Cilia Dysfunction in Lung Disease

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Abstract

A characteristic feature of the human airway epithelium is the presence of ciliated cells bearing motile cilia, specialized cell surface projections containing axonemes composed of microtubules and dynein arms, which provide ATP-driven motility. In the airways, cilia function in concert with airway mucus to mediate the critical function of mucociliary clearance, cleansing the airways of inhaled particles and pathogens. The prototypical disorder of respiratory cilia is primary ciliary dyskinesia, an inherited disorder that leads to impaired mucociliary clearance, to repeated chest infections, and to the progressive destruction of lung architecture. Numerous acquired lung diseases are also marked by abnormalities in both cilia structure and function. In this review we summarize current knowledge regarding airway ciliated cells and cilia, how they function to maintain a healthy epithelium, and how disorders of cilia structure and function contribute to inherited and acquired lung disease.

INTRODUCTION

The human airway—a dichotomous, hollow-tube, branching structure of up to 23 generations from trachea to the alveoli—is lined by a continuous layer of pseudostratified epithelium composed of approximately 10^{10} cells covering a surface area of 2,500 cm^2 (1). The airway epithelium is composed of four major cell types lining a continuous basement membrane, including ciliated, secretory, and undifferentiated intermediate and basal cells (2). The basal cells function as the stem/progenitor cells, responding to cell senescence and injury by generating intermediate undifferentiated cells, which then differentiate to ciliated and secretory cells in a ratio of 7–8 to 1 in both the large airways (0 to 5 generations) and the small airways ($\geq$6 generations).

The ciliated and secretory cells are the first line of defense against inhaled pathogens and particulates. Elements of this defense include tight junctions linking the cells, providing a physical barrier; receptors that sense the environment, signaling the cells to secrete defense-related molecules in response to inhaled pathogens, particulates, and xenobiotics; and, importantly, the mucociliary escalator, a layer of fluid and mucins that lines the epithelium and that cleans the airways by moving continuously from the lower respiratory tract cephalad, where it is insensibly swallowed (2–4). The effectiveness of the mucociliary escalator depends on hydration, on mucins produced by secretory cells, and on the coordinated function of the ciliated cells that provides the force and direction of the escalator (4). When there is dysfunction of the mucociliary escalator, the defenses of the epithelium are markedly weakened, resulting in lung disease.

This review focuses on the role of ciliated cells and cilia in the normal function of the airway epithelium and how abnormalities in the structure and function of airway cilia result in disease. We first summarize what is known about human airway ciliated cells and cilia, then provide an overview of the role of ciliated cells in the mucociliary escalator, and thereafter detail how airway cilia structure and function are assessed in humans. Such discussion provides the background to describe the current state of knowledge of the inherited and acquired disorders of airway cilia dysfunction.

AIRWAY CILIATED CELLS

The airway ciliated cell, the dominant cell type of the airway epithelium, has a columnar shape that tapers toward the surface, and rests on the basement membrane (**Figure 1*a*,*b***). The percentage of ciliated cells increases with airway branching, from 47 $\pm$ 2% in trachea to 73 $\pm$ 1% in the small airway epithelium (5). Ciliated cells are easily distinguishable from other cell types by the presence of cilia (~200 to 300 cilia per cell) on the luminal surface; cilia are 0.2 to 0.3 μm in diameter and range in length from 6 to 7 μm in the upper airways to 4 μm in the smaller airways (6). The apical surface of ciliated cells also contains numerous microvilli, which play a role in the transepithelial movement of fluid and electrolytes (6). Adjacent ciliated cells are connected via tight junctions, specialized multiprotein structures that regulate the passage of solutes and ions across the epithelial barrier and that separate the apical and basolateral epithelial compartments, and E-cadherin-based adherens junctions that provide firm cell-to-cell adhesion (3, 7). At the basolateral pole, ciliated cells connect to the airway epithelial basement membrane directly or through desmosome-mediated attachment to basal cells (8).

The major function of airway ciliated cells is to mediate propulsion of the mucus gel layer in a cephalad direction, thus maintaining the mucociliary escalator (**Figure 1*c***) (4, 9). The ciliated cells accomplish this by the highly coordinated in-plane beating of cilia across multiple cells, generating a wavelike movement across the epithelial surface (4). The forward stroke of the cilia is rapid and powerful such that it allows for penetration into the mucus layer and for propulsion of the mucus gel layer in a cephalad direction (4).

