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Review article

Cystic fibrosis and primary ciliary dyskinesia: Similarities and differences

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ABSTRACT

Cystic fibrosis (CF) and Primary ciliary dyskinesia (PCD) are both rare chronic diseases, inherited disorders associated with multiple complications, namely respiratory complications, due to impaired mucociliary clearance that affect severely patients' lives. Although both are classified as rare diseases, PCD has a much lower prevalence than CF, particularly among Caucasians. As a result, CF is well studied, better recognized by clinicians, and with some therapeutic approaches already available. Whereas PCD is still largely unknown, and thus the approach is based on consensus guidelines, expert opinion, and extrapolation from the larger evidence base available for patients with CF. Both diseases have some clinical similarities but are very different, necessitating different treatment by specialists who are familiar with the complexities of each disease.This review aims to provide an overview of the knowledge about the two diseases with a focus on the similarities and differences between both in terms of disease mechanisms, common clinical manifestations, genetics and the most relevant therapeutic options. We hoped to raise clinical awareness about PCD, what it is, how it differs from CF, and how much information is still lacking. Furthermore, this review emphasises the fact that both diseases require ongoing research to find better treatments and, in particular for PCD, to fill the medical and scientific gaps.

1. Introduction

The human respiratory epithelium (or airway epithelium) is responsible for critical functions such as air transport, mucociliary clearance (MCC), and the regulation of pulmonary immune responses. MCC is the lung's primary innate defence mechanism [1]. In a healthy system, MCC is responsible for trapping foreign bodies in mucus and transporting them out of the lungs via cilia-generated flow to the nasopharynx, where they are expectorated or swallowed [1].

The respiratory epithelium is classified as ciliated pseudostratified columnar epithelium and contains a wide range of cell types. Ciliated, mucus secretory (also known as club cells), and basal stem cells are the most abundant cell types. Other rare cell types found in the respiratory epithelium include brush cells (also known as tuft cells), pulmonary neuroendocrine cells, goblet cells, and ionocytes [2,3]. Ciliated epithelial cells are the predominant cell type in the airways, with about 100–300 cilia per cell [4]. The coordinated fast sweeping movement, with forward power stroke and a backward recovery stroke, generated by ciliated cells is critical for the MCC [5,6].

The airway epithelium (Fig. 1) is covered by an airway surface layer (ASL), which is divided into two distinctive gel-like layers: (1) a mucus layer that entraps inhaled particles and foreign pathogens; and (2) a periciliary layer (PCL) located beneath the mucus layer, which is a Newtonian fluid with a dynamic viscosity similar to water [7,8].

Mucus is primarily composed of water (~ 95%), but it also contains salts, lipids such as fatty acids, cholesterol and proteins, which serve a defensive purpose. Mucin glycoprotein is the main component responsible for the viscous and elastic gel-like properties of mucus [9]. According to the gel-on-brush model [10], the PCL contains membrane-spanning mucins and large mucopolysaccharides that are tied to cilia, microvilli, and epithelial surface tethered macromolecules forming a brush-like structure. The mucus and PCL forms two separate layer as a result of its brush-like structure, which prevents mucin and inhaled particles from penetrating the PCL. PCL offers lubricant on the epithelial surfaces of the airways and permits ciliary beating for an effective MCC. Moreover, the densely tethered macromolecules in the PCL generate biophysical forces that regulate the hydration of both the

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Abbrevations

Adenosine triphosphate (ATP)-binding cassette (ABC)
Airway surface layer (ASL)
Antisense oligonucleotides (ASO)
Central pair complex (CPC)
CF transmembrane conductance regulator (CFTR)
Chloride ions (Cl^-)
Congenital absence of the vas deferens (CBAVD)
Computed tomography (CT)
Cyclic adenosine monophosphate (cAMP)
Cystic fibrosis (CF)
Dynein arms (DA)
Dynein regulatory complex (DRC)
Endothelin receptor type A (EDNRA)
Epithelial sodium channel (ENaC)
European medicines agency (EMA)
Food and Drug Administration (FDA)
Inner dynein arm (IDA)
Kartagener syndrome (KS)
Micro RNA (miRNA)
Mucociliary clearance (MCC)
Nitric Oxide (NO)
Nonsense-mediated decay (NMD)
Nucleotide-binding domain 1 (NBD1)
Outer dynein arm (ODA)
Pancreatic enzyme therapy (PERT)
Pancreatic insufficiency (PI)
Pancreatic sufficiency (PS)
Periciliary layer (PCL)
Primary ciliary dyskinesia (PCD)
Protein kinase (PKA)
Radial spokes (RS)
Short interfering RNA (siRNA)
Sodium ions (Na^+)
Bicarbonate ($HCO3^-$)
Potassium (K^+)
Calcium (Ca^{2+})
Whole-exome sequence (WES)

PCL and mucus layer by osmotic pressures [10,11]. PCL hydration is of upmost importance.When not sufficiently hydrated, the mucus layer compresses the PCL, and cilia will become trapped and stop moving mucus.

Cystic fibrosis (CF) and Primary ciliary dyskinesia (PCD) are rare genetic diseases that affect multiple organs, with the lungs being the main cause of morbidity, and both are linked to impaired MCC [12,13]. Despite the rarity of both diseases, CF is more prevalent than PCD among Caucasians. In the Caucasian population, CF affects approximately 1:3200 Caucasian live births, while PCD affects about 1:16,000 live births [14]. This discrepancy in prevalence may be due to PCD's significantly lower rate of diagnosis than CF. PCD diagnosis is more challenging than CF and requires specialised centres, thus is probable that many patients with PCD have been misclassified with other respiratory diseases. Moreover, in contrast to CF which is included in most national newborn screening programs, PCD is not, thus requiring that medical doctors have knowledge about this disease and how it manifests. Consequently, knowledge regarding PCD is scarce when compared to CF. There is no specific therapy for PCD, and current PCD management is largely based on CF evidence [15,16]. However, CF and PCD have distinct disease mechanisms, clinical manifestations and thus require different treatment strategies. In order to give a comprehensive picture of the current understanding of the two diseases, this review will highlight their parallels and differences in terms of clinical

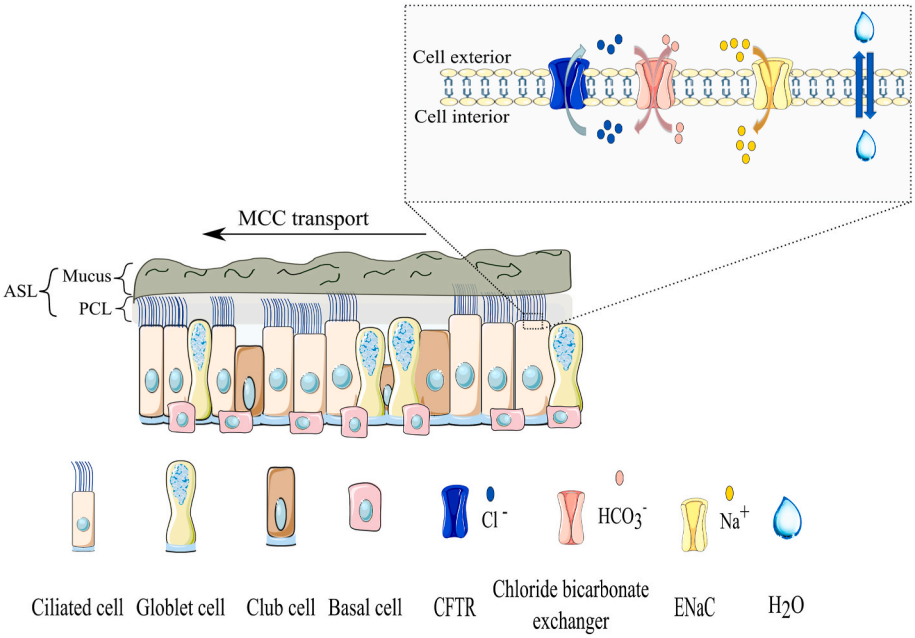

Fig. 1. Schematic representation of the healthy airway epithelium, showing the most predominate cell types, namely the ciliated cells, secretory cells, which includes the goblet cells and club cells (formerly known as Clara cells), as well as, the basal cells (that can generate secretory and ciliated cell lineage) and rare cell types, specifically tuff cells, ionocytes and neuroendocrine cells.
The mucociliary clearance (MCC) and the airway surface layer (ASL) are also represented. The ASL includes a periciliary layer (PCL, represented at light green), that lubricates airway surfaces and facilitates ciliary beating, and mucous layer (represented at dark green, above the PCL).
In the zoom box (box at dotted line) is a representation of a normal ion exchange at the apical membrane of the airway epithelium. The normal CFTR channel allows movement of chloride ions (Cl^-) to the outside of the cell and the epithelial sodium channel (ENaC) allows that the reabsorption of the sodium ions (Na^+). The reabsorption of Na^+ is regulated by the CFTR, that controls the Na^+ influx. The chloride bicarbonate exchanger channel maintains the electrochemical gradient among the bicarbonate ($HCO3^-$) and Cl^- ions. Other ions such as potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and carbonate ($CO3^{2-}$) are also important and balanced in a healthy situation, for simplicity were not represented. The balanced exchange of the ions allows the creation of an isotonic environment, thus the water influx and efflux is constant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

presentations, disease mechanisms, clinical features, genetics, and treatment choices.

2. Disease mechanisms

2.1. Cystic fibrosis mechanisms

Cystic Fibrosis is caused by a defective CF transmembrane conductance regulator (CFTR), which is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter superfamily. All ABC transporters bind to ATP and use its energy to drive the transport of several molecules across cell membranes. A wide variety of epithelial tissues, such as the salivary and sweat glands, gastrointestinal and reproductive tracts, and airways, depend heavily on the CFTR anion channel for transepithelial fluid and electrolyte transfer [17,18].

The CFTR gene was discovered in 1989 by Dr Lap-Chee Tsui's team [19]. Later, in 1991, Anderson and co-workers [20] proved CFTR as a cyclic adenosine monophosphate (cAMP)-regulated chloride (Cl^-) channel. Thereafter, several groups studied CFTR and it is now well established that CFTR drives Cl^- across the cell membrane and is regulated by the cAMP-dependent protein kinase (PKA) [21].

Another particularity of the CFTR is its ability to switch from a conformation permeable to Cl^- to a conformation permeable to bicarbonate (HCO3−), depending on the presence of external Cl^- [22]. Besides, CFTR is also important in the regulation of other transport proteins, including the potassium (K^+) channels [23], ATP-release mechanisms [24], sodium-bicarbonate ($Na^+/HCO3^-$) transporters [25], and the aquaporin water channel [26]. Consequently, disruption in the CFTR causes an ion transport defect, which generates an imbalance in the normal homeostatic ion levels, particularly of Cl^- and Na^+ions. CFTR dysfunction affects primarily the airways and lungs, with lung disease being the major cause of death in CF patients. However, the effects of CFTR dysfunction are not limited to the lungs. CF Patients may have other organs affected such as the kidney, pancreas, intestine, heart, vas deferens and sweat duct [27].

Under normal physiological conditions, CFTR can inhibit the activity of the epithelial sodium channel (ENaC) in the airways, regulating sodium ions (Na^+) [28]. Thus, in a normal airway epithelium, CFTR is responsible for balancing the rates of Na^+ absorption and Cl^- secretion (Fig. 1), allowing the maintenance of proper hydration of airway secretions [17].

There is still no perfect agreement on how CFTR dysfunction leads to CF disease. But so far, two main hypotheses (Fig. 2) have been postulated to explain how CFTR dysfunction affects the airways: the low-volume hypothesis and the high-salt hypothesis (reviewed in detail by Refs. [18,29,30]).

The **low-volume hypothesis**, proposes that CFTR malfunction results in a loss of ENaC activity inhibition, which leads to an intracellular accumulation of Na^+.This causes an intracellular flux of Cl^- (probably through a non-CFTR pathway) and water reabsorption to maintain isotonicity, resulting in dehydration of the PCL. In a cascade effect, the reduction in PCL water volume causes the ASL to become dehydrated, which will entrap the ciliated cells and impair their ability to move, promoting the reduction of MCC and the growth of harmful microbes in the mucus (e.g. Pseudomonas aeruginosa (P. aeruginosa)). The accumulation of microorganisms can be caused by one of two reasons: (1) the plaques create a favourable environment to harbour pathogenic microorganisms such as bacteria; and (2) it renders the major endogenous antimicrobial substances in mucus ineffective [31–33].

The **high-salt hypothesis** relies on the assumption that normal ASL

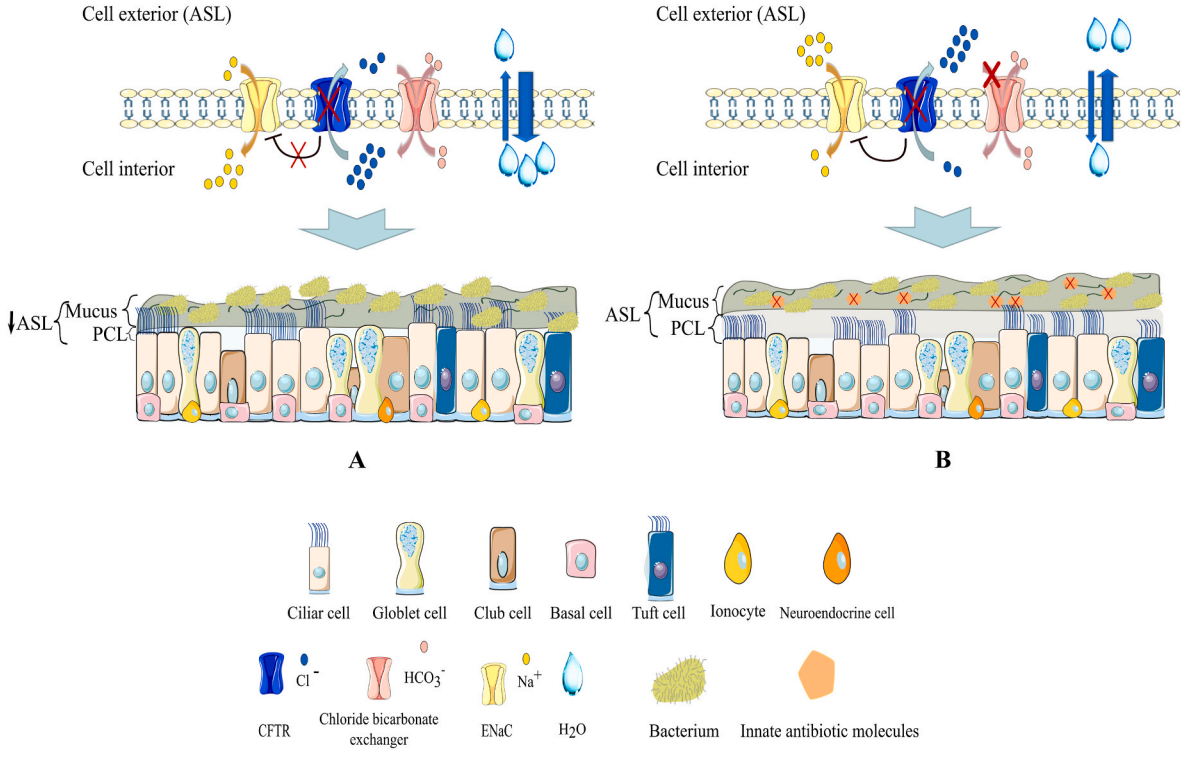

Fig. 2. Schematic representation of the main hypotheses that attempts to explain CF lung disease. A) represents the **low-volume hypothesis,** postulates that because of CFTR dysfunction, the CFTR's ability to regulate the activity of the ENaC is lost, as well as the control of Cl^- efflux. This causes an excess of Na^+ and Cl^- in cell interior, leading to an increase in water reabsorption, resulting in dehydration of airway surface. The subsequent decrease in water volume of the airway surface layer (ASL) results in a reduction in the lubrication and may compromise the ciliary motility and mucociliary clearance, hence allowing bacteria accumulation. **B)** represents the **high-salt hypothesis,** that proposes that the disfunction of CFTR impairs the ability to reabsorb chloride ion from ASL. By other side, the ENaC absorption continues to be regulated, hence is there an excess of Na^+ and Cl^- at the ASL. This increased ion concentration disrupts the function of important innate antibiotic molecules allowing bacteria accumulation.

contains small molecules with broad-spectrum bactericidal activity that protect a healthy epithelium from pathogenic bacteria. This hypothesis states that the absence of a functional CFTR channel imposes an excess of Na^+ and Cl^- in the ASL. This increased ion concentration disrupts the function of innate antibiotic molecules, fostering colonization of airway mucus by bacteria [34,35].

