Chapter 10

Channelopathies in bronchiectasis

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Summary

Channelopathies are diseases caused by dysfunction of ion channel subunits. They result in impaired mucociliary clearance and may therefore lead to bronchiectasis.

The main channelopathy associated with bronchiectasis is cystic fibrosis (CF), an autosomal recessive disease caused by mutations in the CFTR gene, which encodes the chloride CFTR channel.

Bronchiectasis can be associated to channelopathies in following cases: 1) patients with already known typical CF; 2) patients with bronchiectasis who, on investigation, are found to have a single-organ manifestation of CF; 3) patients with only one or none mutation of CFTR with abnormal sweat test or nasal potential difference (PD) where CFTR mutations play the role of a modifier deleterious gene; and 4) patients with only one or no mutation of CFTR with normal sweat test or nasal PD, who may still have an undefined channelopathy. In these last two cases, it may be that, CFTR mutation combined with another ion transport abnormality, in a situation of transheterozygosity, creates the conditions for abnormal airway surface liquid (ASL) hydration regulation and defective mucociliary clearance.

Keywords: Airway surface liquid, bicarbonate, calcium-dependent chloride channel, cystic fibrosis, cystic fibrosis transmembrane conductance regulator, epithelial sodium channel

Bronchiectasis is defined as an abnormal dilation of proximal medium-sized bronchi due to weakening or destruction of the muscular and elastic components of the bronchial walls [1]. It is caused by a vicious cycle of transmural infection and inflammation, resulting in retained secretions that damage the airways and impair mucociliary clearance.

Bronchiectasis can appear as either a local obstructive process or a diffuse disease involving both lungs. In the latter case, a systemic condition must be sought. These can include autoimmune disease, α1-antitrypsin deficiency, connective tissue disorders, immunodeficiency states, allergic...
bronchopulmonary aspergillosis and primary ciliary dyskinesia. Channelopathies, defined as diseases caused by dysfunctioning ion channel subunits, are another possibility. Channels are pore-forming proteins that provide pathways for the controlled movement of ions into or out of cells, and are hence important in regulating mucociliary clearance [2]. The present chapter focuses on the role of channelopathies as causative factors for the development of bronchiectasis.

The link between ion transport and mucus transport in the airways

Two opposing transport systems tailored to controlling the volume of liquid on the epithelial surface

The thin film of liquid covering airway surfaces, called airway surface liquid (ASL), is partitioned into two compartments, the mucus layer, which entraps particles and pathogens and has lubricant activity, and the periciliary liquid (PCL) layer, which facilitates ciliary beating and separates the mucus layer from the mucins tethered to the cell surface [3]. Normal airway surface epithelia can regulate ASL volume by setting the height of the PCL to approximately the height of the extended cilia (∼7 μM) [3]. The coordination of sodium and chloride ion transport regulates ASL homeostasis to provide efficient mucus transport.

Under resting conditions, airway surface epithelia display net salt and fluid absorption (fig. 1), driven by active apical Na\(^+\) absorption through the amiloride-sensitive epithelial sodium channel (ENaC), passively accompanied by Cl\(^-\), in part, via a transcellular pathway, mainly the cystic fibrosis transmembrane conductance regulator (CFTR), and, in larger part, via the paracellular pathway [3]. This absorptive pattern occurs due to basolateral sodium–potassium adenosine triphosphatase (Na\(^+\),K\(^+\)-ATPase), which generates an electrochemical gradient favourable for apical Na\(^+\) absorption. ASL remains isotonic under basal conditions because of the airway epithelium’s permeability to water (due to the relative leakiness of the tight junctions) and the iso-osmotic conditions of ion transport.