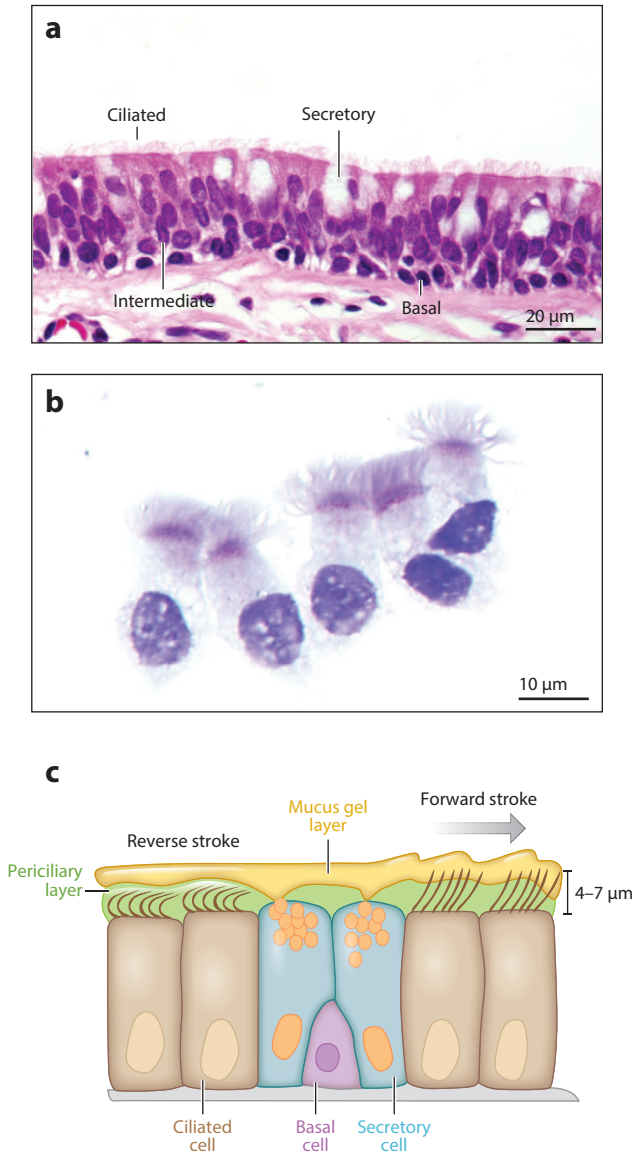

Figure 1

The human airway epithelium. (*a*) Histology of the large airway epithelium (the fourth to fifth generation of bronchi) from a healthy nonsmoker. Shown are ciliated cells, secretory cells, intermediate undifferentiated cells, and basal cells. Cells are visualized by hematoxylin and eosin. (*b*) Ciliated cells isolated from the human small airway epithelium (tenth to twelfth generation of bronchi) obtained by bronchoscopic brushings from a normal, healthy individual. Cells are visualized by Diff-Quik. (*c*) Role of cilia in airway mucociliary clearance. The mucociliary escalator is composed of the mucus gel layer, the periciliary layer, and ciliated cells. The gel-forming mucins produced by mucous secretory (goblet) cells are major constituents of the mucus gel layer, which entraps microorganisms and other inhaled particles and transports them out of the lung through cilia beating. The membrane-bound mucins form a brush-like pericellular niche around the cilia that controls the distribution of water between the two layers. During the power forward stroke, the cilia tips extend upward into the mucus gel layer, propelling the mucus forward. During the slow return stroke, the cilia recede and are contained completely in the periciliary layer. Normal cilia length (4–7 µm, depending on the airway region) is critical for effective mucociliary clearance.

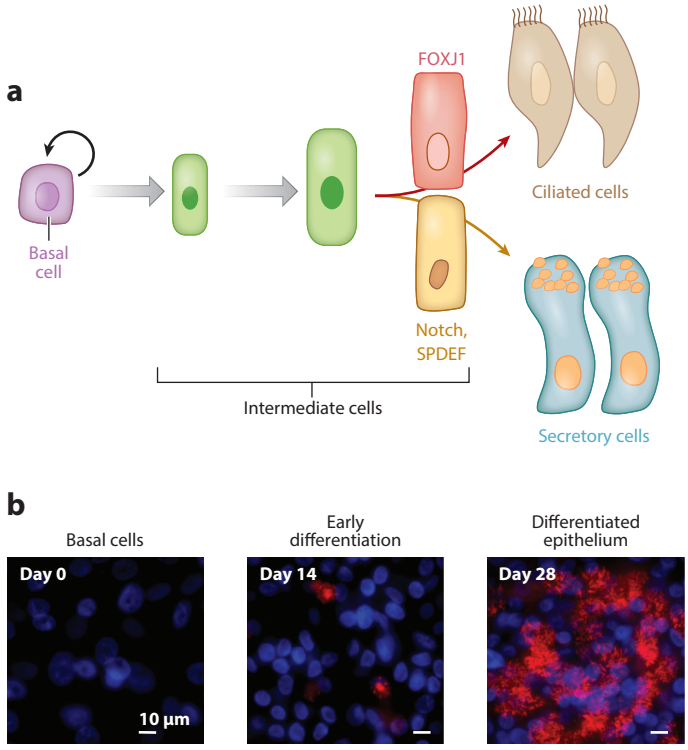

Figure 2

Ciliated cell differentiation in the human airway epithelium. (*a*) Differentiation pathways. The basal stem/progenitor cells are capable of self-renewal and are responsible for the generation of intermediate progenies, which, upon distinct activation programs, differentiate into ciliated or secretory cells. Ciliated cell differentiation depends on the transcription factor forkhead box J1 (FOXJ1), whereas the generation of secretory cells requires Notch signaling and/or activation of SPDEF (SAM pointed domain–containing ETS transcription factor). (*b*) Differentiation of human airway basal cells (day 0) into ciliated airway epithelium (day 28) in air-liquid interface culture. Appearance of ciliated cells is demonstrated by expression of β-tubulin IV (*red*; visualized by immunofluorescence). Cell nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI) (*blue*).

Generation of Airway Ciliated Cells

Airway ciliated cells are terminally differentiated cells incapable of self-renewal (10). The ciliated cells turn over slowly; turnover time is estimated at 1–4 months in humans (11). They are replenished by the basal cells, which function as stem/progenitor cells for airway epithelial ciliated and secretory cells (**Figure 2*a***) (12). This process is accelerated in response to injury, to which ciliated cells are sensitive (11). In human subjects who underwent mechanical injury of large airways with a cytology brush, the ciliated cell population was replenished by 14 days after injury (13). Exposure of basal cell stem/progenitor cells to the luminal air is critical for basal cell differentiation toward the ciliated cell lineage (14). This differentiation process can be modeled in vitro; when purified human airway basal cells are cultured on type IV basement membrane collagen with the apical surface exposed to air, the basal cells differentiate to a mucociliated epithelium (**Figure 2*b***).