These two hypotheses are widely accepted to explain the CF phenotype in lung disease. However, some groups have proposed other hypotheses. One of them suggested that the CF phenotype in lung disease is due to changes in the pH of ASL, resulting in a decrease in the bactericidal activity of the innate immune system [36]. Other hypotheses are related to a dysregulation of the host's inflammatory response [37,38] or a primary predisposition to infection [39]. Regardless of the hypotheses to explain the CF phenotype, what they all have in common is the fact that colonization of pathogenic microorganisms is promoted, leading to chronic infection.

Recently a rare cell type from the airway epithelium, named ionocyte, was found to be responsible for more than 50% of CFTR expression, despite comprising less than 1% of the respiratory epithelium [2,3,40]. Although there hasn't been any research on this topic published to date, it's plausible that abnormal ionocytes may also be linked to CFTR malfunction and contribute to the CF phenotype in the mucus of lung diseases.

2.2. Primary ciliary dyskinesia mechanisms

Primary Ciliary Dyskinesia is a ciliopathy caused by dysfunction of the motile cilia leading to respiratory, reproductive, and laterality issues [13,41] and, in rare cases, can also lead to hydrocephalus [42]. The main cellular structure responsible for ciliary motility is the axoneme [43]. The axoneme (Fig. 3) is the structural core of the cilium. It is formed by a hollow cylinder composed of 9 peripheral microtubule doublets and two central single microtubules (9d+2s pattern). Doublets are linked by a dynein regulatory complex (DRC) and contain a pair of projections, named dynein arms (DA), which are nominated for their position as inner (IDA) and outer (ODA) in the A-microtubule of each peripheral doublet [43]. The two central microtubules are linked by a central bridge and surrounded by a central fibrillar sheath, which constitutes the central pair complex (CPC). Doublets bind to the CPC by radial projections, called radial spokes (RS). The coordinated action of DA, which are motor proteins, generate the main mechanical forces and the bending forces required for ciliary motility [44]. The DA activity is regulated by the mechano-chemical signals initiated at the CPC and transmitted to DAs by the RS [45–47].

When the axoneme structure is normal, motile cilia beat rhythmically and their movement is critical for cell locomotion and for driving fluid transport over epithelia. Multiple motile cilia are located in the epithelial cells from the Fallopian tubes, where they are important for

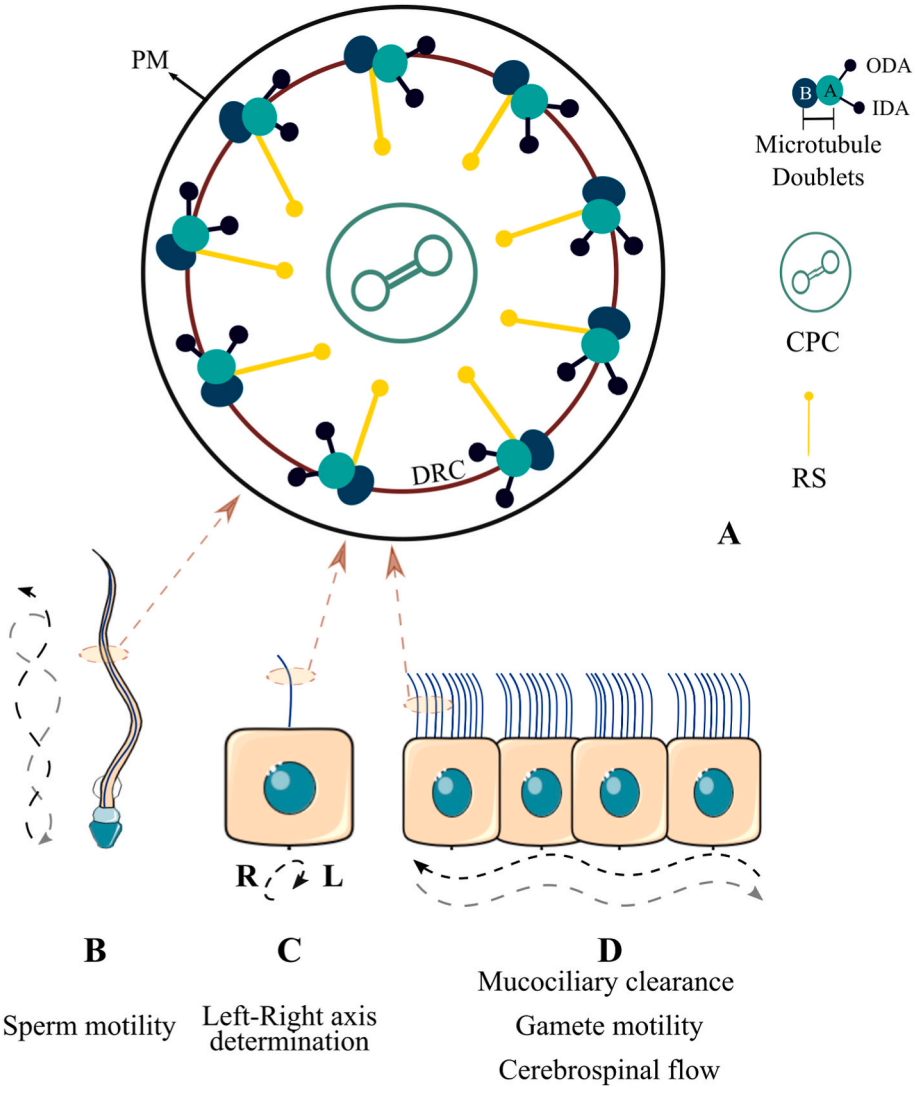

Fig. 3. Schematic representation of the axoneme structure (A) representing the different motile cilia, with representation of the different types of ciliary motion and the main biological function associated to each movement (**B-D**). **A.** The axoneme is the central core common to all motile cilia and is composed by 9 peripheral doublets of microtubules (Mt) linked by the dynein regulatory complex (DRC) and connected by radial spokes (RS) to a single pair of central Mt (central tubules that are surrounded by a fibrillar central sheath, constituting the central pair complex (CPC). Each pair of peripheral Mt has an inner (A) and an outer (B) Mt. From each A-Mt arises two dynein arms: the outer (ODA) and inner (IDA) dynein arms. **B.** Representation of the flagellar motion. It is characterized by a rhythmic and asymmetrical movement, which contains forward stroke (black dotted line) and recovery stroke (grey dotted line). **C.** Node and their clockwise rotation, which generates a leftward effective stroke and rightward recovery stroke vital to define mammalian left-right axis. **D.** Drawing of ciliary sweeping movement with a forward power stroke (black dotted line) and a backward recovery stroke (grey dotted line), which is critical for mucociliary clearance, gamete motility in the female reproductive system and for cerebrospinal flow.

ovulated oocytes and early embryos transport through the Fallopian tube [48,49]; brain ependymal cells, where they promote the flow of cerebrospinal fluid [50], and respiratory airways, where, as referred above, are critical for MCC [1]. Two other types of motilie cilia are the sperm flagellum and the nodal cilia. Sperm flagellum produces a symmetrical, lower-amplitude waveform (and then an elliptical waveform) that propels sperm through the female reproductive tract [43]. The nodal cilia, located in the embryonic node, are critical for determining the organ arrangement of the left-right asymmetric body axis [51].

The ciliar/axoneme dysfunction are caused by mutations in genes involved in the formation, assembly, and/or function of cilium/axoneme components [43].

3. Genetics

3.1. Cystic fibrosis genetics

Cystic Fibrosis is an autosomal recessive disorder caused by mutations in a single gene that encodes for the CFTR protein [52]. Because CFTR is the only gene with known mutations, the genetics of CF may sound simple. However, the correlation between the CFTR genotype and the phenotype is far from simple.

Currently, according to the CF Mutation Database (available at http://www.genet.sickkids.on.ca/StatisticsPage.html, accessed on February 3, 2023), there are 2111 mutations listed. However, these variants are not evenly distributed among CF patients, with the Phe508del variant being the most common, accounting for 70% of the individuals studied. Furthermore, not all variants have the same clinical significance.The CFTR2 (from Clinical and Functional TRanslation of CFTR) project (accessible at https://cftr2.org/mutations_history, updated at 29 April 2022), have analysed 485 variants from the 88,000 patients to provide clinical information about specific CFTR variants and genotypes and verified that only 450 variants are considered CF-disease-causing variants. However, there is still much work to be done, as only about 22% of the CFTR variants were analysed.

CFTR variations have been divided into seven functional groups to aid in the understanding of molecular and cellular processes involved in the pathogenecity [53] (Table 2). Accordingly, **Class I mutations** include mostly null mutations that lead to shortened and defective proteins. **Class II mutations** include the most common CF variant (Phe508del) as well as other mutations that cause protein misfolding and endoplasmatic reticulum (ER) quality control mechanism retention. **Class III mutations** include mutations that impair the channel regulation (called gating) and thus block the flow of Cl^- ions through the CFTR channel. **Class IV mutations** also cause a defective, although less severe, gating leading to a substantial decrease in CFTR channel conductance of Cl^- and $HCO3^-$. **Class V mutations** include splice site mutations that only partially disturb correct splicing and result in a significant reduction in the levels of normal CFTR protein. **Class VI mutations** destabilize CFTR on the cell surface, activating other alternative molecular pathways, such as stimulation of CFTR endocytosis. The most severe cases, previously included in Class I, that result in the loss of the CFTR protein and are not treatable by the existing pharmacological therapies are included in the more recent **Class VII** mutations, which was introduced primarily for pharmaceutical reasons [53]. In general, patients with Class I, II, III or VII mutations, which impair protein production, tend to have a severe phenotype, whereas individuals with Class IV, V, or VI tend to have a milder disease phenotype [53]. While this mutation classification system is helpful, it has limitations as some mutations, particularly the Phe508del can fall into different classes [54].

In addition to the variety of CF disease-causing gene variants, the same variants can exhibit a variety of clinical severity, especially in light of genetic modifiers, environmental variables, and/or stochasticity [54–57]. For instance, a study by Havasi et al. showed evidence that the endothelin receptor type A (EDNRA) plays a role during the development of the vas deferens and that it may contribute to the congenital bilateral absence of the vas deferens (CBAVD) phenotype associated with CF [58].

Overall, this demonstrates the complexity of CF genetics and the fact that, despite remarkable advancements, much remains to be learned.

3.2. Primary ciliary dyskinesia genetics

Primary Ciliary Dyskinesia is predominantly a rare autosomal recessive disease, with some rare cases of X-linked [59,60] and one gene proposed to be autosomal dominant (the FOXJ1-PCD) [42,61]. Unlike CF, more than 40 genes have been associated with the PCD phenotype (Supplemental Table 1 and regularly updated in Ref. [62]). Recent proteomic studies have emphasized the cilium proteome's high complexity as well as the intricate molecular processes involved in ciliary development and function [63–67] Thus, the genes already associated with PCD may be only a small portion of what might be involved in PCD genetics.

The phenotype in most PCD cases is caused by an ultrastructural ciliary defect in the axoneme structure, with DA (particularly ODA) anomalies affecting about 40% of PCD patients and considered hallmark defects [68]. Ultrastructural defects in other structures such as CPC and RS defects are estimated to justify, no more than 10% of all PCD [62,69]. Therefore, pathogenic variants in genes that code for DA proteins, and more specifically for ODA proteins are a major genetic cause in PCD patients.

The gene *DNAH5 (*dynein axonemal heavy chain 5) is the major disease-causing gene in PCD and has been linked to several PCD cases [13,70–74]. Another relevant gene for PCD is *DNAH11* which encodes a ciliary ODA protein and is considered the second disease-causing gene in PCD [62]. Surprisingly, changes in this gene result in normal axonemal ultrastructure [75,76].

Besides, mutations in other genes that code for chaperones, proteins involved in the cytoplasmatic assemble of axonemal dynein, and axonemal dynein docking, namely *DNAAF1* [77], *HEATR2* [78], *LRRC6* [79], *DNAAF4* [80], *ZMYND10* [81]. *CCDC103* [41], and *TTC25* [82] have also been linked to PCD.

Given the high genetic complexity, genetic testing is critical in PCD diagnosis, and high-throughput techniques, particularly whole-exome sequencing (WES), are required [83,84]. Current guidelines define that a biallelic pathogenic mutation or hemizygous X-linked mutation in a PCD gene can confirm a diagnosis [16,85]. Genetic testing in PCD is difficult due to the existence of several mutations described, each with a low prevalence, and even those patients who possess the same harmful variant may exhibit different manifestations. In addition, for about 30% of PCD patients, the genetic information remains elusive and thus also provide an inconclusive result. Consequently, PCD genetic screening can be a delicate and prolonged process [64]. Consequently, unlike in CF, a quicker genetic screening test is not available for PCD. The inability to gather a sufficient number of people with the same genetic variant(s) reduces the statistical power of genotype-phenotype association studies. Therefore, coordinated efforts are required to collect more data from PCD patients, which could enhance the genotype-phenotype association. This analysis would be crucial for helping with the diagnosis.

4. Clinical manifestations

As described in the previous section, in both, CF and PCD, the MCC system is compromised, resulting in recurrent and chronic respiratory inflammation and infection, which causes progressive lung disease. Although there are certain respiratory symptoms that are similar, the overall clinical characteristics and the major systems affected are different (Fig. 4). Having one or more of the clinical characteristics indicated in Table 1 a clinical suspicion of CF or PCD is raised, or if a family member was considered a positive case. Several laboratory tests are needed to confirm a diagnosis [16,86] and the main diagnostic

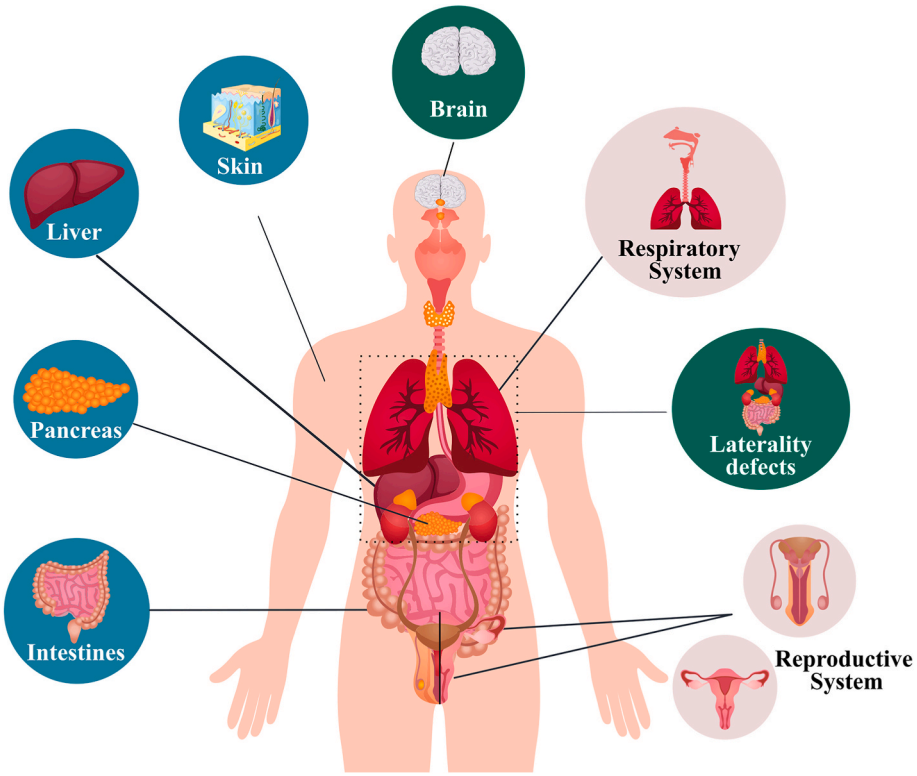

Fig. 4. Schematic representation of main organs/systems affected in CF (blue circle) and PCD (green circle). Systems affected in both diseases are noted by a light pink circle. See text for more details. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

criteria for both diseases are summarized in Fig. 5.

