize systemic toxicity. The technique enables transient expression of mature and functional CFTR on the cell surface, as shown in cell cultures and in mice[244]. Human trials initially focused on adenoviral vectors owing to their high transfection efficiency *in vitro*. Single-dose trials involving nasal or intratracheal administration showed transient expression of CFTR without substantial adverse effects at low doses[245,246]. However, repeated dosing is associated with an immune response that reduces transfection efficiency and clinical benefit has not yet been shown[247]. Modifications of adenoviral vectors might help to reduce their immunogenic potential.

Using adeno-associated virus as the vector is less immunogenic than adenoviral vectors and initial studies have suggested prolonged expression and positive trends in clinical outcome measures[248]. However, this finding was not subsequently supported in a longer term multidose trial[249]. Lentiviral vectors are currently being investigated in preclinical studies, in which they have shown high transfection efficiency and prolonged activity of CFTR expression[250].

Liposomal vectors could potentially overcome these limitations of viral vectors. Liposomes are less immunogenic and therefore better suited for repeated dosing; unfortunately, this positive attribute is associated with a lower level of transfection efficiency. A multicentre multiple dose trial has recently been completed by The UK Cystic Fibrosis Gene Therapy Consortium and results are expected soon[251]. Delivery of RNA rather than DNA is another strategy that is currently being explored[252].

Translational readthrough therapy. Class I mutations include premature termination codons (PTCs) that lead to a truncated protein and mRNA transcripts that undergo nonsense-mediated decay. Aminoglycosides, such as gentamicin, can bind to the ribosome and enable readthrough of PTCs, which leads to some production of the full-length protein and partial restoration of channel function. Both *in vitro* studies and studies using topical application to the nasal epithelium in patients carrying stop mutations on at least one allele have shown evidence of CFTR expression after gentamicin treatment[253]. Clinical applicability is limited as prolonged administration of gentamicin is required, which would probably cause renal and/or ototoxicity.

Ataluren was developed as a compound with similar translational readthrough properties but it lacks the potential adverse effects of aminoglycosides[254]. Initial uncontrolled studies in patients showed improvement in chloride conductance measured by nasal potential difference, but a larger placebo controlled trial failed to show clinical benefit[19,255]. However, the subgroup of patients not receiving inhaled antibiotics and without chronic exposure showed less lung function decline after 12 months, an effect that could be explained by competitive inhibition at the level of the ribosome. One study[256] is under way to prospectively assess efficacy in this subgroup. Overall, the benefits observed so far are

Box 3 | **Domains on the Cystic Fibrosis Questionnaire-Revised**

Functional and psychosocial scales

- Physical functioning: ability to walk, climb stairs and carry heavy items, and ability to run, jump and play
- Social and school functioning: going out with friends and engaging in social activities
- Emotional functioning: feeling happy, sad or worried
- Treatment burden: time spent on treatments and fitting treatments into daily activities
- Eating problems: challenges eating and making calorie goals
- Body image: physical appearance and being short or thin
- Vitality*: energy level and extent of fatigue
- Health perceptions*: perceptions of current health and disease severity
- Role functioning*: ability to carry out daily activities (attending school, working and household tasks)

Symptom scales

- Respiratory symptoms: frequency and severity of cough, mucus production and chest congestion
- Digestive symptoms: frequency and severity of abdominal pain, stools and gas
- Sinus symptoms: frequency and severity of sinus headaches, nasal congestion and postnasal drip
- Weight*: perception of current weight

*Not administered to children 6–13 years of age

limited in magnitude, but could probably be increased by combining ataluren with a CFTR potentiator.

CFTR potentiator therapy. Mutations that cause residual surface expression are potentially amenable to drugs that increase channel opening probability. These drugs are called potentiators and the first drug, ivacaftor, has been approved in most countries for clinical use in patients with class III mutations. The most common CFTR class III mutation, G551D, is associated with normal cell surface expression but reduced gating. Ivacaftor improves CFTR function, as shown by improvement in ion channel measurements[24,25,257]. Notably, sweat chloride concentrations fell below the diagnostic threshold in most treated patients — a result that has been confirmed in an observational study[258]. This drop in sweat chloride is accompanied by marked improvement in lung function as well as improvements in other clinical measures (weight, symptoms and pulmonary exacerbations)[257]. Similar effects have been described in other gating mutations, which underlines the potential of CFTR pharmacotherapy in a broader range of *CFTR* mutations[258,259].