Basal cell–derived progenitors mature into fully differentiated ciliated cells as a result of activation of the transcription factor forkhead box J1 (FOXJ1), with contribution from the regulatory factor X (RFX) family (15–17). FOXJ1 specifically supports formation of ciliated cells, whereas

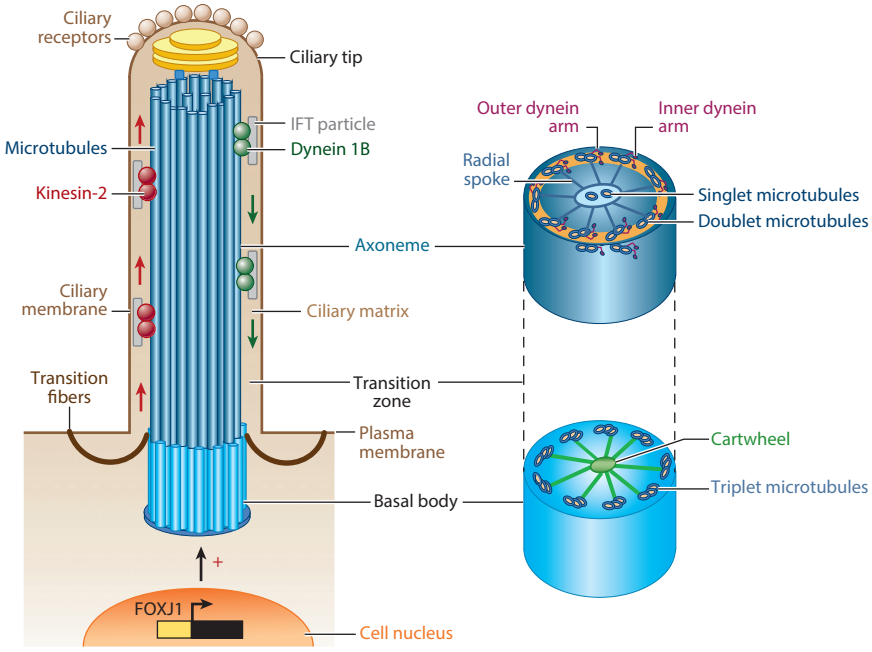

Figure 3

Structure and maintenance of human airway cilia. Each airway cilium is composed of a 9 + 2 axoneme with nine doublet microtubules and a central pair of microtubule singlets that are surrounded by a specialized cilia membrane. The cilia membrane is contiguous with, but distinct from, the plasma membrane and harbors a number of receptors that are critical for sensing environmental signals. Cilia formation is initiated and coordinated by a distinct gene expression program, primarily by activation of the transcription factor forkhead box J1 (FOXJ1). Cilia assembly begins with formation of the basal body from the centrosome, which migrates to and docks on the cell surface. The basal body is a specialized centriole with a 9 + 3 microtubule structure and a cartwheel embedded in pericentriolar material and anchored to the plasma membrane by transition fibers. Axonemal microtubule doublets arise from the inner two microtubules of the basal body microtubule triplets, extend from the basal body, and form the cilia membrane by pushing out an extension of the plasma membrane. Axonemal microtubules are elongated distally via intraflagellar transport (IFT) of proteins, which are synthesized in the cell and moved as IFT particles by kinesin-2 motors from the basal body to the cilia tip (anterograde IFT, *left side*) and by the cytoplasmic dynein motors back to the basal body (retrograde IFT, *right side*). The cilia tip contains the microtubule plus ends, from which the axonemes grow, and a number of signaling components responsible for the sensory function of cilia. The force needed for cilia beating is produced by the outer and inner dynein arms of the axonemal microtubule doublets that are connected to the central pair of microtubules by radial spokes.

Notch signaling and activation of SPDEF (SAM pointed domain–containing ETS transcription factor) are critical for secretory cell differentiation (18, 19).

Airway Cilia Structure

Cilia are evolutionarily conserved, hairlike, cellular organelles that project from the cell surface (20). The basic structure consists of a centriole-derived microtubule core termed the axoneme that protrudes continuously from the plasma membrane (**Figure 3**) (21). Cilia are subdivided into two classes, immotile or motile, on the basis of the physical ultrastructural characteristics of the axoneme (21–23). Airway cilia are in the motile cilia class, as are cilia found on ciliated cells of

sinuses, brain ventricle ependyma, oviducts, and epididymal ducts (24–26). Immotile cilia (referred to as primary cilia) are solitary structures on most cell types, where they sense the environment (20, 24, 27).

Supplemental Material

Airway cilia have components typical of motile cilia (see **Figure 3** for an overview and **Supplemental Text** for further details). Proteomic analysis of cilia isolated from in vitro–generated human airway epithelial cells on air-liquid interface culture identified >200 axonemal proteins (28). Some, such as α and β tubulin, are conserved with axonemal proteins identified in other motile cilia. However, some—including the sperm- or testis-associated proteins SPA17 and SPAG6 and retinitis pigmentosa protein 1, which associates with photoreceptor axonemal structures—are unique (28). Characterization of the transcriptome of murine tracheal epithelial cells identified similarities among components of motile and primary cilia (29), and many genes identified as part of the mouse ciliated cell transcriptome correspond to human airway cilia proteins, suggesting conservation across species.

Cilia Growth and Maintenance

Ciliogenesis is a complex, multistage process coupled to the cell cycle. Cilia cannot synthesize protein, and all proteins required for ciliogenesis and cilia maintenance are moved into and out of cilia by intraflagellar transport. Cilia length, a process affected by smoking, is regulated by multiple genes (see **Figure 3** and **Table 1** for an overview and **Supplemental Text** for details).

Transcriptional Control of Cilia Genes

It is well documented that FOXJ1 is central to airway epithelial ciliogenesis, with the RFX family transcription factors contributing. However, the transcriptional control of both ciliogenesis and the maintenance of human airway cilia is not well understood and likely involves a complex interaction of several transcription factors (see **Supplemental Text** for further details).