4.1. Gastrointestinal system

In contrast to PCD, gastrointestinal and nutritional disorders are early symptoms of CF [16,85]. Approximately 15% of CF children have *meconium ileus* (obstruction of the terminal ileum) at birth and about 90% of CF children will develop pancreatic insufficiency (PI) during the first year of age [87]. It is possible to distinguish two important groups of CF patients: those with PI (CF-PI) and those with pancreatic sufficiency (PS). CF with PS (CF-PS) patients have an exocrine pancreatic function capable of digesting and absorbing food without the need for supplemental pancreatic enzyme therapy (PERT). Nevertheless, they do not have a completely normal pancreatic function, it may worsen and become patients with PI later on [87]. CF-PS is likely caused by mutations of class IV and V, whereas, CF-PI is caused by CFTR mutations of class I, II, and III [88].

In healthy individuals, the apical $Cl^-/HCO3^-$ exchanger relies on the CFTR protein, which is in the apical membrane of the epithelial cells of the pancreatic ducts [89,90]. CFTR dysfunction impairs electrolyte transport and leads to reduced fluid secretion. As a result, pancreatic juice is significantly more concentrated, causing obstruction of the pancreatic ducts, inflammation, and injury, and ultimately leads to PI [91]. Fat malabsorption, steatorrhea (the presence of excess fat in faeces), decreased weight, changes in bone metabolism, decreased serum albumin, and blood urea nitrogen levels are all consequences of PI that can be observed in CF-PI patients [91]. Further, CF-PI patients are also prone to develop carbohydrate intolerance and insulin insufficiency, which can result in CF-related *diabetes mellitus*, with symptoms distinct from typical type I or type II *diabetes mellitus* [87]. Patients with CF-related *diabetes mellitus* frequently have more severe pulmonary disease, with recurring pulmonary exacerbations and/or worse nutritional status [92]. Fortunately, the introduction of PERT made gastrointestinal and nutritional disorders manageable, allowing people to live longer lives.

Gastrointestinal complications are not considered a suggestive clinical feature of PCD. Nevertheless, in a survey of a heterotaxy population (i.e. individuals with laterality disorders) authors have identified a probable case of PCD, without chronic respiratory symptoms, and with duodenal atresia and *imperforate anus* [93]. Therefore, gastrointestinal issues should not be fully ruled out in PCD patients, especially those with laterality disorders, and physicians should be mindful of this possibility while diagnosing their patients.

4.2. Respiratory system

Although CF and PCD exhibit a wide variety of clinical symptoms, lung illness remains to be the leading cause of morbidity and mortality in both conditions. But by distinct mechanisms.

In PCD, the mucus accumulation is consequence of the motile cilia dysfunction, which is primarily caused by ciliar or axoneme anomalies, that cause ciliary immotility. Whereas in CF, abnormal ciliary motility is consequence of PCL dehydratation and mucus accumulation.

CFTR dysfunction creates a perfect environment for the colonization of the airways and lungs by opportunistic pathogens that lead to chronic infection [94,95]. Despite the high levels of infiltrating cells, especially neutrophils, lymphocytes and macrophages, and the elevated levels of inflammatory cytokines to fight pathogens, this defence is incapable of successfully deal with the infection [38]. This results in the progression of the chronic airway infection to bronchiectasis, and ultimately can lead to respiratory failure.

In addition to recurrent bronchogenic infections, nasal polyps and sinusitis are recognized diagnostic criteria for CF [96].

In contrast to CF, where the airways and lungs are usually not compromised at birth, neonatal respiratory distress is a typical clinical presentation in PCD. Most children with PCD develop, from birth, recurrent respiratory infections, including rhinitis, sinusitis, otitis media, bronchitis and pneumonia which may progress to bronchiectasis.

Table 1
Comparison of the main clinical features between Cystic Fibrosis and Primary Ciliary Dyskinesia, by system, affected.

System affected	Cystic Fibrosis	Primary Ciliary Dyskinesia
Respiratory	**Common features:**	
	Chronic pansinusitis/sinusitis	
	Bronchiectasis	
	Recurrent respiratory infection	
	Respiratory failure	
	Distinctive features:	
	Nasal polyposis (+)	Nasal polyposis (−)
	Chronic otitis media (−)	Chronic otitis media (+) and hearing loss
	Allergic bronchopulmonary aspergillosis	Neonatal respiratory distress
Gastrointestinal	Meconium ileus	Gastrointestinal disorders are not a major issue in PCD, although some punctual cases were reported (see text for further details)
Endocrinological	Pancreatic insufficiency	
	Rectal prolapse	
	Malabsorption of fat-soluble vitamins	
	CF-related diabetes (CFRD)	
	Distal Intestinal Obstruction Syndrome	
	Liver disease	
Reproductive	**Males**	
	Obstructive azoospermia due to congenital absence of the vas deferens	Sperm immotility due to structural deficiencies in the axoneme
	Sperm capacitation due to the variations in the $HCO3^-$ levels	Azoospermia caused by immotility of cilia in the *rete testis*
	Females	
	Cyclical changes in hydration or pH of cervical mucus	Dysfunction of motile cilia in the Fallopian tube
	Anovulation	
Laterality defects	Laterality disorders not reported in CF	*Situs inversus totalis*
		Situs ambiguous
		Kartagener Syndrome
		Congenital heart defects
Other complications	Bone and Joint disease	Retinitis pigmentosa
	Excessively salty-sweet skin	Hydrocephalus (rare)

(+)/(−): means more or less frequently reported than in the other disease, respectively. Neonatal refers to the first 4 weeks of a child's life.

Another distinctive feature of PCD is the presence of a productive (wet) cough that begins in infancy and persists during adult life. Otitis media are more frequent in PCD patients, likely related to changes in middle ear clearance.

Distinct clinical features between CF and PCD have been highlighted by few studies [97–105]. Cohen-Cymberknoh et al. compared PCD patients with the two distinct groups of CF patients (CF-PI and CF-PS). These researchers showed that the severity of lung disease in patients with PCD and CF-PS was comparable and less severe than in patients with CF-PI [98]. Furthermore, using high-resolution computed tomography (CT), they observed that all patients develop severe injuries in distinct lung territories: in PCD the upper lung lobes were more spared; in CF-PI, the upper lung lobes were more attained; and in CF-PS the lung injuries appeared to be dispersed [98]. Other authors have also identified differences in CT scans between CF and PCD, which led the authors to suggest that PCD should have a specific CT scoring system to properly describe the changes seen in PCD [105].

Despite similar clinical severity and decreases in lung function, Ratjen and colleagues discovered differences in airway inflammation between CF and PCD patients during pulmonary exacerbations and in response to antibiotic treatment [104]. According to these authors, during the exacerbation stage, absolute neutrophil counts and interleukin-8 levels were higher in PCD patients, but bacterial density was higher in CF patients. Additionally, they observed that CF patients who received oral antibiotics for pulmonary exacerbations had better outcomes than PCD patients, with a substantial decrease in bacterial density [104]. In agreement, Mackerness et al. described that the levels of neutrophil chemoattractants were higher in PCD patients compared to CF [100].

However, the studies comparing the clinical features between CF and PCD are not always consensual. Maglione et al. [101], observed no discernible variations in lung shape or function between CF and PCD patients, contrary to prior reports [98,104,106], but highlighted that PCD patients are diagnosed much later than CF, rendering PCD individuals to suffer long periods of inadequate management. Further, two other studies on the exhaled molecular profiles produced contradictory results, with one demonstrating notable variations between patients with CF and PCD [102] and the other not noticing group differences [104]. These disparities clearly demonstrate the need for more studies comparing both diseases, as well as the need for collaborative efforts to increase the number of patients in order to obtain robust data.

Advanced culture-based and genomic sequencing techniques have allowed an increase in the knowledge regarding respiratory pathogens previously undetected, helping to disclose the differences between both diseases (Fig. 6). But this knowledge is still far from full, particularly in the case of PCD, where this kind of analysis is only getting started.

In CF, the Gram-positive bacteria *Staphylococcus aureus* (*S. aureus)* was the first to be identified as an important respiratory pathogen. *S. aureus* and its subtypes have a high prevalence among CF children and

Table 2
Classes of CF transmembrane conductance regulator (CFTR) mutations.

Class	Molecular defect	Functional CFTR in apical membrane	Main Mutation type	Examples	Current therapy and main active compounds used
I	Non CFTR protein production	No	Nonsense, frameshift	Tyr304X, Gly542X, 621+1G > T, Trp361CysfsX8	Not approved yet
II	CFTR abnormal processing and trafficking	No	Missense and amino acid deletions	Phe508del, Asn1303Lys, Gly85Glu, Ile507del, Leu1065Pro, Asp1507	Mostly correctors: Lumacaftor, Ivacaftor and Tezacaftor
III	Defective CFTR channel regulation (Gating defect)	No	Missense	Gly551Asp, Ser492Phe, Val520Phe, Arg553Gly, Arg560Thr, Arg560Ser	Potentiators: Ivacaftor
IV	Defective channel conductance	Yes, but with reduced Cl^- transport	Missense	Arg117Cys, Leu227Arg, Arg347Pro, Ala455Glu, Asp1152His	
V	Reduced protein production	Yes, but reduced	Missense and splicing	c.3717 + 12191C > T, c.1679 + 1643G > T, c.1766+5G > T, 789+5G > A	Not approved yet
VI	Destabilization of CFTR at the cell surface	Yes, but unstable	Missense and Indels	4326delTC, Gln1412X, 4279insA, c. 120del23	
VII	Non CFTR protein production	No	Nonsense, frameshift	dele2,3(21 kb), 1717-1G→A	

CFTR: cystic fibrosis transmembrane conductance regulator; Indels- Insertion–deletion mutations, refer to insertion and/or deletion of nucleotides into genomic DNA with less than 1 kb in length; Large indels-indels with more than 1 kb in length, such an entire exon deletion. Arrow at right side of table indicating a decrease in disease severity.

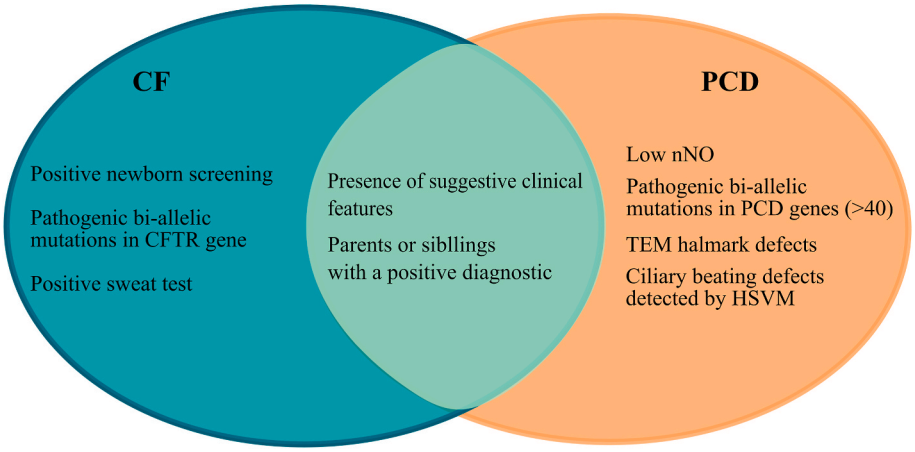

Fig. 5. Summary of diagnostic criteria for both CF and PCD. To confirm the diagnosis, in addition to suspicious clinical features and/or relatives with a positive diagnostic, laboratory tests are needed. For CF it includes the sweat test, which remains the gold standard for the diagnosis, a positive newborn screening, and pathogenic bi-allelic mutations in CFTR gene. In contrast, for PCD the diagnostic tests include nasal nitric oxide (NO) measurement, with low levels of NO being suspicious of PCD; evaluation of cilia motility by high-speed video microscopy analysis (HSVMA) or determination of the variation in ciliary beat axis and ciliary deviation; evaluation of the ultrastructure of the axoneme of cilia by transmission electron microscopy (TEM); and genetic analysis. According to the European Respiratory Society guidelines, a definite diagnosis corresponds to the presence of an ultrastructural defect in the cilia axoneme or of bi-allelic mutations in PCD-related genes; and a highly likely diagnosis is made by the combined presence of low nasal NO levels and a dysfunction in ciliary motility [191]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

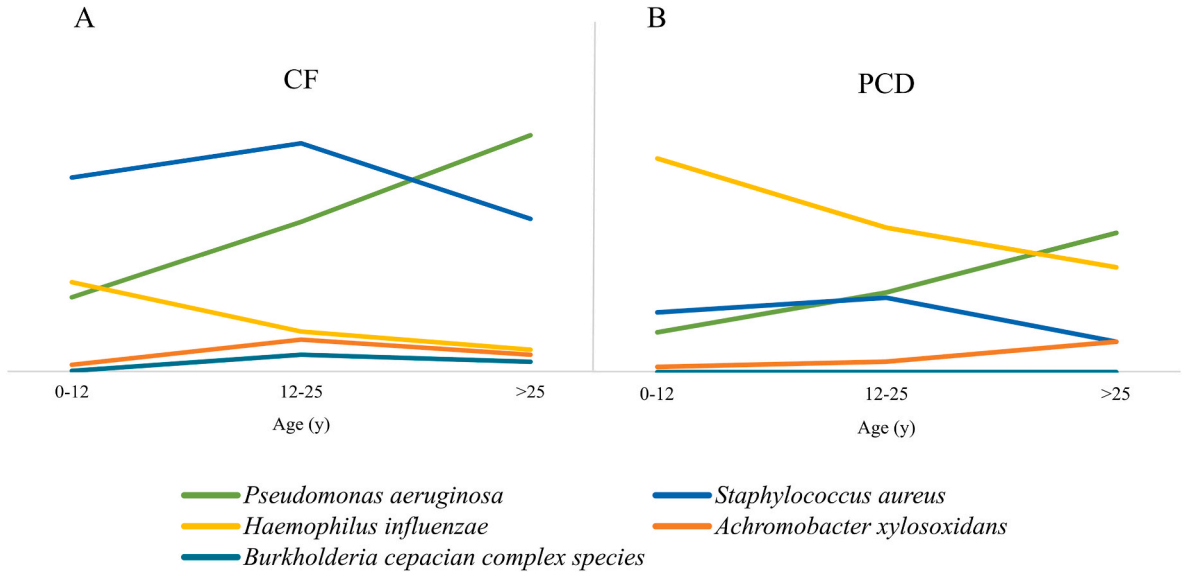

Fig. 6. Graphical representation of the overall prevalence of respiratory organisms commonly isolated in different age periods of patients with CF (A) and PCD (B). This graphical representation aims to highlight the main differences concerning the most prevalent respiratory organisms in each age period according with references [107,114]. Therefore, the specific percentages of the pathogens are not indicated, as they are not the scope of the graphic.

have been associated with worse clinical outcomes [107,108]. The Gram-negative bacteria *Pseudomonas aeruginosa (P. aeruginosa)* is the leading cause of morbidity and mortality in CF patients, especially among the ones that are co-infected with *S. aureus* [109]. In CF patients, *P. aeruginosa* can form highly heterogeneous micro-populations within the lungs, making this bacterium highly resistant, dynamic and dangerous [110]. Other microorganisms, such as *Burkholderia cepacia complex*, *Stenotrophomonas maltophilia (S. maltophilia), Haemophilus influenzae (H. influenzae), Achromobacter xylosoxidans (A. xylosoxidans), Nontuberculous Mycobacteria* have also been detected in CF patients, with a lower prevalence than *S. aureus* and *P. aeruginosa* but also causing serious clinical consequences [111]. For instance, the *Burkholderia cepacia complex* (comprised of about 24 related opportunistic pathogenic species) has infected less than 4% of CF patients but is particularly dangerous, as they are naturally resistant to different classes of antibiotics used and are known to cause severe decline in lung function, which can progress to uncontrolled bronchopneumonia and cachexia with the development of a life-threatening systemic infection known as Cepacia syndrome (fatal combination of necrotizing pneumonia, worsening of respiratory failure, and bacteremia) [112].

In contrast, *H. influenza* is the most commonly isolated organism in PCD [113]. Besides *H. influenza*, PCD patients are also chronically infected with *S. aureus, Streptococcus pneumoniae (S. pneumoniae), A. xylosoxidans, Aspergillus fumigatus* and *Moraxella catarrhalis (M. catarrhalis)*, with infection prevalence varying with age. Alanin and co-workers found that *H. influenzae* and *M. catarrhalis* predominate in children, whearas *P. aeruginosa* and other Gram-negative bacteria were the most common pathogens in teenagers and adults [114]. In PCD, as in CF, pulmonary *P. aeruginosa* infection is associated with severe disease [115,116]. The incidence of Achromobacter species in PCD patients has recently been discovered to be comparable to that in CF patients, and as has been seen in CF patients, Achromobacter infection can result in a significant persistent lung infection that requires long-term antibiotic treatment [117].