Studies are also underway in other mutation classes that could benefit from potentiation of CFTR function, including class IV and class V mutations, as well as mutations in class VI that are associated with residual CFTR function. In addition, as readthrough and corrector therapy is unlikely to normalize CFTR function alone, potentiators might be needed for combination therapy in patients carrying class I or class II mutations. Potentiation of CFTR function could also be of benefit in diseases with secondary CFTR dysfunction, such as chronic bronchitis, as smoke exposure has been found to cause secondary CFTR dysfunction[260].

Intracellular trafficking. The most common CFTR mutation, F508del, is associated with defective protein folding that results in proteosomic degradation with very little or no CFTR reaching the apical membrane (class II). Indeed, as little or no CFTR is expressed on the cell surface in F508del homozygous patients, potentiators such as ivacaftor have little effect on chloride transport in bronchial epithelial cells; this finding has recently been confirmed in a Phase II study[261]. Drugs affecting CFTR transport have been called 'correctors' even though they do not necessarily correct the folding defect. Some correctors alter cell chaperones and other quality control mechanisms; nevertheless, their use generally increases trafficking to the cell surface. The best-studied compounds have evolved from high-throughput screening programmes, with two small molecules — lumacaftor and VX-661 — having undergone clinical studies. Given alone, both compounds have little clinical benefit in patients with F508del; in fact, lung function worsened in patients on lumacaftor alone[262]. However, when ivacaftor was added to lumacaftor, lung function improved above baseline[262]. The magnitude of the effect was significantly less than that observed in response to ivacaftor alone in patients harbouring the G551D mutation (class III), which corroborates results from *in vitro* experiments in human bronchial epithelial cells[263]. Recent *in vitro* data suggest that ivacaftor-mediated CFTR activation might destabilize F508del CFTR and might decrease the beneficial effects of some correctors, including lumacaftor; whether this finding is relevant *in vivo* is currently unclear, but this interaction could be one explanation for the fairly small clinical effect of combination therapy with ivacaftor and lumacaftor in F508del homozygous patients[264,265]. Although the clinical efficacy data of combination therapy is promising, it has become clear that the search for additional CFTR corrector compounds needs to continue. Adding a second corrector has been shown to improve the efficacy of lumacaftor and ivacaftor *in vitro*[266] by addressing distinct cellular mechanisms.

Ion channel-directed therapy. Cystic fibrosis is associated with decreased chloride and bicarbonate secretion through CFTR and increased sodium absorption by ENaC. Another potential mechanism to address the ion dysbalance on the epithelial surface is to target other apical ion channels. Activation of the apical calcium-activated chloride channel using derivatives of its natural activator ATP has been studied using the P2Y purinoceptor agonist denufosol. Although initial studies showed some promise, beneficial effects could not be confirmed in subsequent studies[267,268]. Inhibition of sodium absorption was initially examined using amiloride, but limited clinical efficacy was shown[269]. More potent and specific inhibitors with longer half-lives are currently being developed.

Quality of life

There is growing recognition of the importance of patient-reported outcomes (PROs) in health outcomes research and clinical care[270]. These instruments, which