Cilia Function

In the healthy human lung, cilia beat at 12 to 15 Hz in coordinated waves of metachronal motion that propel mucus cephalad at 4 to 20 mm/min (30). Cilia tips contact the mucus layer only on the forward stroke; on the reverse stroke, a bend in the cilia shaft causes the tip to pass underneath the mucus layer such that the mucus is propelled only in the forward direction on the forward stroke (**Figure 1*c***) (31, 32). The contents are propelled through the vocal cords into the pharynx, where an estimated 30 mL of respiratory mucus are expectorated or swallowed daily (30).

Multiple signaling molecules, including cAMP, Ca^{2+}, nitric oxide, and progesterone, regulate airway cilia beat frequency (33–36). In an important observation, Welsh and coworkers (37) identified sensory bitter taste receptors (T2R) on cilia of differentiated human airway epithelium, providing a mechanism by which airway cilia sense the environment. Several other receptors responsible for sensing environmental signals have been identified in cilia. Ciliated cells also express structures that maintain normal periciliary fluid osmolarity, including the epithelial sodium channel (ENaC) and the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) (38, 39; see **Supplemental Text** for further details).

Cilia Function in the Mucociliary Escalator

Mucociliary clearance requires highly coordinated synchronized beating of cilia across multiple ciliated cells. Under normal conditions, the cilia machinery functions in two modes to generate

Table 1 Genes encoding major components of airway motile cilia[a]

Gene name[b]	Gene symbol	Association with primary cilia dyskinesia (PCD)[c]	Smoking effect[d]
Axoneme (outer dynein arm) genes			
Dynein, axonemal, heavy chain 5	*DNAH5*	+	↓
Dynein, axonemal, heavy chain 9	*DNAH9*	+	↓
Dynein, axonemal, heavy chain 11	*DNAH11*	+	↓
Dynein, axonemal, intermediate chain 1	*DNAI1*	+	
Dynein, axonemal, intermediate chain 2	*DNAI2*	+	
Dynein, axonemal, light chain 1	*DNAL1*	+	
Axoneme (inner dynein arm) genes			
Dynein, axonemal, heavy chain 3	*DNAH3*		
Dynein, axonemal, heavy chain 6	*DNAH6*		
Dynein, axonemal, heavy chain 12	*DNAH12*		
Dynein, axonemal, light intermediate chain 1	*DNALI1*		↓
WD repeat domain 63	*WDR63*		
WD repeat domain 78	*WDR78*		
Dynein assembly and docking genes			
Dynein, axonemal, assembly factor 1	*DNAAF1*	+	
Dynein, axonemal, assembly factor 2	*DNAAF2*		
Dynein, axonemal, assembly factor 3	*DNAAF3*	+	
Dyslexia susceptibility 1 candidate 1	*DYX1C1*	+	
Armadillo repeat containing 4	*ARMC4*	+	
Coiled-coil domain containing 39	*CCDC39*	+	
Coiled-coil domain containing 40	*CCDC40*	+	
Coiled-coil domain containing 114	*CCDC114*	+	
Dynein regulatory complex subunit 1 homolog	*DRC1*	+	
Leucine rich repeat containing 6	*LRRC6*	+	
HEAT repeat containing 2	*HEATR2*	+	
Zinc finger, MYND-type containing 10	*ZMYND10*	+	
Central pair genes			
HYDIN, axonemal central pair apparatus protein	*HYDIN*	+	
Sperm-associated antigen 6	*SPAG6*		↓
Sperm-associated antigen 16	*SPAG16*		
Sperm-associated antigen 17	*SPAG17*	+	
Sperm-associated antigen 1	*SPAG1*	+	
Primary ciliary dyskinesia protein 1	*PCDP1*		
Radial spoke genes			
Radial spoke head 1 homolog	*RSPH1*	+	
Radial spoke head 3 homolog	*RSPH3*		
Radial spoke head 4a homolog	*RSPH4A*	+	
Radial spoke head 9 homolog	*RSPH9*	+	

(*Continued*)

Table 1 (*Continued*)

Gene name[b]	Gene symbol	Association with primary cilia dyskinesia (PCD)[c]	Smoking effect[d]
Tubulins and other microtubule-associated genes			
Tubulin, alpha 1A	*TUB1A1*		
Tubulin, alpha 3	*TUBA3*		
Tubulin, beta 2C	*TUBB2C*		
Tubulin, beta 4	*TUBB4*		
Tektin 1	*TEKT1*		↓
NME/NM23 family member 8	*NME8*	+	
Anterograde intraflagellar transport (IFT) genes			
Kinesin family member 3A	*KIF3A*		
Kinesin family member 3B	*KIF3B*		
IFT protein 20 homolog	*IFT20*		
IFT protein 46 homolog	*IFT46*		
IFT protein 52 homolog	*IFT52*		
IFT protein 57 homolog	*IFT57*		↓
IFT protein 80 homolog	*IFT80*		↓
IFT protein 81 homolog	*IFT81*		
IFT protein 88 homolog	*IFT88*		
IFT protein 172 homolog	*IFT172*		↓
Clusterin-associated protein 1	*CLUAP1*		↓
RAB, member RAS oncogene family-like 5	*RABL5*		
TNF receptor–associated factor 3–interacting protein 1	*TRAF3IP1*		↓
Tetratricopeptide repeat domain 26	*TTC26*		↓
Tetratricopeptide repeat domain 30B	*TTC30B*		
Retrograde IFT genes			
Dynein, cytoplasmic 2, heavy chain 1	*DYNC2H1*		↓
Dynein, cytoplasmic 2, light intermediate chain 1	*DYNC2LI1*		
IFT protein 122 homolog	*IFT122*		
IFT protein 140 homolog	*IFT140*		
Tetratricopeptide repeat domain 21B	*TTC21B*		
WD repeat domain 19	*WDR19*		↓
WD repeat domain 35	*WDR35*		↓
Basal body and BBSome[e] genes			
Bardet-Biedl syndrome 1	*BBS1*		
Bardet-Biedl syndrome 2	*BBS2*		
Bardet-Biedl syndrome 4	*BBS4*		
Bardet-Biedl syndrome 5	*BBS5*		↓
Bardet-Biedl syndrome 6	*BBS6*		
Bardet-Biedl syndrome 7	*BBS7*		
Bardet-Biedl syndrome 8	*BBS8*		
Bardet-Biedl syndrome 9	*BBS9*		↓
BBSome-interacting protein 1	*BBIP1*		↓