Another distinction between CF and PCD patients is that, to date, no species of the Burkholderia cepacia complex have been identified in the

airways of PCD patients [118]. This corroborates the fact that both diseases are distinct, despite their clinical similarities. Further studies are needed to investigate why *Burkholderia cepacia complex* species were not found in PCD patients, whether due to causality or if, for some reason, PCD patients are protected against these infections. Microbiological data in PCD are still incomplete in contrast to CF, namely how these pathogens are associated with lung disease and if they are, as in CF, associated with a decline in lung function. The answer to those questions may open doors to newer therapeutic approaches.

4.3. Reproductive system

Infertility is a common feature of both diseases, with the majority of men suffering from CF and PCD being infertile. In CF, the major cause of male infertility is obstructive azoospermia (absence of sperm in the ejaculate) caused by CBAVD [119]. Besides, a recent study, using the CF mouse model $Cftr^{tm1Unc}$ (S489X), proposed that CFTR is important to the regulation of Dicer1 (the core microRNA (miRNA) processing protein) in germ cells. This authors show that CFTR mutations can also affect spermatogenesis due to the dysregulation of the miRNA, particularly the miR-15b [120].

In contrast, the majority of males with PCD show asthenozoospermia (that is, total or partial sperm immotility), which is mainly explained by structural or functional deficiencies of the sperm flagellum axoneme [41,121]. Aprea and colleagues proposed that PCD patients may also have azoospermia, albeit through a different mechanism than CF patients. In PCD, azoospermia is likely caused by the dysfunction of the cilia present in the *rete testis (*conducts testicular sperm to the efferent ducts and these to the epididymis). These cilia are responsible for fluid flow and sperm transport, as sperm is immotile in this stage. Therefore, if the function of these cilia is compromised, sperm are not transported to the epididymis, and the patient presents obstructive azoospermia [122].

Women with CF and PCD can also have infertility or subfertility, although less than men. In contrast to men, who had an average subfertility rate of 87%, only roughly 59% of the 119 women with documented fertility outcomes were subfertile, according to a recent systematic analysis that summarises the prevalence of subfertility in PCD [123]. It is yet unclear why women's fertility is less affected, but some reasons have already been proposed.

In women with PCD, dysfunction of the motile cilia in the Fallopian tube is the most consensual explanation. The dysfunction in cilia from the Fallopian tube was proposed to favour the occurrence of ectopic pregnancy and/or cause abnormal embryo transport towards the uterine cavity [48,49]. However, the consequences of motile cilia dysfunction in the Fallopian tube may be compensated by the muscle contractions and peristaltic movements of the Fallopian tubes reducing the incidence of female infertility [124] and by the fact that the ciliary motility is not mandatory for sperm and embryo transport [49]. A study by Vanaken and co-workers has evaluated the fertility status of adults with definite PCD diagnoses. Authors have shown that PCD implies an increased risk of fertility problems in both sexes, with more than 50% of patients being infertile, and propose a potential link between fertility status, the ultrastructural findings, and the patient's genotype [125].

For women with CF, it has been postulated that CFTR dysfunction impairs the cyclical hydration changes of cervical mucus during the menstrual cycle, reducing the water content and making the cervical mucus thick, viscous, and tenacious, which blocks the passage of sperm through the cervical canal [126,127]. A further hypothesis is that in CF, the CFTR dysfunction may also affect sperm capacitation due to variations in $HCO3^-$ levels, which will affect the pH within the female reproductive tract [128,129]. Anovulation, small ovarian and uterine size, aberrant estrous cycles, and decreased oocyte ovulation rates have also been proposed as reasons for subfertility in CF women [130,131].

4.4. Laterality defects

Nodal cilia cells are located in the embryonic node (Hensen's node). The nodal cilium moves in a clockwise rotation and is critical to creating a leftward flow across the embryo ventral nodes (ventral surface of Hensen's node). Leftward nodal flow, working together with cascades of signalling molecules and ions, establishes the left-right axis of the internal organs [132,133]. Laterality defects, including *situs solitus, situs inversus totalis* and *situs ambiguous,* are a feature of PCD, being *situs inversus totalis* the most frequent laterality defect observed, with an estimated prevalence of 1:10,000 and occurring in about 50% of PCD cases [134]. This phenomenon can result in other associated problems such as congenital heart defects. Kartagener syndrome (KS) is a particular type of PCD, characterized by the combination *of situs inversus*, chronic sinusitis and bronchiectasis, occurring in about 50% of PCD cases [13].

Laterality disorders, so far, were not reported in CF patients.

5. Therapeutic options

5.1. Cystic fibrosis therapeutic options

Significant progress in CF therapeutics, particularly regarding gastrointestinal and nutritional disorders with advances in PERT [135], has significantly improved the survival of CF patients. However, respiratory diseases continue to be the leading cause of morbidity and mortality. As a result, intensive research has been focused on finding better therapies.

CF therapies can be simplistically divided into **symptomatic treatments**, which aim to reduce the signs and symptoms to increase the comfort and well-being of the patient, and the so-called **new therapies**, which aim to treat the disease by targeting the molecular defect in the CFTR gene/protein. However, those new therapies should be referred to as **Target therapies** because several new components are also being developed for symptomatic treatments.

So far, none of the new therapies have proved successfully enough to be used as a stand-alone treatment. Even the most successful new treatment, Ivacaftor, a CFTR potentiator and used primarily in patients with gating mutations (class III mutation), must be accompanied by standard therapies [136]. An extensive description of the available therapeutic options is beyond the scope of the present review, as already presented in detail elsewhere [53,137–139]. In this section, we will do a brief overview.

5.1.1. Symptomatic treatments

In CF patients, airway dehydration is a significant problem affecting lung function. **Hypertonic saline** and **mannitol** are currently used as hydrators to improve airway clearance [136]. The administration of mannitol *per se* has been associated with an improvement in lung function [140].

Dornase alfa is a recombinant human DNase-1 that hydrolyses the DNA present in the sputum/mucus of patients, with a subsequent decrease in sputum viscosity and increased MCC. The therapy with Dornase alfa has also been shown to improve lung function and lead to a decrease in pulmonary exacerbations [141]. To further restore MCC, **ENaC inhibitors** are under investigation [142], but so far any have been successfully translated into clinical efficacy [136].

As discussed above, a hallmark of CF lung disease is infection by pathogens, a situation where a significant inflammation is associated with an ineffective clearness of pathogens. Unfortunately, the use of common **anti-inflammatory drugs**, such as corticosteroids, have no proven efficacy in CF patients [137]. Novel compounds are being studied in clinical trials, such as Acebilustat (CTX-4430) and Lenabasum (JBT-101). Briefly, both are small molecules whose ultimate goal is to reduce inflammation either by blocking excessive neutrophil influx and activation or by triggering the production of pro-inflammatory

mediators [143,144].

To deal with a chronic lung infection, **antibiotic treatments** are the first line of intervention. *S. aureus* and *P. aeruginosa* are the most common agents and *P. aeruginosa*, in particular, is connected to worse clinical conditions [110]. Consequently, antibiotics against *S. aureus* and *P. aeruginosa* have been a cornerstone of CF clinical care for many years [145]. However, the risk of widespread antibiotic resistance is increasingly evident. Thus, efforts have been made to find alternatives to the use of antibiotics, using a diverse range of approaches [146–158]. So far, all are still in pre-clinical or in the initial stages of clinical trials. For instance, to prevent biofilm formation by *P. aeruginosa,* two **saccharides**, the liposomal β glycan [146] and OligoG [147,148], and *de novo* engineered **antimicrobial peptides** were proposed [149–151]. Although studies with *de novo*-engineered antimicrobial peptides showed promising results *in vitro*, some years of research are still needed before discussing their clinical applicability.

Nitric oxide (NO) concentrations are decreased in the exhaled air from patients with CF or PCD. Increasing NO levels by inhalation of exogenous NO has been shown to have potent antimicrobial activity in preclinical models against a broad range of bacteria [159,160]. Thus, studies were developed to further study this hypothesis [152]. Another line of research in the search for safer alternatives to antibiotics is **bacteriophage** (phage) **therapy** [161]. Bacteriophages (viruses) are natural bacteria predators that infect and replicate within bacteria and kill a specific bacterial strain. Although promising results have been reported for other diseases, robust clinical data are still missing for CF [162,163]. In CF, the clinical evidence is limited to a few case studies in unrelated CF patients, with satisfactory efficacy and no adverse effects [153–158].

5.1.2. CFTR molecular defects correction treatments

The ultimate goal in CF research is to develop therapies that could correct CFTR and not just alleviate the symptoms. To date, only a few CFTR modulators have demonstrated clinical efficacy and have been approved for use [164,165]. The **Ivacaftor** (VX-770), known as a **potentiator**, i.e. it increases the likelihood of the defective channel opening, was designed specially for patients with the Gly551Asp mutation (class III) and was the first to be approved by the FDA in 2015 [166,167]. It has also been shown to be beneficial in patients with the Arg117His mutation (class IV) [168].

Currently, the most satisfactory pharmacological treatment results in patients with the Phe508del mutation have been obtained using compounds referred to as "**correctors**", with some compounds already approved by the FDA for use in clinical practise [169,170]. CFTR correctors try to fix the current deficiency so that the protein can still be delivered to cell membranes without being degraded, even while the gene mutation is still there. In the specific case of the Phe508del mutation, CFTR correctors can either bind directly to the Phe508del-CFTR protein (chaperones) or work by creating conditions in the cell so that higher concentrations of CFTR can be made without being degraded (proteostasis regulators). The **Lumacaftor** is a corrector that acts as a chaperone during protein folding and increases the number of CFTR proteins that are trafficked to the cell surface. Lumacaftor, alone, is ineffective in restoring Cl^- transport. However, when combined with Ivacaftor, the therapy had a greater effectiveness in the management of CF in patients with the Phe508del homozygous mutation. Thus was approved by the FDA [171]. Gradually, newer correctors were identified and approved by the FDA for the treatment of patients with Phe508del mutations, such as Tezacaftor [172]. Elexacaftor is considered a next-generation corrector of the CFTR protein. A triple combination composed of two correctors and one potentiator, **Elexacaftor–Tezacaftor–Ivacaftor**, has proven to be effective in patients with Phe508del mutation [166,173], and already approved by FDA and EMA [165].

Despite the proven value of these new therapies, they act exclusively on the defective protein, not in CFTR mutations. Therefore, investigations into genetic approaches to correct CFTR are being developed. These include alternative ion channel approaches by RNA silencing of the ENaC regulators, antisense oligonucleotides (ASO) for correcting CFTR mutations, a nanoparticle/short interfering RNA (siRNA) approach to regulate CFTR, and genetic therapies either by non-viral or viral vectors to correct CFTR mutations [174–176]. Most of these novel therapies are still in the pre-clinical stage, being evaluated *in vitro*, but a few are already in clinical trials after collaboration between pharmaceutical companies and academic laboratories. We will briefly describe the main therapies that are being developed.

For patients with nonsense mutations (class I), the use of an ASO (single-stranded RNA complementary to the target mRNA) are being studied. The downregulation of a molecular target is the main objective of the ASO strategy. Nevertheless, it can also play additional roles, including as correcting splicing events or controlling RNA translation (further reviewed by Ref. [177]). For instance, using human bronchial epithelial cell lines homozygous for the Trp1282X mutation (also common in CF), it was been shown that the inhibition of the nonsense-mediated decay (NMD) pathway with ASO can upregulate the expression of CFTR-nonsense alleles (Trp1282X) [178]. However, since the NMD pathways might not be unique to a particular gene and is a component of universal regulatory networks, more research is required to prevent catastrophic repercussions.

Classes III and IV mutations affect CFTR channel gating and conductance, respectively. For patients with these types of mutations, and despite the success of the potentiator **Ivacaftor** [176], novel potentiators are currently in clinical trials. For instance, the potentiator **VX-561** (formerly CTP-656), a deuterated form of Ivacaftor (i.e. some of the hydrogen atoms of Ivacaftor have been replaced by its heavier stable isotope, named deuterium), and two other CFTR potentiators **GLPG1837** and **GLPG2451** have demonstrated improved stability *in vitro* compared to Ivacaftor [179,180]. Another potentiator is **QBW251**, which has been shown to be safe and well-tolerated in both healthy volunteers and CF patients, as well as to improve lung function and sweat Cl-levels in CF patients with Class III and IV CFTR mutations [181].

For patients with mutations that affect CFTR protein abundance (such as class V mutations), a novel class of small molecules has been identified and named **amplifiers**. Amplifiers can increase the expression of the immature CFTR protein. They are being tested for clinical use in combination with other CFTR modulators such as potentiators (ivacaftor and PTI-808), and correctors (lumacaftor, tezacaftor, and PTI-801) [182]. Currently, a clinical trial (NCT03500263) is ongoing to assess the safety, tolerability and pharmacokinetics of a pharmacological combination with **PTI-428** (amplifier), **PTI-808** (potentiator) and **PTI-801** (corrector). Hopefully, it could be helpful for a broader type of mutations (from class II to V) and thus be beneficial for a higher percentage of individuals.

Some CFTR mutated proteins are functional but unstable in the plasma membrane (as in Class VI mutations, but not exclusively). **CFTR stabilizers** are designed to anchor the functional channel in the membrane and reduce CFTR degradation [183]. So far, only one CFTR stabiliser, cavosonstat (N91115), has entered in clinical trials, and it has failed to show any additional benefit in lung function [184]. Bioengineered nanobodies that target the nucleotide-binding domain 1 (NBD1) of human CFTR have recently been shown able to stabilize both isolated NBD1 and the full-length CFTR protein, thus, are a new promise for CF [185].

Despite recent advances in the development of CFTR molecular therapies, a significant number of patients are still ineligible for modulator therapy. Personalized therapies and gene editing therapies (such as CRISPR-CAS9) have been proposed as the most effective treatment options for these patients. But, unfortunately, challenges emerged regarding genotoxicity, safety, carrying capacity, gene transfer and editing efficacy and, despite some therapies having shown great pre-clinical results, their clinical efficacy was still not proven [186,187].

Furthermore, the lung is not an easy organ to apply gene therapy, given the many extracellular barriers, such as the airways mucus, MCC and mucopurulent sputum, and also cellular immune responses have great importance in gene therapy success [188]. Nevertheless, several studies are ongoing to improve the safety and gene transfer efficiency of gene therapy, increasing the hope to find an efficient gene therapy for CF [189]. For instance, **MRT5005** is the first clinical-stage mRNA product candidate designed to treat all patients with CF, regardless of the underlying genetic mutation, as it delivers the fully functional mRNA of CFTR to the lung epithelial cells via nebulization.

5.2. Primary ciliary dyskinesia therapeutic options

Currently, such as for CF patients, for PCD patients there is no available cure, and management focuses on careful monitoring, physiotherapy and symptomatic treatments regarding respiratory symptoms and infections. However, for PCD, unfortunately, less investment towards the development of disease-specific new therapies has been made and PCD treatments remain based on extrapolations from CF. However, as we have discussed throughout this paper, CF and PCD are clinically distinct, making this difficult. For instance, in patients with CF, the use of Dornase Alfa is associated with an improvement in lung function and with a reduction in pulmonary exacerbations [190], but, for PCD patients, this treatment is not recommended [191].

Management of PCD is hindered by the existence of large variability of clinical presentations that require a multidisciplinary study, and by the fact that the diagnosis is not straightforward as is for CF. It is complex, expensive and the report of the clinical symptoms and management approaches is not standardized. Despite the existence of several guidelines for diagnosis and treatment [15,16,85,191,192], these are not considered definitive and thus therapeutic options may vary from center to center. The **FOLLOW-PCD project**, resulting from a network between 12 countries, developed by a multidisciplinary and international working group of the BEAT-PCD (Better Experimental Approaches to Treat PCD), aims to standardize data collection during routine clinical follow-up of PCD patients, and promote a uniformization in the report of the clinical symptoms and management approaches [193].