When ASL volume is depleted, normal airway epithelium exerts dynamic regulation by switching its status from net NaCl absorption to net secretion (fig. 2) [3, 4]. This requires the accumulation of Cl\(^-\) within the cell through the action of the Na\(^+\)/K\(^+\)/2Cl\(^-\) cotransporter located in the basolateral membrane. Cl\(^-\) then exits the cell across the apical Cl\(^-\) channels, at the same time as apical Na\(^+\) absorption slows and Na\(^+\) moves paracellularly to maintain electroneutrality. Adenosine triphosphate (ATP), released on to the airway surface, is the main sensor for this regulation [5]. Its actions are mediated by two purinergic receptor subtypes, the pertussis-toxin-insensitive G-protein (Gq)-coupled ATP/uridine triphosphate (UTP)-sensing P2Y\(_2\) P2Y receptor and the stimulatory G-protein (Gs)-coupled A\(_{2B}\) adenosine receptor. Activation of the A\(_{2B}\) purinoreceptor raises cell cyclic adenosine monophosphate (cAMP), which, in turn, activates the CFTR sufficiently to provide CFTR-dependent Cl\(^-\) secretion and negative ENaC

**Figure 1.** Cellular models of electrolyte secretion: absorption pathway. In airway epithelial cells, under resting conditions, Na\(^+\) is taken up by a luminal epithelial sodium channel (ENaC); Cl\(^-\) is transported via the paracellular shunt and probably via cystic fibrosis transmembrane conductance regulator (CFTR) Cl\(^-\) channels. Na\(^+\) is pumped out of the cell by the basolateral sodium–potassium adenosine triphosphatase (Na\(^+\),K\(^+\)-ATPase), whereas Cl\(^-\) and K\(^+\) leave the cell via Cl\(^-\) and K\(^+\) channels, respectively. PKA: protein kinase A, -: inhibition; +: stimulation.
Regulation by the CFTR. Higher ATP concentrations then activate the P2Y2 receptor, promoting, on the one hand, the inhibition of Na\(^+\) absorption and, on the other, Cl\(^-\) secretion, mediated by another apical channel, the calcium-activated chloride channel (CaCC).

Finally, when ASL volumes are depleted, the epithelium rehydrates airway surfaces by: 1) inhibiting absorption (in the surface epithelium); and 2) activating secretion (in the submucosal glands).

**Ion transporters involved in mucociliary clearance**

Consistent with these fundamental observations, most of the channelopathies identified as possible causes of the impaired clearance of bronchial tree secretions appear to involve Cl\(^-\), Na\(^+\) and bicarbonate transport.

**Cl\(^-\) transporters**

Cystic fibrosis transmembrane conductance regulator

The CFTR is a member of the ATP-binding cassette transporter superfamily, principally expressed in the apical membrane of epithelia. It plays a fundamental role in transepithelial fluid and electrolyte transport because it functions as an anion channel and a regulator of ion transporters in epithelial cells. The CFTR is a cAMP- and ATP-regulated Cl\(^-\) channel that permits Cl\(^-\) to be released from the cell [6]. Recent data also suggest that the CFTR pore may switch dynamically from a conformation permeable to Cl\(^-\) to a conformation permeable to large anions, such as glutathione and HCO\(_3\)\(^-\), and may, therefore, be involved in pH regulation of the ASL and mucus [7].

Apart from its secretary function, the CFTR has the regulatory function of other epithelial channels. The CFTR inhibits ENaC activity and, therefore, conveys reduction in Na\(^+\) resorption [8]. The CFTR upregulates an outwardly rectifying chloride channel (ORCC) following its activation by protein kinase A (PKA) [9]. The CFTR can also interact via its extreme C-terminal amino acid sequence with PDZ-domain-containing proteins, which are important organisers for receptors, ion transporters and regulatory elements present in airway epithelium [10]. For example, reciprocal activation between the CFTR and the solute carrier (SLC) 26 transporter (SLC26T) family of HCO\(_3\)\(^-\)/Cl\(^-\) exchangers has been shown to depend upon PDZ domain interaction and binding of the sulfate transporter and anti-\(\sigma\) factor antagonist (STAS) domain of SLC26T family proteins to the CFTR regulatory (R) domain [11].
Calcium-activated chloride channels

Airway epithelial cells display Ca\(^{2+}\)-dependent Cl\(^{-}\) secretion through CaCCs in response to mucosal nucleosides. The mechanism relies on the stimulation by ATP or UTP of the G\(_{q}\)-coupled P2Y\(_{2}\) purinergic receptors, which increases inositol 1,4,5-trisphosphate (IP\(_{3}\)) production and subsequently cytosolic Ca\(^{2+}\) release [12].