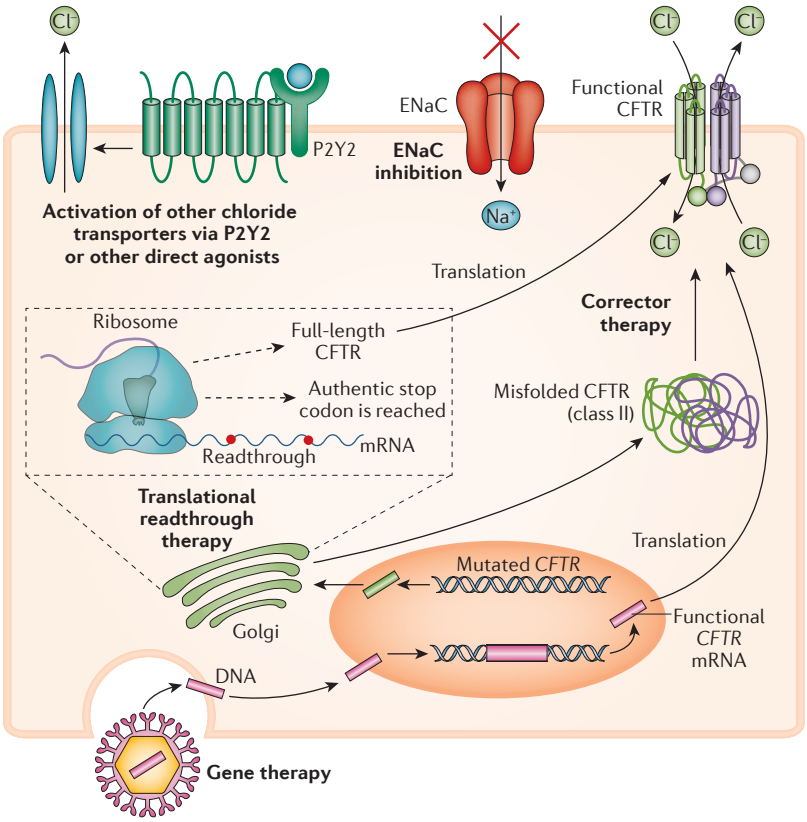

Figure 6 | **Emerging approaches to address the ion channel abnormalities in cystic fibrosis.** The main target for therapeutics is cystic fibrosis transmembrane conductance regulator (CFTR), which can be replaced through gene therapy or increased in concentration on the cell surface. Increased CFTR concentration could be achieved through translational readthrough therapy (class I mutations), correction of intracellular trafficking (class II mutations) or potentiation of its function (class III mutations and potentially class IV and class V). Alternatively, a calcium-activated chloride channel could be a therapeutic target; this channel is activated through the P2Y purinoceptor 2 (P2Y2; the natural ligand being ATP). Finally, as sodium absorption is upregulated in the airways of patients with cystic fibrosis, blocking the epithelial sodium channel (ENaC) could be of benefit to increase airway surface liquid.

include health-related quality-of-life (HRQOL) measures, can be used for several purposes: as primary or secondary outcomes in clinical trials of new medications or behavioural interventions[219,271,272]; to document the natural progression of the disease[273]; to describe the effect of an illness on patient functioning[274]; to analyse the costs and the benefits of medical interventions; and to aid in communication and clinical decision making[275].

Quality-of-life studies in cystic fibrosis over the past 10 years have highlighted three key issues: the importance of patient-reported respiratory symptoms as an outcome measure for clinical trials; the growing perceptions among patients and families that the prescribed treatment regimen is burdensome[276]; and the considerable differences in HRQOL according to socioeconomic and racial and/or ethnic minority status. Indeed, prior research on HRQOL in cystic fibrosis has shown that generic measures are insensitive and that disease-specific tools are needed[277]. Efforts to develop reliable and valid HRQOL instruments in cystic fibrosis have been successful[228,270,278] and are now routinely used in clinical trials of new medications and in studies of patient functioning. Currently, the most widely used HRQOL measure for cystic fibrosis is the Cystic Fibrosis Questionnaire-Revised (CFQ-R) (BOX 3), with developmentally appropriate versions for preschooler children, school-aged children and their parents, and adolescents and adults[279]. The CFQ-R has been translated into more than 36 languages and is being used in several multinational trials.

Randomized controlled trials of new therapies in cystic fibrosis have used the CFQ-R as a primary or secondary outcome and have shown benefits in terms of reduction in respiratory symptoms and improvements in other domains, such as physical functioning, vitality and health perceptions[228,271,273,279]. Interestingly, improvements in respiratory symptoms have been found across trials of medications with very different mechanisms of actions (for example, inhaled antibiotics, hypertonic saline and potentiators), increasing confidence that this is an important outcome.

Although a primary reason that health outcomes and lifespan have markedly improved over the past 20 years has been the development of new long-term therapies for patients with cystic fibrosis, treatment regimens now take 2–3 hours per day for most patients[279]. In several studies using the CFQ-R, which has a treatment burden scale, patients and parents have reported increasing perceptions of burden[274,280]. In a recent US epidemiological study over 3 years, both treatment complexity and perceived burden were highest among adults and those with severe lung disease, but increased in all age groups over the course of the study[281].