Despite the lack of standardized management approaches, it is consensual that monitoring lung function, pulmonary physiotherapy coupled with physical exercise, and active follow-up and treatment of infections with antibiotics are important in PCD patients [191,192]. In this sense, **Chest physiotherapy** was proposed as a valuable approach to promoting better drainage of secretions, delaying the accumulation of mucus and pathogens and has been one of the bases of PCD therapy. However, the influence of chest physiotherapy on lung function is not clear [194]. A recent study, have found no significant short-term effect of chest physiotherapy on lung function parameters in stable children with CF and PCD [195] The study's weaknesses, according to the authors, include the small sample size, the absence of adult patients and/or patients with severe lung illness, and the study's unusually long intervals between visits for a cross-over design. Therefore, more study is required.

Additionally, **Physical exercise** in PCD treatments has been proposed to be beneficial to pulmonary clearance, lung function, and overall quality of life [196].

Recurrent or persistent otitis media with effusion may lead to chronic otitis media and hearing loss, which has a higher incidence in PCD in comparison with CF patients. Its management includes antibiotic therapy, as well as hearing aids and myringotomy with or without the insertion of ventilation tubes [197].

As with CF, **lung transplantation** can be the last resort for PCD cases, but it requires experienced professionals and facilities. The presence of *situs inversus* in PCD patients can be a barrier in donor lung selection, and extra careful lung transplant evaluation and advanced surgical planning is needed for these cases.

Current evidence on PCD therapy is limited, inconclusive and conflicting. Compared with CF, PCD has only 10 interventional studies (clinical trials) listed at the moment (in CF there are 159 interventional studies active studies ongoing, updated on 6 February 2023). PCD studies are typically observational studies with small sample sizes and limited follow-up periods, which impose challenges in designing studies that yield statistically powerful results [191]. This could be related to the difficulties with the recruitment of patients or due to the potentially higher cases of misdiagnosed patients.

Currently, there is no specific therapy was developed for PCD. Promising and attractive approaches are expected for gene therapy in PCD. However, research in this field is scarce. In addition to the limitations already described for gene therapy, PCD presents another limitation that CF does not have, that is the larger number of genes and mutations involved, each with lower prevalence, with still about 30% of PCD patients remaining without an identified genetic etiology [64]. This means that, for PCD, much research is still needed on molecular mechanisms and pathways. Thus, an approach such as the MRT5005 which is being evaluated for CF is not as straightforward nor cost-effective for PCD patients. Therefore, any genetic therapy that comes to be applied to PCD must be personalized for a particular small group of patients with the same mutations or mutations in the same gene. Another limitation is related to the mRNA full size, which influences the efficacy of gene delivery. Most of the PCD-associated genes are larger than the CFTR, for instance, the *DNAH5* full mRNA (15781bp) has more than the double size of the CFTR full mRNA (6070 bp). Nevertheless, advances towards PCD gene therapy have also been described recently [198–201]. For instance, studies are being made to develop lipid nanoparticles that carry the mRNA of the mutated gene, which is then nebulized, and delivered as an aerosol directly into the airway. So far, ReCode Therapeutics is testing this system with the DNAI1 mRNA with positive results in animal models (oral communication in Cilia 2022 conference).

Hopefully and with the benefit from the knowledge learned from extensive work done over the past decades to develop efficient gene and cell therapies for CF, some of those studies might lead to efficient gene therapies for PCD that pass to clinical trials testing in the next years.

6. Conclusions

Through this reviwe, we highlighted the clinical disparities between the two diseases as well as their distinct disease mechanisms and genetic features.

PCD is caused by defects in motile cilia, imparing MCC flow, whearas, CF is caused by mutations in the *CFTR* gene, which leads to an imbalance in H_20 and Cl^- flow across cell membranes, making the mucus become sticky. This review intended to highlight the main differences and similarities between CF and PCD whose differences are frequently not considered. A distinctive example is a fact that both CF and PCD patients have sinus-related diseases, but chronic rhinitis and recurrent otitis media are clinical hallmark features of PCD patients. Furthermore, while infertility is a problem in both CF and PCD, the underlying causes of infertility are very different. Men with CF frequently suffer from CBAVD, meaning that they produce viable sperm cells which is not properly transported and thus sperm cells are absent in semen from CF patients. In contrast, semen from PCD men often have sperm cells, but these cells are immotile, due to ultrastructural anomalies in the development of the axoneme. Another distinctive characteristic of PCD is that about half of the patients have *situs inversus* due to defects in the motility of the embryonic node cilia. Dyskinetic nodal cilia lead to laterality defects, which can be also associated with complex congenital heart disease and polysplenia. In a similar vein, CF also exhibits traits that PCD patients lack, notably, gastro-intestinal disorders. These issues used to be the primary cause of CF morbidity and mortality, but thanks to PERT's success, they are no longer the main contributing factor. Gastrointestinal complications are not considered a suggestive clinical feature of PCD.

PCD is caused by mutations in many genes as opposed to CF, which is

caused by mutations in a single gene.

Some studies have been performed to compare both diseases but they are still limited, and the results are not always consensual. The discrepancies shown by the studies that compared both diseases emphasized the need for more studies comparing both diseases and the importance of collaborative efforts among health professionals and researchers to better understand these diseases. However, is accepted that PCD is much more complex to diagnose and consequently patients are highly misdiagnosed or diagnosed much later than CF, rendering PCD individuals to suffer long periods of inadequate management.

Altogether, we evidenced that both diseases require different management by specialists with experience in the peculiarities of each disease, especially for PCD which, in comparison to CF, knowledge is still scarce and requires more attention for both clinicians and scientists. It is urgent to increase clinical awareness about what is PCD, to uniformize diagnostic approaches and to create guidelines to orient and assist clinicians in the treatments.

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Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Author contributions

Rute Pereira: Conceptualization, Writing - Original Draft preparation, Writing- Reviewing and Editing; **Telma Babosa and Ana Cardoso**: Writing - Original Draft preparation, Writing- Reviewing and Editing; **Rosália Sá:** Writing- Reviewing and Editing; Mário Sousa: Supervision, Funding acquisition, Writing - Review & Editing.

Ethics approval

The authors declare that they have followed all the rules of ethical conduct regarding originality, data processing and analysis, duplicate publication, and biological material.

Consent for publication

Not applicable.

Declaration of competing interest

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Appendix A. Supplementary data

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References

[1] X.M. Bustamante-Marin, L.E. Ostrowski, Cilia, Mucociliary Clearance, Cold Spring Harbor Perspect. Biol. 9 (2017) a028241, https://doi.org/10.1101/cshperspect.a028241.
[2] L.W. Plasschaert, R. Zilionis, R. Choo-Wing, V. Savova, J. Knehr, et al., A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte, Nature 560 (7718) (2018) 377–381, https://doi.org/10.1038/s41586-018-0394-6.
[3] D.T. Montoro, A.L. Haber, M. Biton, V. Vinarsky, B. Lin, et al., A revised airway epithelial hierarchy includes CFTR-expressing ionocytes, Nature 560 (7718) (2018) 319–324, https://doi.org/10.1038/s41586-018-0393-7.
[4] R.R. Mercer, M.L. Russell, V.L. Roggli, J.D. Crapo, Cell number and distribution in human and rat airways, Am. J. Respir. Cell Mol. Biol. 10 (6) (1994) 613–624, https://doi.org/10.1165/ajrcmb.10.6.8003339.
[5] C. Shingyoji, in: S.M. King (Ed.), Regulation of Dynein in Ciliary and Flagellar Movement, Dyneins, Academic Press, Boston, 2012, pp. 366–393, https://doi.org/10.1016/b978-0-12-382004-4.10013-5.
[6] J. Lin, K. Okada, M. Raytchev, M.C. Smith, D. Nicastro, Structural mechanism of the dynein power stroke, Nat. Cell Biol. 16 (5) (2014) 479–485, https://doi.org/10.1038/ncb2939.
[7] M. Vanaki S, D. Holmes, S.C. Saha, J. Chen, R.J. Brown, et al., Muco-ciliary clearance: a review of modelling techniques, J. Biomech. 99 (2020), 109578, https://doi.org/10.1016/j.jbiomech.2019.109578.
[8] D. Song, D. Cahn, G.A. Duncan, Mucin biopolymers and their barrier function at airway surfaces, Langmuir 36 (43) (2020) 12773–12783, https://doi.org/10.1021/acs.langmuir.0c02410.
[9] R. Bansil, B.S. Turner, The biology of mucus: composition, synthesis and organization, Adv. Drug Deliv. Rev. 124 (2018) 3–15, https://doi.org/10.1016/j.addr.2017.09.023.
[10] B. Button, L.-H. Cai, C. Ehre, M. Kesimer, D.B. Hill, et al., A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia, Science 337 (6097) (2012) 937–941, https://doi.org/10.1126/science.1223012.
[11] M. Kesimer, C. Ehre, K.A. Burns, C.W. Davis, J.K. Sheehan, et al., Molecular organization of the mucins and glycocalyx underlying mucus transport over mucosal surfaces of the airways, Mucosal Immunol. 6 (2) (2013) 379–392, https://doi.org/10.1038/mi.2012.81.
[12] K. De Boeck, Cystic fibrosis in the year 2020: a disease with a new face, Acta Paediatr. 109 (5) (2020) 893–899, https://doi.org/10.1111/apa.15155.
[13] R. Pereira, T. Barbosa, L. Gales, E. Oliveira, R. Santos, et al., Clinical and genetic analysis of children with kartagener syndrome, Cells 8 (8) (2019) 900–919, https://doi.org/10.3390/cells8080900.
[14] NOfRD. Nord, Rare Disease Database, 2020. https://rarediseases.org/?s=C&post_type=rare-diseases. (Accessed 19 March 2021).
[15] A. Barbato, T. Frischer, C.E. Kuehni, D. Snijders, I. Azevedo, et al., Primary ciliary dyskinesia: a consensus statement on diagnostic and treatment approaches in children, Eur. Respir. J. 34 (6) (2009) 1264–1276, https://doi.org/10.1183/09031936.00176608.
[16] J.S. Lucas, A. Barbato, S.A. Collins, M. Goutaki, L. Behan, et al., European respiratory society guidelines for the diagnosis of primary ciliary dyskinesia, Eur. Respir. J. 49 (1) (2017), 1601090, https://doi.org/10.1183/13993003.01090-2016.
[17] V. Saint-Criq, M.A. Gray, Role of CFTR in epithelial physiology, Cell. Mol. Life Sci. 74 (1) (2017) 93–115, https://doi.org/10.1007/s00018-016-2391-y.
[18] B.P. O'Sullivan, S.D. Freedman, Cystic fibrosis, Lancet 373 (9678) (2009) 1891–1904, https://doi.org/10.1016/S0140-6736(09)60327-5.
[19] J.R. Riordan, J.M. Rommens, B.-S. Kerem, N. Alon, R. Rozmahel, et al., Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, Science 245 (4922) (1989) 1066–1073, https://doi.org/10.1126/science.2475911.
[20] M. Anderson, R. Gregory, S. Thompson, D. Souza, S. Paul, et al., Demonstration that CFTR is a chloride channel by alteration of its anion selectivity, Science 253 (5016) (1991) 202–205, https://doi.org/10.1126/science.1712984.
[21] C. Mihályi, I. Iordanov, B. Töröcsik, L. Csanády, Simple binding of protein kinase A prior to phosphorylation allows CFTR anion channels to be opened by nucleotides, Proc. Natl. Acad. Sci. USA 117 (35) (2020) 21740–21746, https://doi.org/10.1073/pnas.2007910117.
[22] N. Shcheynikov, K.H. Kim, K-m Kim, M.R. Dorwart, S.B.H. Ko, et al., Dynamic control of cystic fibrosis transmembrane conductance regulator Cl−/HCO3− selectivity by external Cl−*, J. Biol. Chem. 279 (21) (2004) 21857–21865, https://doi.org/10.1074/jbc.M313323200.
[23] A.-A. Konstas, J.-P. Koch, S.J. Tucker, C. Korbmacher, Cystic fibrosis transmembrane conductance regulator-dependent up-regulation of Kir1.1 (ROMK) renal K+ channels by the epithelial sodium channel, J. Biol. Chem. 277 (28) (2002) 25377–25384, https://doi.org/10.1074/jbc.M201925200.
[24] Y.C. Ruan, W.W.C. Shum, C. Belleannée, N. Da Silva, S. Breton, ATP secretion in the male reproductive tract: essential role of CFTR, J. Physiol. 590 (17) (2012) 4209–4222, https://doi.org/10.1113/jphysiol.2012.230581.

[25] D. Borowitz, CFTR, bicarbonate, and the pathophysiology of cystic fibrosis, Pediatr. Pulmonol. 50 (S40) (2015) 2S4–S30, https://doi.org/10.1002/ppul.23247.

[26] J. Llinares, A. Cantereau, L. Froux, F. Becq, Quantitative phase imaging to study transmembrane water fluxes regulated by CFTR and AQP3 in living human airway epithelial CFBE cells and CHO cells, PLoS One 15 (5) (2020), e0233439, https://doi.org/10.1371/journal.pone.0233439.

[27] P.M. Polgreen, A.P. Comellas, Clinical phenotypes of cystic fibrosis carriers, Annu. Rev. Med. 73 (1) (2022) 563–574, https://doi.org/10.1146/annurev-med-042120-020148.

[28] R.C. Rubenstein, S.R. Lockwood, E. Lide, R. Bauer, L. Suaud, et al., Regulation of endogenous ENaC functional expression by CFTR and ΔF508-CFTR in airway epithelial cells, Am. J. Physiol. Lung Cell Mol. Physiol. 300 (1) (2011) L88–L101, https://doi.org/10.1152/ajplung.00142.2010.

[29] M.A. Mall, D. Hartl, CFTR, Cystic fibrosis and beyond, Eur. Respir. J. 44 (4) (2014) 1042–1054, https://doi.org/10.1183/09031936.00228013.

[30] S.M. Rowe, S. Miller, E.J. Sorscher, Cystic fibrosis, N. Engl. J. Med. 352 (19) (2005) 1992–2001, https://doi.org/10.1056/NEJMra043184.

[31] R.C. Boucher, Airway surface dehydration in cystic fibrosis: pathogenesis and therapy, Annu. Rev. Med. 58 (1) (2007) 157–170, https://doi.org/10.1146/annurev.med.58.071905.105316.

[32] H. Matsui, B.R. Grubb, R. Tarran, S.H. Randell, J.T. Gatzy, et al., Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease, Cell 95 (7) (1998) 1005–1015, https://doi.org/10.1016/S0092-8674(00)81724-9.

[33] H. Matsui, V.E. Wagner, D.B. Hill, U.E. Schwab, T.D. Rogers, et al., A physical linkage between cystic fibrosis airway surface dehydration and Pseudomonas aeruginosa biofilms, Proc. Natl. Acad. Sci. USA 103 (48) (2006) 18131–18136, https://doi.org/10.1073/pnas.0606428103.

[34] J.J. Smith, S.M. Travis, E.P. Greenberg, M.J. Welsh, Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid, Cell 85 (2) (1996) 229–236, https://doi.org/10.1016/S0092-8674(00)81099-5.

[35] J. Zabner, J.J. Smith, P.H. Karp, J.H. Widdicombe, M.J. Welsh, Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro, Mol. Cell 2 (3) (1998) 397–403, https://doi.org/10.1016/S1097-2765(00)80284-1.

[36] D. McShane, J.C. Davies, M.G. Davies, A. Bush, D.M. Geddes, et al., Airway surface pH in subjects with cystic fibrosis, Eur. Respir. J. 21 (1) (2003) 37–42, https://doi.org/10.1183/09031936.03.00027603.

[37] T.L. Bonfield, M.W. Konstan, M. Berger, Altered respiratory epithelial cell cytokine production in cystic fibrosis, J. Allergy Clin. Immunol. 104 (1) (1999) 72–78, https://doi.org/10.1016/S0091-6749(99)70116-8.

[38] A.C. Tang, S.E. Turvey, M.P. Alves, N. Regamey, B. Tümmler, et al., Current concepts: host–pathogen interactions in cystic fibrosis airways disease, Eur. Respir. Rev. 23 (133) (2014) 320–332, https://doi.org/10.1183/09059180.00006113.

[39] L. Imundo, J. Barasch, A. Prince, Q. Al-Awqati, Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface, Proc. Natl. Acad. Sci. USA 92 (7) (1995) 3019–3023, https://doi.org/10.1073/pnas.92.7.3019.