Transmembrane protein 16A (TMEM16A), which generates Ca\(^{2+}\)-activated Cl\(^{-}\) currents with similar biophysical and pharmacological properties to those in native epithelial tissues, is a very likely candidate for these CaCCs [13].

Chloride channel-2

Chloride channel (ClC)-2 is a member of the pH- and voltage-activated chloride channel family and is present on the apical membranes of airway epithelial cells [14]. Activation of ClC-2 is hypothesised to provide a parallel pathway for Cl\(^{-}\) secretion [15].

Indirect activation of Cl\(^{-}\) secretion by K\(^{+}\) channels

Activation of K\(^{+}\) channels at the basolateral side of the epithelium causes hyperpolarisation of the basolateral membrane, which electrically drives Cl\(^{-}\) to the luminal side of the epithelium and stimulates Cl\(^{-}\) secretion through the CFTR and/or CaCCs. At least two different populations of K\(^{+}\) channel are located on the basolateral side of airway epithelial cells that are activated by an increase in either intracellular cAMP (cAMP-dependent potassium channel (K\(_{\alpha}7.1\)) or Ca\(^{2+}\) (calcium-activated potassium channel (K\(_{Ca}3.1\))) [16].

Na\(^{+}\) transporters

The ENaC is a heteromultimer composed of distinct but homologous \(\alpha\)-, \(\beta\)- and \(\gamma\)-subunits known to be activated by selective endoproteolysis [17]. As pointed out above, it provides the main pathway for apical Na\(^{+}\) absorption at the apical membrane [3, 4]. The ENaC and the CFTR physically associate in mammalian cells [18], an interaction that may impede ENaC proteolytic cleavage and inhibit stimulation of the channel open probability [19].

HCO\(_{3}\(^{-}\) transport

HCO\(_{3}\) plays a critical role in determining the viscosity of mucins and mucus by decondensing mucin granules. Intracellularly, mucins are condensed in granules by high concentrations of Ca\(^{2+}\) and H\(^{+}\) that shield the repulsive forces of the anionic sites of mucin glycoproteins. As granules are secreted, Ca\(^{2+}\) and H\(^{+}\) have to dissociate quickly from the mucus to unshield the negative sites, so that Na\(^{+}\) can replace Ca\(^{2+}\) to allow mucin network hydration, swelling and dispersion. HCO\(_{3}\) is critical for sequestration of Ca\(^{2+}\) and H\(^{+}\) and maintenance of a low concentration of these free cations in solution, which, in turn, favour their disassociation from mucins [20, 21]. Moreover, a normal pH is necessary for effective mucociliary clearance, as assessed by several observations. For example, a reduction in extracellular pH of 0.5 reduces mucociliary beat frequency by 22% in bronchi and 16% in bronchioles [22].

As stated above, the CFTR clearly plays a role in HCO\(_{3}\) transport. Cell membrane ion transporters besides CFTR may also be involved in ASL and/or gland fluid pH regulation [23]. These include the following.

Na\(^{+}\)/HCO\(_{3}\) cotransporters

The basolaterally located isoform sodium bicarbonate cotransporter (NBC) 1 permits the basal influx of HCO\(_{3}\) followed by efflux through the apical CFTR [24].
Cl⁻/HCO₃⁻ exchangers

Based on an analogy to SLC26A3 function in HCO₃⁻ secretion by the pancreatic duct epithelium, Wheat et al. [25] proposed a model for HCO₃⁻ transport in the airway epithelium: Cl⁻/HCO₃⁻ exchange activity, governed by SLC26A3 in the apical membrane, might secrete HCO₃⁻ into the ASL, with Cl⁻ recycling through the CFTR. However, experiments in polarised airway epithelial cells failed to confirm this hypothesis [26]. The role of Cl⁻/HCO₃⁻ exchangers in ASL pH regulation at the apical membrane therefore remains speculative.