Using a national database in the United States, differences on the CFQ-R were shown by socioeconomic and racial and/or ethnic minority status. In a sample of 4,751 patients and 1,826 parents, after controlling for disease severity, people with low socioeconomic status reported significantly lower scores on the CFQ-R (children, parents and teen/adults) across the majority of domains. After controlling for both disease severity and socioeconomic status, African-American and Hispanic families reported lower scores on the social and emotional functioning scales compared with their white counterparts[282]. These differences might be related to access to care, ability to afford medical insurance and other ancillary costs of optimal care (for example, good nutrition). Whether similar disparities are found in countries with national healthcare systems remains unclear.

Another important issue that affects HRQOL is comorbid depression and anxiety. An international psychological screening study of >6,000 patients with cystic fibrosis and 4,000 parent caregivers in nine countries recently found a significantly higher prevalence of these symptoms than in community samples[283]. Psychological distress has a direct negative effect on therapy adherence, attendance at clinic, hospitalizations and healthcare costs[284–287]. As we move towards more patient-centred care, standardized assessments of HRQOL and psychological symptoms will be increasingly integrated into clinical care.

Outlook

Understanding the underlying genetic abnormalities and the mechanisms by which *CFTR* mutations cause disease has led to CFTR-specific therapies that are either already available to patients or that are close to clinical use (FIG. 6). Additional compounds are being assessed in ongoing studies; thus, the armamentarium of treatment options will probably expand. Although genotype-specific therapy is being referred to as personalized medicine, it will not provide the same benefit to each patient. Some studies have already shown that response to interventions is not homogeneous even in patients carrying the same *CFTR* mutation[262]. Exploring modifier genes of involved intracellular pathways or other ion channels might help to better understand this variability and to define additional therapeutic targets. In addition, current studies focus on patients with the *CFTR* mutations with known functional consequences on CFTR processing and function, and will not be applicable to patients with rare mutations.

An individualized treatment approach will probably be needed for CFTR-directed pharmacotherapy, particularly for patients with rare or difficult to treat alleles. Individualized treatment will require test systems or biomarkers that have a high precision-predicting treatment response. Potential assays could involve cells harvested from patients that can be used to test the most promising treatment combination *in vitro*. On the basis of their accessibility, nasal epithelial cells and intestinal cells transformed into organoids are currently being investigated as potential models[134]. Preliminary data using stem cells derived from skin fibroblasts or blood cells transformed into airway epithelial cells could offer an alternative in the near future[288]. Alternatives or additions to these *in vitro* assays could be used topically *in vivo* to test drug response by applying them to the sweat gland or to the nasal epithelium. In addition, sensitive clinical outcome measures will need to be developed to enable individual patient monitoring of drug response.

CFTR-directed therapy should ultimately prevent lung damage; to do so will require treatment early in the disease process, ideally as soon as the diagnosis is established. Newborn screening offers a unique opportunity, but further work is needed to establish robust outcome measures in young children to provide evidence for efficacy of early treatment interventions. Prevention of deterioration rather than improvements in disease manifestations will probably need to be the outcome measure of clinical trials in this age group. If this can be achieved, CFTR modulation could potentially maintain lung health and preserve lung function and structure. In addition, early application of CFTR modulators could also affect other disease manifestations, such as exocrine and endocrine pancreatic function. Conversely, in patients with established disease, the best scenario might be to transform cystic fibrosis into non-cystic-fibrosis bronchiectasis — a disease that is much less severe but often still progressive. Thus, even with optimal CFTR-directed treatment, it will take decades until we can expect patients to not need treatment for the prominent disease manifestations of mucus obstruction, infection and inflammation. Although targeting CFTR is likely to make a major difference, finding better therapeutic strategies for these aspects of the disease will continue to be important to maintain lung health in patients with cystic fibrosis.

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Author contributions

Introduction (F.R.); Epidemiology (C.H.G.), Mechanisms/pathophysiology (S.M.R. and S.C.B.); Diagnosis, screening and prevention (A.B., F.R. and S.M.R.); Management (S.C.B. and F.R.); Quality of life (A.L.Q.); Outlook (F.R. and A.B.); overview of the Primer (F.R.).

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