[40] P. Scudieri, I. Musante, A. Venturini, D. Guidone, M. Genovese, et al., Ionocytes and CFTR chloride channel expression in normal and cystic fibrosis nasal and bronchial epithelial cells, Cells 9 (9) (2020), https://doi.org/10.3390/cells9092090.

[41] R. Pereira, M.E. Oliveira, R. Santos, E. Oliveira, T. Barbosa, et al., Characterization of CCDC103 expression profiles: further insights in primary ciliary dyskinesia and in human reproduction, J. Assist. Reprod. Genet. 36 (2019) 1683–1700, https://doi.org/10.1007/s10815-019-01509-7.

[42] J. Wallmeier, D. Frank, A. Shoemark, T. Nöthe-Menchen, S. Cindric, et al., De novo mutations in FOXJ1 result in a motile ciliopathy with hydrocephalus and randomization of left/right body asymmetry, Am. J. Hum. Genet. 105 (5) (2019) 1030–1039, https://doi.org/10.1016/j.ajhg.2019.09.022.

[43] R. Pereira, R. Sa, A. Barros, M. Sousa, Major regulatory mechanisms involved in sperm motility, Asian J. Androl. 19 (1) (2017) 5–14, https://doi.org/10.4103/1008-682X.167716.

[44] S.M. King, in: S.M. King (Ed.), Composition and Assembly of Axonemal Dyneins, Dyneins, Academic Press, Elsevier, 2018, pp. 162–201, https://doi.org/10.1016/B978-0-12-809471-6.00005-X.

[45] I. Grossman-Haham, N. Coudray, Z. Yu, F. Wang, N. Zhang, et al., Structure of the radial spoke head and insights into its role in mechanoregulation of ciliary beating, Nat. Struct. Mol. Biol. 28 (1) (2021) 20–28, https://doi.org/10.1038/s41594-020-00519-9.

[46] Z. Samsel, J. Sekretarska, A. Osinka, D. Wloga, E. Joachimiak, Central apparatus, the molecular kickstarter of ciliary and flagellar nanomachines, Int. J. Mol. Sci. 22 (6) (2021) 1–22, https://doi.org/10.3390/ijms22063013.

[47] M. Gui, M. Ma, E. Sze-Tu, X. Wang, F. Koh, et al., Structures of radial spokes and associated complexes important for ciliary motility, Nat. Struct. Mol. Biol. 28 (1) (2021) 29–37, https://doi.org/10.1038/s41594-020-00530-0.

[48] J. Raidt, C. Werner, T. Menchen, G.W. Dougherty, H. Olbrich, et al., Ciliary function and motor protein composition of human fallopian tubes, Hum. Reprod. 30 (12) (2015) 2871–2880, https://doi.org/10.1093/humrep/dev227.

[49] S. Yuan, Z. Wang, H. Peng, S.M. Ward, G.W. Hennig, et al., Oviductal motile cilia are essential for oocyte pickup but dispensable for sperm and embryo transport, Proc. Natl. Acad. Sci. USA 118 (22) (2021), e2102940118, https://doi.org/10.1073/pnas.2102940118.

[50] K. Sawamoto, H. Wichterle, O. Gonzalez-Perez, J.A. Cholfin, M. Yamada, et al., New neurons follow the flow of cerebrospinal fluid in the adult brain, Science 311 (5761) (2006) 629–632, https://doi.org/10.1126/science.1119133.

[51] S. Nonaka, S. Yoshiba, D. Watanabe, S. Ikeuchi, T. Goto, et al., De novo formation of left–right asymmetry by posterior tilt of nodal cilia, PLoS Biol. 3 (8) (2005) e268, https://doi.org/10.1371/journal.pbio.0030268.

[52] J.R. Riordan, J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, et al., Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA, Science 245 (4922) (1989) 1066–1073, https://doi.org/10.1126/science.2475911.

[53] K. De Boeck, M.D. Amaral, Progress in therapies for cystic fibrosis, Lancet Respir. Med. 4 (8) (2016) 662–674, https://doi.org/10.1016/S2213-2600(16)00023-0.

[54] G.R. Cutting, Cystic fibrosis genetics: from molecular understanding to clinical application, Nat. Rev. Genet. 16 (1) (2015) 45–56, https://doi.org/10.1038/nrg3849.

[55] W.K. O'Neal, M.R. Knowles, Cystic fibrosis disease modifiers: complex genetics defines the phenotypic diversity in a monogenic disease, Annu. Rev. Genom. Hum. Genet. 19 (1) (2018) 201–222, https://doi.org/10.1146/annurev-genom-083117-021329.

[56] A. Sepahzad, D.J. Morris-Rosendahl, J.C. Davies, Cystic fibrosis lung disease modifiers and their relevance in the new era of precision medicine, Genes 12 (4) (2021), https://doi.org/10.3390/genes12040562.

[57] S. Shanthikumar, M.N. Neeland, R. Saffery, S. Ranganathan, Gene modifiers of cystic fibrosis lung disease: a systematic review, Pediatr. Pulmonol. 54 (9) (2019) 1356–1366, https://doi.org/10.1002/ppul.24366.

[58] V. Havasi, S.M. Rowe, P.N. Kolettis, D. Dayangac, A. Şahin, et al., Association of cystic fibrosis genetic modifiers with congenital bilateral absence of the vas deferens, Fertil. Steril. 94 (6) (2010) 2122–2127, https://doi.org/10.1016/j.fertnstert.2009.11.044.

[59] W.B. Hannah, S. DeBrosse, B. Kinghorn, S. Strausbaugh, M.L. Aitken, et al., The expanding phenotype of OFD1-related disorders: hemizygous loss-of-function variants in three patients with primary ciliary dyskinesia, Mol. Genet. Genomic Med 7 (9) (2019) e911, https://doi.org/10.1002/mgg3.911.

[60] T. Paff, N.T. Loges, I. Aprea, K. Wu, Z. Bakey, et al., Mutations in PIH1D3 cause X-linked primary ciliary dyskinesia with outer and inner dynein arm defects, Am. J. Hum. Genet. 100 (1) (2017) 160–168, https://doi.org/10.1016/j.ajhg.2016.11.019.

[61] A.J. Shapiro, K. Kaspy, M.L.A. Daniels, J.R. Stonebraker, V.H. Nguyen, et al., Autosomal dominant variants in FOXJ1 causing primary ciliary dyskinesia in two patients with obstructive hydrocephalus, Mol. Genet. Genomic Med (2021), https://doi.org/10.1002/mgg3.1726.

[62] M. Zariwala, M. Knowles, M. Leigh, Primary Ciliary Dyskinesia, 2013. https://www.ncbi.nlm.nih.gov/books/NBK1122/#. (Accessed 30 June 2021), 2021.

[63] K. Blackburn, X. Bustamante-Marin, W. Yin, M.B. Goshe, L.E. Ostrowski, Quantitative proteomic analysis of human airway cilia identifies previously uncharacterized proteins of high abundance, J. Proteome Res. 16 (4) (2017) 1579–1592, https://doi.org/10.1021/acs.jproteome.6b00972.

[64] R. Pereira, T. Barbosa, A. Alves, R. Santos, J. Oliveira, et al., Unveiling the genetic etiology of primary ciliary dyskinesia: when standard genetic approach is not enough, Adv. Med. Sci. 65 (1) (2020) 1–11, https://doi.org/10.1016/j.advms.2019.10.003.

[65] M. Failly, L. Bartoloni, A. Letourneau, A. Munoz, E. Falconnet, et al., Mutations in DNAH5 account for only 15% of a non-preselected cohort of patients with primary ciliary dyskinesia, J. Med. Genet. 46 (4) (2009) 281–286, https://doi.org/10.1136/jmg.2008.061176.

[66] M. Geremek, E. Ziętkiewicz, M. Bruinenberg, L. Franke, A. Pogorzelski, et al., Ciliary genes are down-regulated in bronchial tissue of primary ciliary dyskinesia patients, PLoS One 9 (2) (2014), e88216, https://doi.org/10.1371/journal.pone.0088216.

[67] K. Boldt, J. van Reeuwijk, Q. Lu, K. Koutroumpas, T.M. Nguyen, et al., An organelle-specific protein landscape identifies novel diseases and molecular mechanisms, Nat. Commun. 7 (2016), 11491, https://doi.org/10.1038/ncomms11491.

[68] A. Shoemark, M. Boon, C. Brochhausen, Z. Bukowy-Bieryllo, M. Margherita De Santi, et al., International consensus guideline for reporting transmission electron microscopy results in the diagnosis of Primary Ciliary Dyskinesia (BEAT PCD TEM Criteria), Eur. Respir. J. 57 (4) (2020), 1900725, https://doi.org/10.1183/13993003.00725-2019.

[69] E. Kott, M. Legendre, B. Copin, J.-F. Papon, F. Dastot-Le Moal, et al., Loss-of-Function mutations in< i> rsph1</i> cause primary ciliary dyskinesia with central-complex and radial-spoke defects, Am. J. Hum. Genet. 93 (2013) 561–570, https://doi.org/10.1016/j.ajhg.2013.07.013.

[70] N. Hornef, H. Olbrich, J. Horvath, Zariwala Ma, M. Fliegauf, et al., DNAH5 mutations are a common cause of primary ciliary dyskinesia with outer dynein arm defects, Am. J. Respir. Crit. Care Med. 174 (2006) 120–126, https://doi.org/10.1164/rccm.200601-084OC.

[71] G. Kano, H. Tsujii, K. Takeuchi, K. Nakatani, M. Ikejiri, et al., Whole-exome sequencing identification of novel DNAH5 mutations in a young patient with primary ciliary dyskinesia, Mol. Med. Rep. 14 (6) (2016) 5077–5083, https://doi.org/10.3892/mmr.2016.5871.

[72] A. Kispert, M. Petry, H. Olbrich, A. Volz, U.P. Ketelsen, et al., Genotype-phenotype correlations in PCD patients carrying DNAH5 mutations, Thorax 58 (6) (2003) 552–554, https://doi.org/10.1136/thorax.58.6.552-b.

[73] H. Olbrich, K. Haffner, A. Kispert, A. Volkel, A. Volz, et al., Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry, Nat. Genet. 30 (2) (2002) 143–144, https://doi.org/10.1038/ng817.

[74] X. Xu, P. Gong, J. Wen, Clinical and genetic analysis of a family with Kartagener syndrome caused by novel DNAH5 mutations, J. Assist. Reprod. Genet. 34 (2017) 275–281, https://doi.org/10.1007/s10815-016-0849-3.

[75] M.R. Knowles, M.W. Leigh, J.L. Carson, S.D. Davis, S.D. Dell, et al., Mutations of DNAH11 in patients with primary ciliary dyskinesia with normal ciliary ultrastructure, Thorax 67 (5) (2012) 433–441, https://doi.org/10.1136/thoraxjnl-2011-200301.

[76] G.C. Schwabe, K. Hoffmann, N.T. Loges, D. Birker, C. Rossier, et al., Primary ciliary dyskinesia associated with normal axoneme ultrastructure is caused by DNAH11 mutations, Hum. Mutat. 29 (2) (2008) 289–298, https://doi.org/10.1002/humu.20656.

[77] N.T. Loges, H. Olbrich, A. Becker-Heck, K. Häffner, A. Heer, et al., Deletions and point mutations of LRRC50 cause primary ciliary dyskinesia due to dynein arm defects, Am. J. Hum. Genet. 85 (2009) 883–889, https://doi.org/10.1016/j.ajhg.2009.10.018.

[78] A. Horani, T.E. Druley, M.A. Zariwala, A.C. Patel, B.T. Levinson, et al., Whole-exome capture and sequencing identifies HEATR2 mutation as a cause of primary ciliary dyskinesia, Am. J. Hum. Genet. 91 (4) (2012) 685–693, https://doi.org/10.1016/j.ajhg.2012.08.022.

[79] A. Horani, T.W. Ferkol, D. Shoseyov, M.G. Wasserman, Y.S. Oren, et al., LRRC6 mutation causes primary ciliary dyskinesia with dynein arm defects, PLoS One 8 (3) (2013), e59436, https://doi.org/10.1371/journal.pone.0059436.

[80] A. Tarkar, N.T. Loges, C.E. Slagle, R. Francis, G.W. Dougherty, et al., DYX1C1 is required for axonemal dynein assembly and ciliary motility, Nat. Genet. 45 (2013) 995–1003, https://doi.org/10.1038/ng.2707.

[81] G.R. Mali, P.L. Yeyati, S. Mizuno, D.O. Dodd, P.A. Tennant, et al., ZMYND10 functions in a chaperone relay during axonemal dynein assembly, Elife 7 (2018), https://doi.org/10.7554/eLife.34389.

[82] J. Wallmeier, H. Shiratori, G.W. Dougherty, C. Edelbusch, R. Hjeij, et al., TTC25 deficiency results in defects of the outer dynein arm docking machinery and primary ciliary dyskinesia with left-right body asymmetry randomization, Am. J. Hum. Genet. 99 (2) (2016) 460–469, https://doi.org/10.1016/j.ajhg.2016.06.014.

[83] R. Pereira, J. Oliveira, L. Ferraz, A. Barros, R. Santos, et al., Mutation analysis in patients with total sperm immotility, J. Assist. Reprod. Genet. 32 (6) (2015) 893–902, https://doi.org/10.1007/s10815-015-0474-6.

[84] A. Gileles-Hillel, H. Mor-Shaked, D. Shoseyov, J. Reiter, R. Tsabari, et al., Whole-exome sequencing accuracy in the diagnosis of primary ciliary dyskinesia, ERJ Open Res 6 (4) (2020), https://doi.org/10.1183/23120541.00213-2020.

[85] A.J. Shapiro, S.D. Davis, D. Polineni, M. Manion, M. Rosenfeld, et al., Diagnosis of primary ciliary dyskinesia. An official American thoracic society clinical practice guideline, Am. J. Respir. Crit. Care Med. 197 (12) (2018) e24–e39, https://doi.org/10.1164/rccm.201805-0819ST.

[86] C. Wallis, N.J. Simmonds, Diagnosis of the symptomatic patient, in: A. Bush, D. Bilton, M. Hodson (Eds.), Hodson and Geddes' Cystic Fibrosis, CRC Press, Taylor & Francis Group, LLC, 2015, pp. 141–154.

[87] V.K. Singh, S.J. Schwarzenberg, Pancreatic insufficiency in cystic fibrosis, J. Cyst. Fibros. 16 (Suppl 2) (2017) S70–S78, https://doi.org/10.1016/j.jcf.2017.06.011.

[88] E. Kerem, B. Kerem, Genotype-phenotype correlations in cystic fibrosis, Pediatr. Pulmonol. 22 (6) (1996) 387–395, https://doi.org/10.1002/(SICI)1099-0496(199612)22:6<387::AID-PPUL7>3.0.CO;2-G.

[89] H. Shumaker, H. Amlal, R. Frizzell, D. Charles, I. Ulrich, M. Soleimani, CFTR drives Na+- n HCO 3 − cotransport in pancreatic duct cells: a basis for defective HCO 3 − secretion in CF, Am. J. Physiol. Cell Physiol. 276 (1) (1999) C16–C25, https://doi.org/10.1152/ajpcell.1999.276.1.C16.

[90] M.G. Lee, J.Y. Choi, X. Luo, E. Strickland, P.J. Thomas, et al., Cystic fibrosis transmembrane conductance regulator regulates luminal Cl−/HCO3 −Exchange in mouse submandibular and pancreatic ducts, J. Biol. Chem. 274 (21) (1999) 14670–14677, https://doi.org/10.1074/jbc.274.21.14670.

[91] M. Wilschanski, I. Novak, The cystic fibrosis of exocrine pancreas, Cold Spring Harbor Perspect.Med. 3 (5) (2013), https://doi.org/10.1101/cshperspect.a009746.

[92] K. Kayani, R. Mohammed, H. Mohiaddin, Cystic fibrosis-related diabetes, Front. Endocrinol. 9 (20) (2018) 1–11, https://doi.org/10.3389/fendo.2018.00020.

[93] A.J. Shapiro, S. Tolleson-Rinehart, M.A. Zariwala, M.R. Knowles, M.W. Leigh, The prevalence of clinical features associated with primary ciliary dyskinesia in a heterotaxy population: results of a web-based survey, Cardiol. Young 25 (4) (2015) 752–759, https://doi.org/10.1017/S1047951114000912.

[94] M.R. Kiedrowski, J.M. Bomberger, Viral-bacterial Co-infections in the cystic fibrosis respiratory tract, Front. Immunol. 9 (3067) (2018) 3067, https://doi.org/10.3389/fimmu.2018.03067.