Investigation of ion transport in airway epithelium

Transepithelial potential difference (PDₑ) results from ion movements across both the basolateral and apical membrane and leakiness of tight junctions. Its assessment has been applied in vivo to both nasal and bronchial mucosa [27]. Nasal potential difference (PD)-based outcomes include the stable maximum baseline (basal PD) and the successive net voltage changes after perfusion of the mucosa with: 1) amiloride (an ENaC inhibitor), to assess Na⁺ transport (Δamiloride); 2) low-chloride solution, to drive Cl⁻ secretion (Δlow-chloride); and 3) isoproterenol in low-chloride solution (Δisoproterenol), to stimulate the cAMP-dependent Cl⁻ conductance related to the CFTR (fig. 3). The sum of Δlow-chloride and Δisoproterenol serves as an index of CFTR function [28].

This PDₑ can also be measured in Ussing chambers, using either epithelial biopsy specimens or airway epithelial cells in culture. This system measures transepithelial ion transport by evaluating PDₑ in volts [29], by either applying a PD and measuring the resulting change in current (technique of voltage clamping) or short-circuiting the tissue, i.e. clamping PDₑ at 0 V and measuring the amount of current required.

Channelopathies: cystic fibrosis

Pathophysiology

Cystic fibrosis (CF) is one of the principal channelopathies resulting in abnormal mucus clearance. It is an autosomal recessive disease caused by mutations in the CFTR gene (CFTR), which encodes the CFTR Cl⁻ channel [30].

In CF, defects in the mechanisms governing both Na⁺ absorption and Cl⁻ secretion severely disrupt ASL volume regulation on airway surfaces. Specifically, they accelerate the basal rate of net epithelial Na⁺ absorption in CF airway epithelia, causing isotonic volume hyperabsorption that reflects the absence of the tonic inhibitory effect of CFTR on ENaC activity [31, 32]. The mechanism linking the missing CFTR and increased Na⁺ absorption in CF airway epithelia may be the failure to protect ENaC from proteolytic cleavage and consequent activation [33].

CF airway epithelia also lack the capacity to enhance Cl⁻ transport [34]. Therefore, whereas non-CF epithelium can rehydrate when ASL volumes are depleted, by activating secretion and inhibiting absorption, CF epithelium cannot switch from net absorption to net secretion [31]. This inability may be due to its dependence on ATP signalling alone, in contrast to the dual signalling (ATP and adenosine) systems that control ASL volume in normal epithelia [35]. In this model, ATP can inhibit ENaC and activate CaCC, via the P2Y₂ receptor, but the A₂B pathway is blocked because the CFTR is not functional. Under resting conditions, the P2Y₂ pathway may be sufficient to produce an ASL volume consistent with mucus transport. It may, however, be overwhelmed in a context of respiratory infections, e.g. virus infections, which are frequent in early life. These infections and their effect on this system might, therefore, be the initiating event of CF disease [31].

The resultant reduction in ASL volume is shared by the two layers: 1) the water content of the mucus layer is reduced, producing a highly viscoelastic adhesive material; and 2) the water content of the periciliary environment is depleted, causing the collapse of this layer and a loss of its
lubricant activity. The combination of the PCL and ASL defects causes the mucus to adhere to the airway [36]. Evidence of adhesion is available from early pathological studies of CF airways, which reveal bronchiolar mucous plugs within 48 hours of birth [37], and from radioparticle deposition studies that show the inability of the cough manoeuvre to clear mucus adhering to airway surfaces [38].