(*Continued*)

Table 1 (*Continued*)

Gene name[b]	Gene symbol	Association with primary cilia dyskinesia (PCD)[c]	Smoking effect[d]
Oral-facial-digital syndrome 1	*OFD1*	+	↓
Outer dense fiber of sperm tails 2	*ODF2*		↓
Ezrin	*EZR*		↓
Transition zone genes			
Nephronophthisis 1 (juvenile)	*NPHP1*		↓
Retinitis pigmentosa GTPase regulator	*RPGR*	+	
Transmembrane protein 67	*TMEM67*		↓
Genes encoding receptors, ion channels, and signaling molecules			
Taste receptor, type 2, member 4	*TAS2R4*		
Taste receptor, type 2, member 38	*TAS2R38*		
Taste receptor, type 2, member 43	*TAS2R43*		
Taste receptor, type 2, member 46	*TAS2R46*		
Sodium channel, non-voltage-gated 1 alpha subunit	*SCNN1A*		
Nitric oxide synthase 3 (endothelial cell)	*NOS3*	+	
Protein kinase, cGMP dependent, type I	*PRKG1*		
A kinase (PRKA) anchor protein 14	*AKAP14*		
Progesterone receptor	*PGR*		
Serum response factor	*SRF*		
Transcription factors: inducers of airway cilia formation			
Forkhead box J1	*FOXJ1*		
Regulatory factor X, 3	*RFX3*		
v-myb avian myeloblastosis viral oncogene	*MYB*		
Multiciliate differentiation– and DNA synthesis–associated cell cycle protein	*MCIDAS*		

[a]For detailed references regarding individual genes associated with PCD and genes downregulated by smoking, see **Supplemental Table 1**.
[b]Genes that encode the major structural components of airway motile cilia and/or that are relevant to airway ciliogenesis and regulation of ciliated cell function according to the available literature are included.
[c]Genes known to cause PCD or associated with PCD pathogenesis according to the available literature are indicated by a plus sign (+). A blank cell denotes no known association between the gene and PCD.
[d]Effect of cigarette smoking on the expression of individual genes related to airway motile cilia structure and/or function according to the available literature is shown. A down arrow (↓) denotes downregulation. A blank cell denotes no known effect of smoking on the expression of the gene.
[e]The BBSome is a complex of Bardet-Biedl syndrome (BBS) proteins.

two different rates (slow and high) of beat frequency. The slow beat frequency is modulated by the inherent dynein ATPase activity of the axoneme, whereas the high beat frequency involves increasing dynein ATPase activity in response to specific signaling molecules (35, 36, 40). The mechanisms controlling the switch from a slow to a high rate of frequency are not fully elucidated, but extensive posttranslational modifications, including phosphorylation and dephosphorylation of axonemal components, play a role (35). In addition to cAMP, Ca^{2+}, and nitric oxide, mechanical forces (e.g., shear stress) can regulate cilia beat frequency by modulating the levels of apical ATP release and subsequent influx of Ca^{2+} into cells (41, 42). The mechanisms regulating synchronization of the beat across multiple cilia are less understood, and no common control mechanism has been identified (35, 36, 40).

Supplemental Material

Most human in vivo studies of cilia beat frequency have been limited to the nasal epithelium. These studies have demonstrated that cilia beat frequency is affected by age, exercise, and environmental stimuli such as cigarette smoke (43–46). Cilia beat frequency and mucociliary clearance slow with aging (43, 44, 47).

METHODS OF EVALUATING CILIA STRUCTURE AND FUNCTION

Several methods can be used to evaluate cilia structure and function in humans. Samples of airway ciliated cells for study can be obtained postmortem or from living subjects directly from the lung via bronchoscopic brushing or biopsy or using nasal samples as a proxy (48). Light microscopy can be used to assess cilia length (49, 50) and phase contrast microscopy to examine cilia beating (51). Transmission electron microscopy is used to evaluate cilia ultrastructure for features including orientation of the central microtubule pair, number and location of dynein arms, and orientation of peripheral tubules (51). Electron tomography relies on transmission electron microscopy images for 3D visualization of structures (52). The 3D structure of the respiratory epithelium can also be assessed by scanning electron microscopy for the presence and number of cilia per cell and for cilia orientation (51).

Cilia beat frequency can be assessed by cine photography (32, 53) or photoelectric methods (54, 55) or by the measurement of light reflected by the moving cilia via microphotometry (56). Methods using optical coherence tomography to measure cilia activity have been described; optical coherence tomography allows for visualization of cilia movement (57). Optical flow analysis has also been used to evaluate cilia motion in cultured human cells (58).