[95] G. Hery-Arnaud, S. Boutin, L. Cuthbertson, S.J. Elborn, M.M. Tunney, The lung and gut microbiome: what has to be taken into consideration for cystic fibrosis? J. Cyst. Fibros. 18 (1) (2019) 13–21, https://doi.org/10.1016/j.jcf.2018.11.003.

[96] C. Wallis, Diagnosis and presentation of cystic fibrosis, in: R Deterding RW Wilmott, A. Li, F. Ratjen, P. Sly, A Bush HJ Zar (Eds.), Kendig's Disorders of the Respiratory Tract in Children, Elsevier, Philadelphia, 2019, pp. 769–776.e2, https://doi.org/10.1016/B978-0-323-44887-1.00050-X.

[97] M. Cockx, M. Gouwy, J. Van Damme, S. Struyf, Chemoattractants and cytokines in primary ciliary dyskinesia and cystic fibrosis: key players in chronic respiratory diseases, Cell. Mol. Immunol. 15 (4) (2018) 312–323, https://doi.org/10.1038/cmi.2017.118.

[98] M. Cohen-Cymberknoh, N. Simanovsky, N. Hiller, A.G. Hillel, D. Shoseyov, et al., Differences in disease expression between primary ciliary dyskinesia and cystic fibrosis with and without pancreatic insufficiency, Chest 145 (4) (2014) 738–744, https://doi.org/10.1378/chest.13-1162.

[99] O. Joensen, T. Paff, E.G. Haarman, I.M. Skovgaard, P.Ø. Jensen, et al., Exhaled breath analysis using electronic nose in cystic fibrosis and primary ciliary dyskinesia patients with chronic pulmonary infections, PLoS One 9 (2014), e115584, https://doi.org/10.1371/journal.pone.0115584.

[100] K. Mackerness, P. Jose, A. Bush, Differences in airway inflammation in cystic fibrosis and primary ciliary dyskinesia, pediatric asthma, Allerg. Immunol. (Paris) 22 (4) (2009) 163–168, https://doi.org/10.1089/pai.2009.0022.

[101] M. Maglione, S. Montella, C. Mollica, V. Carnovale, P. Iacotucci, et al., Lung structure and function similarities between primary ciliary dyskinesia and mild cystic fibrosis: a pilot study, Ital. J. Pediatr. 43 (1) (2017) 34, https://doi.org/10.1186/s13052-017-0351-2.

[102] T. Paff, M.P. van der Schee, J.M.A. Daniels, G. Pals, P.E. Postmus, et al., Exhaled molecular profiles in the assessment of cystic fibrosis and primary ciliary dyskinesia, J. Cyst. Fibros. 12 (2013) 454–460, https://doi.org/10.1016/j.jcf.2012.12.010.

[103] A. Radine, C. Werner, J. Raidt, G.W. Dougherty, L. Kerschke, et al., Comparison of nocturnal cough analysis in healthy subjects and in patients with cystic fibrosis and primary ciliary dyskinesia: a prospective observational study, Respiration 97 (1) (2019) 60–69, https://doi.org/10.1159/000493323.

[104] F. Ratjen, V. Waters, M. Klingel, N. McDonald, S. Dell, et al., Changes in airway inflammation during pulmonary exacerbations in patients with cystic fibrosis and primary ciliary dyskinesia, Eur. Respir. J. 47 (3) (2016) 829–836, https://doi.org/10.1183/13993003.01390-2015.

[105] P. Robinson, L. Morgan, Bronchiectasis in PCD looks different to CF on CT scan, Multidisciplinary Resp.Med. 13 (1) (2018) 24, https://doi.org/10.1186/s40248-018-0139-2.

[106] J.S. Lucas, M. Carroll, Primary ciliary dyskinesia and cystic fibrosis: different diseases require different treatment, Chest 145 (4) (2014) 674–676, https://doi.org/10.1378/chest.13-2590.

[107] C. Gagliardo, L. Saiman, Microbiology of cystic fibrosis: epidemiology of cystic fibrosis pathogens and clinical microbiology laboratory methods, in: A. Bush, D. Bilton, M. Hodson (Eds.), Hodson and Geddes' Cystic Fibrosis, CRC Press, Taylor & Francis Group, LLC, 2015, pp. 174–185.

[108] D. Caudri, L. Turkovic, J. Ng, N.H. de Klerk, T. Rosenow, et al., The association between Staphylococcus aureus and subsequent bronchiectasis in children with cystic fibrosis, J. Cyst. Fibros. 17 (4) (2018) 462–469, https://doi.org/10.1016/j.jcf.2017.12.002.

[109] A.J. Fischer, S.B. Singh, M.M. LaMarche, L.J. Maakestad, Z.E. Kienenberger, et al., Sustained coinfections with Staphylococcus aureus and Pseudomonas aeruginosa in cystic fibrosis, Am. J. Respir. Crit. Care Med. 203 (3) (2021) 328–338, https://doi.org/10.1164/rccm.202004-1322OC.

[110] C.M. Waters, J.B. Goldberg, Pseudomonas aeruginosa in cystic fibrosis: a chronic cheater, Proc. Natl. Acad. Sci. USA 116 (14) (2019) 6525–6527, https://doi.org/10.1073/pnas.1902734116.

[111] E.T. Zemanick, L.R. Hoffman, Cystic fibrosis: microbiology and host response, Pediatr. Clin. 63 (4) (2016) 617–636, https://doi.org/10.1016/j.pcl.2016.04.003.

[112] V.C. Scoffone, L.R. Chiarelli, G. Trespidi, M. Mentasti, G. Riccardi, et al., Burkholderia cenocepacia infections in cystic fibrosis patients: drug resistance and therapeutic approaches, Front. Microbiol. 8 (1592) (2017), https://doi.org/10.3389/fmicb.2017.01592.

[113] A. Shoemark, Haemophilus influenzae biofilms in primary ciliary dyskinesia: a moving story, Eur. Respir. J. 50 (3) (2017), 1701369, https://doi.org/10.1183/13993003.01369-2017.

[114] M.C. Alanin, K.G. Nielsen, C. von Buchwald, M. Skov, K. Aanaes, et al., A longitudinal study of lung bacterial pathogens in patients with primary ciliary dyskinesia, Clin. Microbiol. Infect. 21 (12) (2015) 1093 e1–e7, https://doi.org/10.1016/j.cmi.2015.08.020.

[115] M. Cohen-Cymberknoh, N. Weigert, A. Gileles-Hillel, O. Breuer, N. Simanovsky, et al., Clinical impact of Pseudomonas aeruginosa colonization in patients with Primary Ciliary Dyskinesia, Respir. Med. 131 (2017) 241–246, https://doi.org/10.1016/j.rmed.2017.08.028.

[116] G. Piatti, M.M. De Santi, A. Farolfi, G.V. Zuccotti, E. D'Auria, et al., Exacerbations and Pseudomonas aeruginosa colonization are associated with altered lung structure and function in primary ciliary dyskinesia, BMC Pediatr. 20 (1) (2020) 158, https://doi.org/10.1186/s12887-020-02062-4.

[117] M.G. Holgersen, J.K. Marthin, H.K. Johansen, K.G. Nielsen, A retrospective review of Achromobacter species and antibiotic treatments in patients with primary ciliary dyskinesia, Chron. Respir. Dis. 18 (2021), 14799731211061600, https://doi.org/10.1177/14799731211061600, 14799731211061600.

[118] C.D. Wijers, J.F. Chmiel, B.M. Gaston, Bacterial infections in patients with primary ciliary dyskinesia: comparison with cystic fibrosis, Chron. Respir. Dis. 14 (4) (2017) 392–406, https://doi.org/10.1177/1479972317694621.

[119] S. Teixeira, R. Sa, A. Grangeia, J. Silva, C. Oliveira, et al., Immunohystochemical analysis of CFTR in normal and disrupted spermatogenesis, Syst. Biol. Reprod. Med. 59 (1) (2013) 53–59, https://doi.org/10.3109/19396368.2012.718851.

[120] Y. Chen, X. Li, H. Liao, X. Leung, J. He, et al., CFTR mutation compromises spermatogenesis by enhancing miR-15b maturation and suppressing its regulatory target CDC25A, Biol. Reprod. 101 (1) (2019) 50–62, https://doi.org/10.1093/biolre/ioz062.

[121] M. Sousa, E. Oliveira, A. Alves, M. Gouveia, H. Figueiredo, et al., Ultrastructural analysis of five patients with total sperm immotility, Zygote 23 (6) (2015) 900–907, https://doi.org/10.1017/S0967199414000616.

[122] I. Aprea, T. Nöthe-Menchen, G.W. Dougherty, J. Raidt, N.T. Loges, et al., Motility of efferent duct cilia aids passage of sperm cells through the male reproductive system, Mol. Hum. Reprod. 27 (3) (2021), https://doi.org/10.1093/molehr/gaab009.
[123] L. Newman, J. Chopra, C. Dossett, E. Shepherd, A. Bercusson, et al., The impact of primary ciliary dyskinesia on female and male fertility: a narrative review, Hum. Reprod. Update (2023), https://doi.org/10.1093/humupd/dmad003.
[124] N.P.M. Kuijsters, W.G. Methorst, M.S.Q. Kortenhorst, C. Rabotti, M. Mischi, et al., Uterine peristalsis and fertility: current knowledge and future perspectives: a review and meta-analysis, Reprod. Biomed. Online 35 (1) (2017) 50–71, https://doi.org/10.1016/j.rbmo.2017.03.019.
[125] G.J. Vanaken, L. Bassinet, M. Boon, R. Mani, I. Honoré, et al., Infertility in an adult cohort with primary ciliary dyskinesia: phenotype–gene association, Eur. Respir. J. 50 (5) (2017), 1700314, https://doi.org/10.1183/13993003.00314-2017.
[126] L.-H. Chan, L.L. Tsang, D. Rowlands, L. Rochelle, R.C. Boucher, et al., Distribution and regulation of ENaC subunit and CFTR mRNA expression in murine female reproductive tract, J. Membr. Biol. 185 (2002) 165–176, https://doi.org/10.1007/s00232-001-0117-y.
[127] K.D. Schoyer, F. Gilbert, Z. Rosenwaks, Infertility and abnormal cervical mucus in two sisters who are compound heterozygotes for the cystic fibrosis (CF) DeltaF508 and R117H/7T mutations, Fertil. Steril. 90 (4) (2008) 1201 e19–e22, https://doi.org/10.1016/j.fertnstert.2007.08.063.
[128] K.Y.B. Ng, R. Mingels, H. Morgan, N. Macklon, Y. Cheong, In vivo oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review, Hum. Reprod. Update 24 (1) (2017) 15–34, https://doi.org/10.1093/humupd/dmx028.
[129] L.C. Puga Molina, N.A. Pinto, N.I. Torres, A.L. González-Cota, G.M. Luque, et al., CFTR/ENaC-dependent regulation of membrane potential during human sperm capacitation is initiated by bicarbonate uptake through NBC, J. Biol. Chem. 293 (25) (2018) 9924–9936, https://doi.org/10.1074/jbc.RA118.003166.
[130] C.A. Hodges, M.R. Palmert, M.L. Drumm, Infertility in females with cystic fibrosis is multifactorial: evidence from mouse models, Endocrinology 149 (6) (2008) 2790–2797, https://doi.org/10.1210/en.2007-1581.
[131] M. Johannesson, B.M. Landgren, G. Csemiczky, L. Hjelte, C. Gottlieb, Female patients with cystic fibrosis suffer from reproductive endocrinological disorders despite good clinical status, Hum. Reprod. 13 (8) (1998) 2092–2097, https://doi.org/10.1093/humrep/13.8.2092.
[132] N. Hirokawa, Y. Tanaka, Y. Okada, S. Takeda, Nodal flow and the generation of left-right asymmetry, Cell 125 (1) (2006) 33–45, https://doi.org/10.1016/j.cell.2006.03.002.
[133] Y. Okada, S. Takeda, Y. Tanaka, J.I. Belmonte, N. Hirokawa, Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination, Cell 121 (4) (2005) 633–644, https://doi.org/10.1016/j.cell.2005.04.008.
[134] A.J. Shapiro, S.D. Davis, T. Ferkol, S.D. Dell, M. Rosenfeld, et al., Laterality defects other than situs inversus totalis in primary ciliary dyskinesia: insights into situs ambiguus and heterotaxy, Chest 146 (5) (2014) 1176–1186, https://doi.org/10.1378/chest.13-1704.
[135] D. Gelfond, D. Borowitz, Gastrointestinal complications of cystic fibrosis, Clin. Gastroenterol. Hepatol. 11 (4) (2013) 333–342, https://doi.org/10.1016/j.cgh.2012.11.006, quiz e30-1.
[136] C. Castellani, A.J.A. Duff, S.C. Bell, H.G.M. Heijerman, A. Munck, et al., ECFS best practice guidelines: the 2018 revision, J. Cyst. Fibros. 17 (2) (2018) 153–178, https://doi.org/10.1016/j.jcf.2018.02.006.
[137] O.J. McElvaney, P. Wade, M. Murphy, E.P. Reeves, N.G. McElvaney, Targeting airway inflammation in cystic fibrosis, Expet Rev. Respir. Med. 13 (11) (2019) 1041–1055, https://doi.org/10.1080/17476348.2019.1666715.
[138] K.M. Allan, N. Farrow, M. Donnelley, A. Jaffe, S.A. Waters, Treatment of cystic fibrosis: from gene- to cell-based therapies, Front. Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.639475.
[139] F.A. Almughem, A.M. Aldossary, E.A. Tawfik, M.N. Alomary, W.S. Alharbi, et al., Cystic fibrosis: overview of the current development trends and innovative therapeutic strategies, Pharmaceutics 12 (7) (2020) 1–29, https://doi.org/10.3390/pharmaceutics12070616.
[140] S.J. Nevitt, J. Thornton, C.S. Murray, T. Dwyer, Inhaled mannitol for cystic fibrosis, Cochrane Database Syst. Rev. 2 (2) (2018) CD008649, https://doi.org/10.1002/14651858.CD008649.
[141] C. Yang, M. Montgomery, Dornase alfa for cystic fibrosis, Cochrane Database Syst. Rev. (3) (2021), https://doi.org/10.1002/14651858.CD001127.
[142] P. Couroux, P. Farias, L. Rizvi, K. Griffin, C. Hudson, et al., First clinical trials of novel ENaC targeting therapy, SPX-101, in healthy volunteers and adults with cystic fibrosis, Pulm. Pharmacol. Therapeut. 58 (2019), 101819, https://doi.org/10.1016/j.pupt.2019.101819.
[143] S.H. Burstein, Ajulemic acid: potential treatment for chronic inflammation, Pharm. Res. Perspect. 6 (2) (2018), e00394, https://doi.org/10.1002/prp2.394.
[144] J.S. Elborn, S. Ahuja, E. Springman, J. Mershon, R. Grosswald, et al., EMPIRE-CF: a phase II randomized placebo-controlled trial of once-daily, oral acebilustat in adult patients with cystic fibrosis - study design and patient demographics, Contemp. Clin. Trials 72 (2018) 86–94, https://doi.org/10.1016/j.cct.2018.07.014.
[145] J.F. Chmiel, T.R. Aksamit, S.H. Chotirmall, E.C. Dasenbrook, J.S. Elborn, et al., Antibiotic management of lung infections in cystic fibrosis. I. The microbiome, methicillin-resistant Staphylococcus aureus, gram-negative bacteria, and multiple infections, Annal Am. Thoracic Soc. 11 (7) (2014) 1120–1129, https://doi.org/10.1513/AnnalsATS.201402-050AS.
[146] M. Halwani, H.H. Balkhy, M.A. Khiyami, A. Omri, Antimicrobial activity of liposomal β-glycan against Pseudomonas aeruginosa isolated from cystic fibrosis patients, J. Cyst. Fibros. (2015) S1–S156. Brussels, Belgium.
[147] A. Ermund, C.V. Recktenwald, G. Skjak-Braek, L.N. Meiss, E. Onsoyen, et al., OligoG CF-5/20 normalizes cystic fibrosis mucus by chelating calcium, Clin. Exp. Pharmacol. Physiol. 44 (6) (2017) 639–647, https://doi.org/10.1111/1440-1681.12744.
[148] R. Weiser, P.D. Rye, E. Mahenthiralingam, Implementation of microbiota analysis in clinical trials for cystic fibrosis lung infection: experience from the OligoG phase 2b clinical trials, J. Microbiol. Methods (2021) 181, https://doi.org/10.1016/j.mimet.2021.106133.
[149] L.P. Lashua, J.A. Melvin, B. Deslouches, J.M. Pilewski, R.C. Montelaro, et al., Engineered cationic antimicrobial peptide (eCAP) prevents Pseudomonas aeruginosa biofilm growth on airway epithelial cells, J. Antimicrob. Chemother. 71 (8) (2016) 2200–2207, https://doi.org/10.1093/jac/dkw143.
[150] T. Beaudoin, T.A. Stone, M. Glibowicka, C. Adams, Y. Yau, et al., Activity of a novel antimicrobial peptide against Pseudomonas aeruginosa biofilms, Sci. Rep. 8 (1) (2018), 14728, https://doi.org/10.1038/s41598-018-33016-7.
[151] O. Laselva, T.A. Stone, C.E. Bear, C.M. Deber, Anti-infectives restore ORKAMBI® rescue of F508del-CFTR function in human bronchial epithelial cells infected with clinical strains of P. Aeruginosa, Biomolecules 10 (2) (2020), https://doi.org/10.3390/biom10020334.
[152] L. Bentur, M. Gur, M. Ashkenazi, G. Livnat-Levanon, M. Mizrahi, et al., Pilot study to test inhaled nitric oxide in cystic fibrosis patients with refractory Mycobacterium abscessus lung infection, J. Cyst. Fibros. 19 (2) (2020) 225–231, https://doi.org/10.1016/j.jcf.2019.05.002.
[153] L. Kvachadze, N. Balarjishvili, T. Meskhi, E. Tevdoradze, N. Skhirtladze, et al., Evaluation of lytic activity of staphylococcal bacteriophage Sb-1 against freshly isolated clinical pathogens, Microb. Biotechnol. 4 (5) (2011) 643–650, https://doi.org/10.1111/j.1751-7915.2011.00259.x.
[154] N. Law, C. Logan, G. Yung, C.-L.L. Furr, S.M. Lehman, et al., Successful adjunctive use of bacteriophage therapy for treatment of multidrug-resistant Pseudomonas aeruginosa infection in a cystic fibrosis patient, Infection 47 (4) (2019) 665–668, https://doi.org/10.1007/s15010-019-01319-0.
[155] N. Hoyle, P. Zhvaniya, N. Balarjishvili, D. Bolkvadze, L. Nadareishvili, et al., Phage therapy against Achromobacter xylosoxidans lung infection in a patient with cystic fibrosis: a case report, Res. Microbiol. 169 (9) (2018) 540–542, https://doi.org/10.1016/j.resmic.2018.05.001.
[156] R.M. Dedrick, C.A. Guerrero-Bustamante, R.A. Garlena, D.A. Russell, K. Ford, et al., Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus, Nat. Med. 25 (5) (2019) 730–733, https://doi.org/10.1038/s41591-019-0437-z.
[157] D. Lebeaux, M. Merabishvili, E. Caudron, D. Lannoy, L. Van Simaey, et al., A case of phage therapy against pandrug-resistant achromobacter xylosoxidans in a 12-year-old lung-transplanted cystic fibrosis patient, Viruses 13 (1) (2021), https://doi.org/10.3390/v13010060.
[158] I. Martin, D.T.D. Kenna, S. Morales, E.W.F.W. Alton, J.C. Davies, Variability in bacteriophage and antibiotic sensitivity in serial Pseudomonas aeruginosa isolates from cystic fibrosis airway cultures over 12 months, Microorganisms 9 (3) (2021) 1–12, https://doi.org/10.3390/microorganisms9030660.
[159] K.R. Rouillard, O.P. Novak, A.M. Pistiolis, L. Yang, M.J.R. Ahonen, et al., Exogenous nitric oxide improves antibiotic susceptibility in resistant bacteria, ACS Infect. Dis. 7 (1) (2021) 23–33, https://doi.org/10.1021/acsinfecdis.0c00337.
[160] D.O. Schairer, J.S. Chouake, J.D. Nosanchuk, A.J. Friedman, The potential of nitric oxide releasing therapies as antimicrobial agents, Virulence 3 (3) (2012) 271–279, https://doi.org/10.4161/viru.20328.
[161] S. Trend, A.M. Fonceca, W.G. Ditcham, A. Kicic, A. Cf, The potential of phage therapy in cystic fibrosis: essential human-bacterial-phage interactions and delivery considerations for use in Pseudomonas aeruginosa-infected airways, J. Cyst. Fibros. 16 (6) (2017) 663–670, https://doi.org/10.1016/j.jcf.2017.06.012.
[162] B.K. Chan, G. Stanley, M. Modak, J.L. Koff, P.E. Turner, Bacteriophage therapy for infections in CF, Pediatr. Pulmonol. 56 (S1) (2021) S4–S9, https://doi.org/10.1002/ppul.25190.
[163] L.L. Furfaro, M.S. Payne, B.J. Chang, Bacteriophage therapy: clinical trials and regulatory hurdles, Front. Cell. Infect. Microbiol. 8 (376) (2018), https://doi.org/10.3389/fcimb.2018.00376.
[164] A.R. Habib, M. Kajbafzadeh, S. Desai, C.L. Yang, K. Skolnik, et al., A systematic review of the clinical efficacy and safety of CFTR modulators in cystic fibrosis, Sci. Rep. 9 (1) (2019) 7234, https://doi.org/10.1038/s41598-019-43652-2.
[165] K. Dave, R. Dobra, S. Scott, C. Saunders, J. Matthews, et al., Entering the era of highly effective modulator therapies, Pediatr. Pulmonol. 56 (S1) (2021) S79–S89, https://doi.org/10.1002/ppul.24968.
[166] A. Gramegna, M. Contarini, S. Aliberti, R. Casciaro, F. Blasi, et al., From ivacaftor to triple combination: a systematic review of efficacy and safety of CFTR modulators in people with cystic fibrosis, Int. J. Mol. Sci. 21 (16) (2020), https://doi.org/10.3390/ijms21165882.
[167] I. Sermet-Gaudelus, Ivacaftor treatment in patients with cystic fibrosis and the G551D-CFTR mutation, Eur. Respir. Rev. 22 (127) (2013) 66–71, https://doi.org/10.1183/09059180.00008512.
[168] R.B. Moss, P.A. Flume, J.S. Elborn, J. Cooke, S.M. Rowe, et al., Efficacy and safety of ivacaftor in patients with cystic fibrosis who have an Arg117His-CFTR mutation: a double-blind, randomised controlled trial, Lancet Respir. Med. 3 (7) (2015) 524–533, https://doi.org/10.1016/s2213-2600(15)00201-5.