A recent hypothesis suggests that a defect in HCO$_3^-$ secretion plays a critical role in the pathophysiology of CF [39]. As pointed out above, the level of monovalent cations in ASL in CF patients is normal and constant, whereas it is the concentration of HCO$_3^-$ that is notably subnormal, because of reduced secretion due to the CFTR defect [40]. Several studies have shown a relatively acidic ASL [41] and an intrinsic acidification defect in fluid gland secretion in CF [42]. This reduced HCO$_3^-$ level is associated with increased mucus viscosity due to reduced Ca$^{2+}$ chelation, necessary for rapid mucin swelling and dispersion [21]. Importantly, the extent of these defects correlates with the level of HCO$_3^-$, which suggests a relationship between disease severity and the degree of impairment in HCO$_3^-$ secretion [43, 44].

One consequence of mucus stasis is the formation of thick mucous plaques and plugs, in which microorganisms are embedded. Several features of this thickened adherent CF mucus promote persistent biofilm growth [45]. First, the increased concentration of mucus limits bacterial motility, increases their binding to mucin epitopes and feeds them. Thus bacteria deposited in CF mucus may proliferate densely in the area of droplet deposition. Secondly, the concentrated mucin gel also limits the effectiveness of secondary defence mechanisms that might normally resolve a bacterial infection, such as neutrophil migration or diffusion of antimicrobial substances. Finally, cellular oxygen is consumed at high rates in CF airway epithelium to fuel this increased Na$^+$ transport, thereby creating hypoxic zones in adherent mucous plaques near the cell surface that link the special CF low-oxygen environment and infection [46]. Pseudomonas aeruginosa, specifically, adapts to the hypoxic zones by producing alginate and forming biofilm, thus setting the stage for chronic infection. The persistence of chronic bacterial infection of the airway

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**Figure 3.** Nasal potential difference (PD) trace showing the response to perfusion of various solutions in a) a healthy control and b) a cystic fibrosis (CF) patient. Baseline nasal PD (basal PD) is measured after perfusion of nasal epithelium with saline solution. Nasal PD changes (Δ) were recorded after perfusion with the following solutions: 100 μM amiloride in saline solution (Δamiloride), 100 μM amiloride in low-chloride solution (Δlow-chloride), and 100 μM amiloride plus 10 μM isoproterenol in low-chloride solution (Δisoproterenol). The sum of Δlow-chloride and Δisoproterenol (Δlow-chloride–Δisoproterenol) serves as an index of transepithelial cystic fibrosis transmembrane conductance regulator (CFTR)-dependent Cl$^-$ transport because it reflects the cyclic adenosine monophosphate (cAMP) activation of nasal mucosal Cl$^-$ permeability. In CF patients: 1) basal PD is more negative than in healthy controls because of increased Na$^+$ transport (high depolarisation following amiloride perfusion); and 2) there is no response following low-chloride perfusion and isoproterenol administration, showing the absence of Cl$^-$ permeability.
lumen then stimulates airway defences and induces a chronic hyperinflammatory response, mainly via the nuclear factor (NF)-κB-mediated pathway [47].

Taken together, these findings indicate that the combination of abnormal Na\(^+\) and Cl\(^-\) transport in CF leads to ASL volume regulation failure, mucus stasis, bacterial infection and inflammation. These, in turn, result in inhibition of mucociliary and cough clearance, and, as a final consequence, induction of bronchiectasis.

**Clinical description**

The diagnosis of CF is based on an abnormal sweat test result (sweat Cl\(^-\) level of >60 mM) and the finding of two CF-causing mutations in the CFTR and/or an abnormal PDEe [30]. In the latter case, the response to amiloride is increased because of lack of inhibition of Na\(^+\) resorption, and Cl\(^-\) secretion is absent in the presence of low-chloride solution and isoproterenol. CF clinical presentation can be divided into two types: 1) classic disease, readily diagnosed based on clinical and laboratory data; and 2) less-severe disease that manifests later in life and yields ambiguous genetic testing results [48].