Integrated assessment of mucociliary clearance can be evaluated by placing a saccharine tablet or solution behind the inferior turbinate and measuring the time until a sweet taste is perceived by the subject (59). This may also be done in conjunction with the placement of a drop of blue indigo-carmine solution, allowing for measurement of the time until a blue line can be observed on the posterior pharyngeal wall (59). Other methods involve measuring the clearance of labeled particles from the airways using scintigraphy with technetium-labeled albumin minimicrospheres or macroaggregates (60, 61); aerosolized Fe_2SO_3 particles containing technetium (62); or Teflon particles tagged with technetium, indium, or gold (63, 64). The movement of unlabeled Teflon particles can be visualized by using fiber-optic bronchoscopy videos (65).

INHERITED DISORDERS OF CILIA DYSFUNCTION

The most important inherited disorders of airway cilia dysfunction are primary ciliary dyskinesia (PCD) and CF. Other inherited cilia-related disorders are very rare.

Primary Ciliary Dyskinesia

The classic disorder of respiratory cilia dysfunction is PCD, an autosomal recessive disorder of motile cilia (**Table 1**) (25). The symptoms and signs of this disorder were recognized long before the mechanism was understood (66). It was first referred to as Kartagener syndrome, the triad of chronic sinusitis, bronchiectasis, and situs inversus (25, 66, 67). Subsequently, male infertility was noted to be associated with Kartagener syndrome, and dynein arm defects were observed in both the spermatozoa and respiratory epithelial cells, leading to the syndrome being named immotile cilia syndrome (68). The disorder was renamed PCD when it was recognized that a subgroup of patients with cilia motility but ineffective mucociliary clearance manifest the same clinical syndrome (67, 69). The clinical manifestations of PCD include chronic otitis media, transient

hearing loss/speech delays, nasal congestion, chronic sinusitis, recurrent lower respiratory tract infection, bronchiectasis, male infertility, defects in organ laterality (in 50% of cases), and (in newborns) neonatal respiratory disorders.

Organ laterality in embryogenesis is determined by the normal rotary motion of a single specialized cilium found on each of the cells in the ventral node, which defines right-left symmetry in the developing embryo (25). Without normal directional motion of this specialized cilium, organ placement is random, which is why situs inversus is found in approximately 50% of individuals with PCD.

Cilia structural defects. A variety of cilia structural defects observed by electron microscopy are associated with the PCD clinical phenotype. The most common are (*a*) absent or short outer dynein arms and (*b*) outer dynein arm and inner dynein arm defects (25). Isolated inner dynein arm defects are uncommon and, when causative, are typically associated with abnormalities in the central apparatus of the cilia, such as microtubule disorganization or abnormally placed outer doublets (25).

PCD is also associated with reduced cilia beat frequency, which is most prominent in patients with dynein arm defects (70). Cilia wave form is dyskinetic in PCD, further contributing to the lack of effective mucociliary transport (70). Airway particle clearance is prolonged to 1 week in PCD patients; in contrast, it occurs over 12 h in normal nonsmokers (71).

Genetic basis of primary ciliary dyskinesia. The genetic basis can be identified in approximately 65% of PCD patients (**Table 1**) (25). Many mutations lead to defects in all cilia, but other mutations produce structural abnormalities in only a fraction of cilia or result in no ultrastructural defects. A commercial genetic test for 60 mutant alleles of *DNAI1* and *DNAH5* is available (72). Most (85% of) mutations implicated in PCD are loss-of-function mutations (25). The majority are rare variants found only in a single family or patient (25).

The typical causative genes for PCD encode cilia components, with mutations in specific genes having predictable effects on cilia ultrastructure. Mutations in genes coding for protein components of the outer or the inner dynein arm lead to defects in those structures, and mutations in genes encoding cytoplasmic proteins that participate in cilia assembly lead to defects in both the outer and inner dynein arms. Because the dynein arms are ATP-dependent motors for cilia movement, defects in these structures result in ciliary dyskinesia. Mutations in genes coding for components of the central pair microtubules and radial spokes lead to defects in those structures, which can lead to abnormalities in both cilia beat frequency and beat coordination (73).

Because the specialized cilium present in the ventral node (which, as discussed above, controls organ laterality) has no central complex, mutations leading to central complex defects do not produce laterality abnormalities. A mutation in cyclin O (*CCNO*) causes defective mucociliary clearance and bronchiectasis (74). *CCNO* mutant cells are defective in centriole generation and placement, and affected individuals have reduced numbers of airway cilia. The cilia that are present contain axonemal proteins, and affected individuals do not exhibit laterality defects.

Clinical manifestations. Diagnosis is challenging due to heterogeneity in clinical symptoms and severity and cilia ultrastructural abnormalities identified by electron microscopy, and due to variability in the phenotype associated with the causative mutations (67). Typical initial screening tests include nasal epithelial assessment of cilia motion and mucociliary transport using a saccharine taste test or radioisotope clearance (67, 75). The diagnosis of PCD is conventionally confirmed by identification of cilia ultrastructural defects through electron microscopy. However, 30% of patients exhibit normal ultrastructure (25). Other diagnostic modalities include assessment of

nasal cilia beat frequency and motility and measurements of nasal nitric oxide, which is low in PCD (25). Fluorescently labeled antibodies may be used to evaluate for the absence of specific cilia proteins (25). Caution must be taken to carry out functional and structural studies when the affected individual has had no recent infection to avoid false-positive findings due to secondary ciliary dyskinesia (67).

Lung disease in PCD results from defective mucociliary clearance and worsens over time due to repeated respiratory tract infections (25). Nearly all affected adults develop bronchiectasis. Lung function testing generally reveals an obstructive ventilatory defect with or without air trapping; mixed obstructive and restrictive patterns are also observed (67). Management centers on airway clearance therapies, use of antibiotics for lung infections, routine immunizations, and avoidance of tobacco smoke exposure (25). Surgical resection of severe localized disease is rarely indicated, and lung transplant is an option for end-stage disease (67).