[169] K.W. Southern, J. Murphy, I.P. Sinha, S.J. Nevitt, Corrector therapies (with or without potentiators) for people with cystic fibrosis with class II CFTR gene variants (most commonly F508del), Cochrane Database Syst. Rev. 12 (12) (2020) Cd010966, https://doi.org/10.1002/14651858.CD010966.pub3.
[170] V. Spano, A. Montalbano, A. Carbone, P. Scudieri, L.J.V. Galietta, et al., An overview on chemical structures as DeltaF508-CFTR correctors, Eur. J. Med. Chem. 180 (2019) 430–448, https://doi.org/10.1016/j.ejmech.2019.07.037.
[171] M. Talamo Guevara, S.A. McColley, The safety of lumacaftor and ivacaftor for the treatment of cystic fibrosis, Expet Opin. Drug Saf. 16 (11) (2017) 1305–1311, https://doi.org/10.1080/14740338.2017.1372419.
[172] T. Kirby, Tezacaftor-ivacaftor is safe and efficacious in patients with cystic fibrosis with Phe508del mutations, Lancet Respir. Med. 6 (1) (2018) 13–14, https://doi.org/10.1016/S2213-2600(17)30439-3.
[173] P.G. Middleton, M.A. Mall, P. Dřevínek, L.C. Lands, E.F. McKone, et al., Elexacaftor–Tezacaftor–Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele, N. Engl. J. Med. 381 (19) (2019) 1809–1819, https://doi.org/10.1056/NEJMoa1908639.
[174] M. Marangi, G. Pistritto, Innovative therapeutic strategies for cystic fibrosis: moving forward to CRISPR technique, Front. Pharmacol. 9 (396) (2018) 396, https://doi.org/10.3389/fphar.2018.00396.
[175] A. Christopher Boyd, S. Guo, L. Huang, B. Kerem, Y.S. Oren, et al., New approaches to genetic therapies for cystic fibrosis, J. Cyst. Fibros. 19 (Suppl 1) (2020) S54–S59, https://doi.org/10.1016/j.jcf.2019.12.012.
[176] M. Lopes-Pacheco, C.F.T.R. Modulators, The changing face of cystic fibrosis in the era of precision medicine, Front. Pharmacol. 10 (1662) (2020), https://doi.org/10.3389/fphar.2019.01662.
[177] A.M. Quemener, L. Bachelot, A. Forestier, E. Donnou-Fournet, D. Gilot, et al., The powerful world of antisense oligonucleotides: from bench to bedside, WIREs RNA 11 (5) (2020), e1594, https://doi.org/10.1002/wrna.1594.
[178] M.M. Keenan, L. Huang, N.J. Jordan, E. Wong, Y. Cheng, et al., Nonsense-mediated RNA decay pathway inhibition restores expression and function of W1282X CFTR, Am. J. Respir. Cell Mol. Biol. 61 (3) (2019) 290–300, https://doi.org/10.1165/rcmb.2018-0316OC.
[179] S.L. Harbeson, A.J. Morgan, J.F. Liu, A.M. Aslanian, S. Nguyen, et al., Altering metabolic profiles of drugs by precision deuteration 2: discovery of a deuterated analog of ivacaftor with differentiated pharmacokinetics for clinical development, J. Pharmacol. Exp. Therapeut. 362 (2) (2017) 359–367, https://doi.org/10.1021/acsptsci.9b00060.
[180] M. Gees, S. Musch, S. Van der Plas, A.S. Wesse, A. Vandevelde, et al., Identification and characterization of novel CFTR potentiators, Front. Pharmacol. 9 (1221) (2018) 1221, https://doi.org/10.3389/fphar.2018.01221.
[181] S. Kazani, D.J. Rowlands, I. Bottoli, J. Milojevic, J. Alcantara, et al., Safety and efficacy of the cystic fibrosis transmembrane conductance regulator potentiator icenticaftor (QBW251), J. Cyst. Fibros. 20 (2) (2021) 250–256, https://doi.org/10.1016/j.jcf.2020.11.002.
[182] K.A. Giuliano, S. Wachi, L. Drew, D. Dukovski, O. Green, et al., Use of a high-throughput phenotypic screening strategy to identify amplifiers, a novel pharmacological class of small molecules that exhibit functional synergy with potentiators and correctors, SLAS Discov. 23 (2) (2018) 111–121, https://doi.org/10.1177/2472555217729790.
[183] R. Fukuda, T. Okiyoneda, Peripheral protein quality control as a novel drug target for CFTR stabilizer, Front. Pharmacol. 9 (2018) 1100, https://doi.org/10.3389/fphar.2018.01100.
[184] S.H. Donaldson, G.M. Solomon, P.L. Zeitlin, P.A. Flume, A. Casey, et al., Pharmacokinetics and safety of cavosonstat (N91115) in healthy and cystic fibrosis adults homozygous for F508DEL-CFTR, J. Cyst. Fibros. 16 (3) (2017) 371–379, https://doi.org/10.1016/j.jcf.2017.01.009.
[185] M. Sigoillot, M. Overtus, M. Grodecka, D. Scholl, A. Garcia-Pino, et al., Domain-interface dynamics of CFTR revealed by stabilizing nanobodies, Nat. Commun. 10 (1) (2019) 2636, https://doi.org/10.1038/s41467-019-10714-y.
[186] M. Ensinck, A. Mottais, C. Detry, T. Leal, M.S. Carlon, On the corner of models and cure: gene editing in cystic fibrosis, Front. Pharmacol. 12 (677) (2021), https://doi.org/10.3389/fphar.2021.662110.
[187] K. Mention, L. Santos, P.T. Harrison, Gene and base editing as a therapeutic option for cystic fibrosis-learning from other diseases, Genes 10 (5) (2019) 387, https://doi.org/10.3390/genes10050387.
[188] E. Xia, M.A. Munegowda, H. Cao, J. Hu, Lung gene therapy-How to capture illumination from the light already present in the tunnel, Genes Dis. 1 (1) (2014) 40–52, https://doi.org/10.1016/j.gendis.2014.06.001.
[189] X.M. Anguela, K.A. High, Entering the modern era of gene therapy, Annu. Rev. Med. 70 (1) (2019) 273–288, https://doi.org/10.1146/annurev-med-012017-043332.
[190] C. Yang, M. Montgomery, Dornase alfa for cystic fibrosis, Cochrane Database Syst. Rev. 9 (2018), https://doi.org/10.1002/14651858.CD001127.
[191] B. Rubbo, J.S. Lucas, Clinical care for primary ciliary dyskinesia: current challenges and future directions, Eur. Respir. Rev. 26 (2017) 170023–170034, https://doi.org/10.1183/16000617.0023-2017.
[192] J.S. Lucas, A. Burgess, H.M. Mitchison, E. Moya, M. Williamson, et al., Diagnosis and management of primary ciliary dyskinesia, Arch. Dis. Child. 99 (2014) 850–856, https://doi.org/10.1136/archdischild-2013-304831.
[193] M. Goutaki, J.F. Papon, M. Boon, C. Casaulta, E. Eber, et al., Standardised clinical data from patients with primary ciliary dyskinesia: follow-pcd, ERJ Open Res 6 (1) (2020), 00237, https://doi.org/10.1183/23120541.00237-2019, 2019.
[194] E. Atag, P. Ergenekon, N. Bas Ikizoğlu, I. Bıngol, Y. Gokdemir, et al., The effect of conventional chest physiotherapy and vibratory positive pressure therapy system on lung clearance index and spirometry in patients with primary ciliary dyskinesia, Eur. Respir. J. 52 (suppl 62) (2018) PA4672, https://doi.org/10.1183/13993003.congress-2018.PA4672.
[195] B. Vandervoort, D. De Beuckeleer, E. Huenaerts, M. Schulte, F. Vermeulen, et al., The short term influence of chest physiotherapy on lung function parameters in children with cystic fibrosis and primary ciliary dyskinesia, Front Pediatr. 10 (2022), 858410, https://doi.org/10.3389/fped.2022.858410.
[196] A. Madsen, K. Green, F. Buchvald, B. Hanel, K.G. Nielsen, Aerobic fitness in children and young adults with primary ciliary dyskinesia, PLoS One 8 (8) (2013), e71409, https://doi.org/10.1371/journal.pone.0071409.
[197] R.G. Campbell, C.S. Birman, L. Morgan, Management of otitis media with effusion in children with primary ciliary dyskinesia: a literature review, Int. J. Pediatr. Otorhinolaryngol. 73 (12) (2009) 1630–1638, https://doi.org/10.1016/j.ijporl.2009.08.024.
[198] M.M. Munye, A.D. Tagalakis, J.L. Barnes, R.E. Brown, R.J. McAnulty, et al., Minicircle DNA provides enhanced and prolonged transgene expression following airway gene transfer, Sci. Rep. 6 (2016), 23125, https://doi.org/10.1038/srep23125.
[199] L.E. Ostrowski, W. Yin, M. Patel, J. Sechelski, T. Rogers, et al., Restoring ciliary function to differentiated primary ciliary dyskinesia cells with a lentiviral vector, Gene Ther. 21 (3) (2014) 253–261, https://doi.org/10.1038/gt.2013.79.
[200] M. Lai, M. Pifferi, A. Bush, M. Piras, A. Michelucci, et al., Gene editing of DNAH11 restores normal cilia motility in primary ciliary dyskinesia, J. Med. Genet. 53 (4) (2016) 242–249, https://doi.org/10.1136/jmedgenet-2015-103539.
[201] Z. Bukowy-Bieryllo, M. Dabrowski, M. Witt, E. Zietkiewicz, Aminoglycoside-stimulated readthrough of premature termination codons in selected genes involved in primary ciliary dyskinesia, RNA Biol. 13 (10) (2016) 1041–1050, https://doi.org/10.1080/15476286.2016.1219832.