In the first case, CF is a life-limiting multisystemic disorder that affects the Cl\(^-\) transport system in exocrine tissues. The hallmark is a classic triad of symptoms, most often from infancy or childhood: progressive obstructive lung disease with sputum infected by *Staphylococcus aureus* or *P. aeruginosa*, exocrine pancreatic insufficiency, and a high sweat Cl\(^-\) level. In males, this triad is associated with congenital absence of the vas deferens, leading to sterility. Other specific clinical phenotypes include CF-related liver disease, meconium ileus, CF-related diabetes, pansinusitis and nasal polyps. Mortality occurs mainly due to progression of lung disease and respiratory insufficiency [49]. In children, bronchiectasis is a marker of respiratory disease severity, because it is associated with increased morbidity and accelerated decline in pulmonary function [50]. It can appear as early as 3 months in CF children [38]. In a cohort of 125 Australian children (from birth to 6 years) diagnosed with CF after newborn screening, 22% showed evidence of bronchiectasis, and the prevalence increased with age [51]. In the paediatric (but not adult) population, the presence and severity of bronchiectasis is significantly related to respiratory infection with *P. aeruginosa* [52], and, more specifically, mucoid *P. aeruginosa* [53].

In the second case, advances in basic CF science have broadened the clinical spectrum of CF and highlighted less-severe, so-called CFTR-related, presentations. Most of these patients carry one CF-causing mutation and one or two mutations retaining residual CFTR function [54]. It is not clear whether CFTR-related bronchiectasis, in such cases, is a single-organ manifestation of CF or a condition in which CFTR mutations play the role of a modifier deleterious gene, acting with an environmental contribution.

Several studies [55–66] have investigated the frequency of CFTR mutations in patients with disseminated bronchiectasis (table 1). The prevalence of CFTR mutations in this population is controversial. Four studies [55–58] found no evidence of an increased prevalence of CFTR abnormalities compared with the general population. Other series [57–64] observed very few patients finally diagnosed with CF on the basis of carriage of two CF-causing mutations and/or elevated sweat Cl\(^-\) levels (approximately 7% of all of the patients enrolled in those studies). Most patients had at least one non-CF-causing mutation, including mutations classified as “associated with CFTR-related disorder” [54]. Some of these mutations were associated with normal or borderline sweat Cl\(^-\) levels (substitution of aspartic acid 1152 with histidine (Asp1152His or D1152H), cytosine to thymidine substitution 10 kb downstream of nucleotide 3849 (3849+10 kbC>T), 5T allele of polythymidine tract in intron 8 (IVS8-5T) and Arg117His). It should be pointed out that many of the sequence variations identified are not recognised as CFTR mutations, and still less as CF-disease-causing mutations, mainly because of the lack of established or substantiated knowledge of their pathogenic potential. In these cases, CFTR functional evaluation in epithelium might help in identifying patients with CFTR-related disease [28, 66]. A cohort of patients with bronchiectasis and a sweat Cl\(^-\) level of <60 mM were investigated [66];
15 patients carried two CFTR mutations and exhibited abnormal ion transport in the nasal mucosa (i.e. increased Na$^+$ transport and decreased Cl$^-$ secretion). They were finally diagnosed with a CFTR-related disorder. In the same series, 22 patients carried only one mutation but displayed abnormal ion transport in the nasal mucosa, intermediate between the normal and the CF range. This led to the hypothesis that an as yet unidentified other factor, genetic or environmental, may trigger the pathogenic role of a unique CFTR mutation. Among the possibilities, abnormalities in ion transporters other than CFTR should be considered.

### Other channelopathies

**The epithelial sodium channel**

There are two principal, and rare, human clinical disorders that occur due to ENaC mutations [67]. The first is Liddle’s syndrome, caused by gain-of-function mutations leading to enhanced Na$^+$ resorption in the renal tubule, and characterised by volume-expanded low-renin hypertension and apparently no respiratory disease [68]. The other is pseudohypoaldosteronism (PHA) type I, due to loss-of-function mutations [69]. In addition to kidney impairment, characterised by renal salt wasting, hyperkalaemia and metabolic acidosis, such children also show defective Na$^+$ transport in the sweat gland, which leads to elevated sweat Cl$^-$ and Na$^+$ concentrations. Moreover, children with PHA-I frequently exhibit respiratory tract diseases that involve increased mucociliary clearance and decreased mucus viscosity [69].