Cystic Fibrosis

CF is an inherited disorder due to mutations in the CFTR gene. The disorder affects predominantly Caucasians, with an incidence of 1 in 2,500 newborns of Northern European descent (76). Nearly 2,000 CFTR mutations have been identified (76, 77). Although affected infants are born with seemingly normal lungs, chronic lung disease develops as a result of abnormal mucociliary clearance, leading to repeated infections and bronchiectasis (78).

The *CFTR* gene encodes a cAMP-regulated Cl^- channel expressed apically in epithelial cells (76). The channel modulates Cl^- secretion and regulates other membrane proteins, including ENaC (76). Because both CFTR and ENaC control water movement through the epithelium, CFTR dysfunction leads to increased fluid absorption, dehydration of the epithelial surface, and altered mucin concentration in abnormal airway mucus (76, 79). A gel-on-brush model of the periciliary layer postulates that airway mucus sits atop a dense brush of tethered macromolecules in the periciliary layer that prevent mucus from penetrating the periciliary space (9). In this model, if the airway surface is sufficiently dehydrated, as in CF, the mucus layer compresses the periciliary brush and cilia, interfering with mucus clearance. Alterations in CFTR function may also contribute to lung disease via abnormal modulation of epithelial inflammation and altered bicarbonate transport (76).

The abnormal mucociliary clearance in individuals with CF results from the abnormal biophysical properties of airway mucus, not primarily from ciliopathy (76, 80). Experimental animal data suggest that the most important factor controlling mucus clearance efficiency is airway surface hydration (76). In CF, the increased absorption of Na^+ and reduced secretion of Cl^- lead to reduced water content in both the mucus layer and the periciliary layer (79). This development leads to a mucus layer that is highly adhesive (79, 80). The failure to clear these abnormal secretions is evident shortly after birth, with bronchiolar mucus plugs detected within 48 h of birth in CF neonates (81). In addition to the abnormal hydration status, mucus hypersecretion eventually occurs in response to recurrent infection and persistent inflammation and further worsens the physical properties and the clearance of mucus (79). CF epithelial cells alone, without submucosal glands, generate abnormal airway surface liquid, which may explain the presence of CF lung disease even in distal airways that lack submucosal glands (82).

Electron microscopy of airway cilia from patients with CF shows alterations similar to those seen in a control group of individuals with chronic bronchitis, including compound cilia, excess cytoplasmic matrix, and an abnormal number or arrangement of microtubule doublets (83). With progressive disease, the airways develop squamous metaplasia and dysplasia, regions of missing cilia, cilia with missing inner dynein arms, abnormal numbers and location of microtubule doublets,

compound cilia, single microtubules in place of normal doublets, multiple cilia occupying the central area, and detachment of the axonemal membrane from the groups of cytoplasmic filaments (84).

CF was historically considered a fatal childhood disease, but with improved therapies, including antibiotics and airway clearance treatments, the average life expectancy is now 37 years (76). Because of the abnormal mucus characteristics underlying the disease, treatments to reduce mucus adhesivity are efficacious in CF (80). In part, the sticky nature of CF mucus results from high concentrations of DNA derived from neutrophils recruited in response to chronic infection (85). The use of aerosol recombinant DNase as an effective therapy in CF is directed toward reducing the adhesive properties of the mucus layer (85). In the subset of CF caused by genotype G551D, a missense mutation that is found in 4–5% of CF patients and that affects the function of CFTR channels at the cell surface (86), treatment with a CFTR potentiator, ivacaftor, leads to sustained improvements in lung function, weight, and sweat Cl^- concentration (a measure of CFTR activity) and to decreased exacerbations and chest symptoms. Ivacaftor works by increasing the amount of time that activated CFTR channels at the cell surface remain open and by increasing the Cl^- transport activity of the G551D-CFTR protein (86).

Other Inherited Disorders

Individuals with α1-antitrypsin deficiency with pure emphysema have normal mucociliary clearance (63, 87), although α1-antitrypsin deficiency is associated with bronchiectasis (88). In Usher syndrome—a rare autosomal recessive disorder characterized by sensorineural deafness, vestibular dysfunction, and retinitis pigmentosa with progressive visual loss—case reports have noted bronchiectasis with impaired mucociliary clearance but have noted no ultrastructural cilia abnormalities (89).

Disorders of primary cilia may also have lung manifestations. Jeune syndrome (asphyxiating thoracic dystrophy) is an autosomal recessive disease with skeletal abnormalities as well as variable hepatic, pancreatic, and retinal manifestations (90, 91). Respiratory failure is the most common cause of death due to restrictive disease caused by chest wall abnormalities. Although the genetics are incompletely understood, mutations in both *DYNC1H1*, a dynein heavy chain gene, and the gene encoding IFT80, which is involved in intraflagellar transport, have been linked to Jeune syndrome (90, 91). Roifman syndrome, a rare complex of bone dysplasia, growth retardation, retinal dystrophy, and humoral immunodeficiency associated with recurrent respiratory infections, has been suggested as a possible ciliopathy of primary cilia (92).

ACQUIRED DISORDERS OF AIRWAY CILIA

Abnormalities of mucociliary clearance, and consequent reduced host defenses of the lung, are a common theme in many acquired lung disorders. In many of these disorders, the airway cilia demonstrate acquired structural and/or functional abnormalities with associated abnormalities in mucociliary clearance.

Cigarette Smoking

Smoking has long been recognized to suppress mucociliary clearance in most smokers, and there is documented slowing of mucociliary clearance immediately after smoking cigarettes (93, 94). Individuals with bronchitis have reduced mucociliary clearance (95). Smoking cessation improves measurements of nasal mucociliary clearance compared with baseline values obtained prior to smoking cessation (96).