Recently, ENaCs have been shown to play a critical role in the physiology of mouse airways. Transgenic mice with airway-specific overexpression of the ENaC (β-subunit) develop CF-like lung disease with mucous obstruction and poor bacterial clearance. The airway surfaces of these mice absorb three times more Na$^+$, causing ASL volume depletion, increased mucus concentration, delayed mucus transport and increased mucus adhesion to airway surfaces [70]. These events cause spontaneous and severe lung disease that shares features with CF, including mucous obstruction, goblet cell metaplasia, neutrophilic inflammation and poor bacterial clearance. This outstanding proof-of-concept study demonstrates that increasing airway Na$^+$ absorption creates all of the conditions for the onset of bronchiectasis and initiates a CF-like lung disease [71]. Further support for this mechanism comes from the following two observations: 1) modulation of ENaC activity in CF patients may potentiate disease severity, as suggested by studies showing an enhanced response to amiloride solution in patients with poor respiratory function [72] or chronic *P. aeruginosa* colonisation [66]; and 2) Na$^+$ transport is significantly higher in bronchiectatic patients, even in those with no or only one CFTR mutation, compared with control subjects [66].

<table>
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<tr>
<td>GIRODON [61]</td>
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<td>BOMBIERI [62]</td>
<td>23</td>
<td>Healthy 33</td>
<td>0    11</td>
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<td>HUBERT [63]</td>
<td>601</td>
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<td>45 43</td>
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<td>55</td>
<td>Local historical cohort</td>
<td>0 14; IVS8-5T: 4</td>
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<td>3 8</td>
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<td>BIENVENU [66]</td>
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<td>Healthy 26; obligate heterozygotes 38; typical CF 92</td>
<td>15 22</td>
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COPD: chronic obstructive pulmonary disease; RD: respiratory disease; IVS8-5T: 5T allele of polythymidine tract in intron 8; CF: cystic fibrosis.
The role of ENaCs in non-CFTR-related bronchiectasis has been investigated in a few studies. Sheridan et al. [73] studied 20 patients with diffuse bronchiectasis and elevated sweat Cl⁻ concentrations but without two CFTR mutations and identified four patients with five missense mutations and one splicing mutation in ENaC genes. Moreover, among 55 patients with idiopathic bronchiectasis who did not have two mutations in the CFTR coding regions, 10 were identified with an ENaC mutation [74, 75]. This was higher than the expected frequency, and, as these variants had not been previously described, they are unlikely to be common polymorphisms. Moreover, six patients showed evidence of abnormal ion transport, in either sweat glands or nasal epithelium. Hence, although these variants were each found in a heterozygous state, they might be expected to result in abnormal ENaC function. This hypothesis is further supported by recent evidence of ENaC mutations leading to proved channel dysfunction and associated with atypical CF [76].

Other Cl⁻ channels

The model of ASL homeostasis suggests that dysfunction of other Cl⁻ channels may alter ASL homeostasis. ClC-2 mutations have been identified in people with idiopathic generalised seizures, but they are not associated with a history of lung disease [77]. Moreover, the ClC-2 knockout mouse undergoes normal lung development, possibly because it has multiple alternative Cl⁻ channel conductance pathways. A ClC-2 abnormality may, therefore, not be related to any human lung disease [15].

To date, no human disease has been linked to a defect in Ca²⁺-dependent Cl⁻ channels. However, mice that do not express TMEM16A, the best candidate for CaCCs, show greatly reduced mucociliary clearance [13]. Therefore, the role of this channel in human bronchiectasis requires further investigation.

Indirect inactivation of Cl⁻ transport

A defect in basal K⁺ channels may affect the driving force necessary for Cl⁻ to migrate to the apical membrane, as shown by the strong reduction in Cl⁻ transport in nasal, tracheal and bronchial cells carrying mutations of Kv7.1 and KCa3.1 [16, 78]. However, no lung disease has been reported among patients with these channelopathies [16].

Alternatively, defective interaction between an ion transporter and a mutated protein modulating its function may impair the channel function, as demonstrated for CFTR and SLC26A3. The interaction between these two proteins leads to their reciprocal functional activation [11]. When SLC26A3 displays a mutation identified in humans, i.e. responsible for congenital Cl⁻ diarrhoea, its interaction with the CFTR is altered, and CFTR activation suppressed [11, 79]. Therefore, mutations in proteins that interact with the CFTR, and specifically other SLC26T members, may affect the CFTR and induce a CF-like phenotype.

Bicarbonate

There is evidence that defective HCO₃⁻ secretion is associated with abnormal mucus hydration and impaired mucociliary clearance [20]. The amount of mucus discharged is significantly reduced when HCO₃⁻ secretion is impeded in the intestines [80] and uterine cervix [43]; a similar mechanism might be anticipated in airways. Extracellular acidification also favours inflammation, by inducing neutrophil activation [81] and delaying neutrophil apoptosis [82].

CF is clearly associated with a defect in ASL pH regulation. Defects in HCO₃⁻ transporters other than the CFTR can be envisioned, but require further investigation.

Transheterozygosity

After extensive genetic screening, 33–50% of patients with diffuse bronchiectasis are characterised as heterozygous for the CFTR [66]. As the theoretical frequency of this heterozygosity in the
general population is 3.3%, this highly elevated frequency suggests that heterozygosity for the CFTR may have pathogenic consequences. It may predispose to the development and severity of bronchiectasis by potentiating other genetic factors affecting airway physiology or add to deleterious environmental factors.

Further support for this hypothesis comes from evidence of an abnormal nasal electrophysiological phenotype in patients with bronchiectasis carrying one CFTR mutation, intermediate between control subjects or patients with no CFTR mutations, on the one hand, and patients with two CFTR mutations on the other [66]. However, the absence of any increased prevalence of bronchiectasis in obligate heterozygotes [83], although they display abnormal Cl− transport [84], suggests that carrying a single CFTR mutation is not solely responsible for development of the disease.

A total of 55 patients with diffuse idiopathic bronchiectasis were studied and an unexpectedly high proportion (5%) of heterozygosity was found for both CFTR and ENaC mutations [75]. As the expected frequency of such transheterozygosity in the general population is 0.3%, the finding of so high a prevalence of mutations of both ion transporters suggests that it is clinically relevant. Slight defects in both channels, which separately would not be sufficient to alter ASL homeostasis, are likely to combine their deleterious effects and lead to deficient ENaC/CFTR interaction. Along this line, we speculate that transheterozygosity of a single CFTR mutation and a mutation in another ion channel might create the conditions for abnormal ASL hydration regulation and defective mucociliary clearance.

**Conclusion**

It is likely that the true incidence of cases of ion-transport-related bronchiectasis among all bronchiectasis is underestimated, given the lack of specific symptoms. Although much is now known about the CFTR, the study of other channelopathies is only just beginning. Except for typical CF and CFTR-related syndrome, it is difficult to demonstrate a causal relationship between bronchiectasis and ion transport defects. The continuum of ion transport dysfunction from normal to disease phenotype makes it difficult to define a clear-cut level for the involvement of ion transport defect in the physiopathology of bronchiectasis [85]. Therefore, in order to ascertain the role of channelopathies in the genesis of bronchiectasis, mutations in a given channel and the related ion transport function should be systematically investigated in bronchiectatic patients. Such studies may point to interesting therapeutic pathways aimed at normalising the first cause of the pathogenic cascade resulting in bronchiectasis.

**Statement of interest**

None declared.

**References**