Examination of the airway ciliated cells of both male and female smokers shows patches of atypical nuclei and missing cilia (97). These abnormalities increase with increasing intensity of smoking behavior and are more frequent in smokers using high-tar/nicotine cigarettes (98). Smoking induces expression of epidermal growth factor in ciliated cells, which may shift basal cell fate toward a squamous phenotype and suppress ciliated cell differentiation (99). Healthy smokers have shorter cilia in the large and small airways compared with cilia of nonsmokers, with further shortening observed in smokers with chronic obstructive pulmonary disease (COPD), a common, progressive disorder of the lung characterized by airflow limitation in response to inhaled particles or gases, including cigarette smoke (49, 50). Smoking is associated with suppression of a number of genes in the airway epithelium, likely contributing to slowing the process of regenerating cilia (**Table 1**).

Electron microscopic assessment of cilia ultrastructure demonstrates that smokers with chronic bronchitis have greater numbers of cilia abnormalities than nonsmokers do; such abnormalities include compound cilia and giant cilia and other abnormalities in the microtubules and the axonemal 9 + 2 pattern of organization and in cilia orientation (100–102). These abnormalities may persist after smoking cessation (103).

Data on the effect of smoking on cilia beat frequency are conflicting. Exposure to cigarette smoke extract (104) or direct cigarette smoke (105) leads to reduced cilia beat frequency in in vitro models of the human airway epithelium. In contrast, nasal epithelium grown in culture demonstrated increased cilia beat frequency in samples from smokers or those with passive smoke exposure compared with cilia beat frequency in samples from nonsmokers (106). In one study utilizing freshly obtained nasal samples, there was no difference in cilia beat frequency in smokers, nonsmokers, or nonsmokers who acutely smoked two cigarettes (107). No difference was found in cilia beat frequency of resected tissue from cancer surgery in smokers versus nonsmokers (108) or in cilia beat frequency of samples obtained by biopsy (109). A study including smokers with lung disease found that cilia beat frequency was unaltered in healthy smokers, although decreased in smokers with moderate to severe COPD (46). However, a contradictory study found higher cilia beat frequency in nasal biopsies from both active and passive smokers than in nonsmokers (110).

Exhaled nitric oxide is lower in smokers than in nonsmokers, and smoking cessation is associated with an increase in exhaled nitric oxide; given that nitric oxide is important for normal cilia beating, this relationship may be one mechanism for the effect of cigarette smoking on cilia motion (111). In vitro, cigarette smoke upregulates airway epithelial expression of interleukin (IL)-8, which does not directly decrease cilia beat frequency but does abrogate the increase in cilia beat frequency induced by β-agonists (112).

Passive smoke exposure may also be associated with cilia abnormalities. Among children who were undergoing sinus surgery, those who were exposed to environmental tobacco smoke had reduced regrowth of cilia following the procedure compared with children without passive smoke exposure (113). Examination of nasal mucosa in children passively exposed to smoke showed both patchy and generalized loss of cilia on a background of other epithelial abnormalities (114). Adenoid explants from children with exposure to secondhand smoke showed a blunted cilia beat frequency response to cilia stimulants ex vivo (115). This finding is consistent with data showing an increased risk of respiratory disease in children exposed to passive cigarette smoke (113). Adults exposed to passive smoking have reduced nasal mucociliary clearance compared with clearance in nonsmokers, although the reduction in clearance is less than that in active smokers (116).

Use of Alternative Tobacco Products and Illicit Drugs

Limited data exist on cilia abnormalities in users of alternative tobacco products and illicit drugs. Users of marijuana more frequently have regions of cilia loss and goblet cell hyperplasia, which may

correlate with increased mucus secretion and altered mucociliary clearance, than do nonsmokers (117, 118). Cocaine use is also associated with focal loss of airway cilia (117). The effects of alternative tobacco product use, including shisha (waterpipe, hookah) or electronic cigarettes, on cilia structure or function are not known.

Environmental Pollutants

Several studies link environmental exposure to airborne pollutants to cilia abnormalities and dysfunction. Nasal biopsies from Mexico City residents exposed to high levels of air pollution showed patches of short cilia and regions of cilia loss (119). Healthy nonsmokers exposed to ozone at 0.4 ppm or sulfur dioxide at 0.75 ppm showed no abnormalities in cilia structure assessed in nasal samples, although experimental animals exposed to substantially higher concentrations of ozone (4 ppm) demonstrated blebbing and vesiculation of cilia membranes as well as disruption of trachea cilia structure (120). Exposure of cultured human bronchial epithelial cells to diesel exhaust particles, but not to nitrogen dioxide, reduces cilia beat frequency (121). Compounds present in indoor air pollution, including formaldehyde, acrolein, and ammonia, have effects on cilia beating and structure as well as on mucus flow (122). Cilia dysfunction has also been linked to workplace exposures such as cadmium (reduced cilia beat frequency), nickel (reduced cilia beat frequency, cell damage, and disorganized cilia), hairspray (reduced mucociliary clearance in the noses and trachea of hairdressers), and wood dust (decreased nasal mucociliary clearance and loss of ciliated epithelium) (122).

COPD

Mucociliary clearance is impaired in COPD in association with increased vulnerability to respiratory tract infections (87). This decrease in clearance is attributed to a shortening of cilia caused by cigarette smoke as well as to airway epithelial dysfunction (123). Respiratory cilia are shorter in healthy smokers than in nonsmokers and even shorter in smokers with COPD than in smokers without evidence of airway disease (49, 50). Cilia beating is impaired in nasal cilia from individuals with COPD (46), even after smoking cessation (124).

A new concept in COPD cilia dysfunction is that of ciliophagy, the consumption of cilia components by autophagic mechanisms (125). Lam and colleagues (123) demonstrated that lung autophagic flux increased in the setting of cigarette smoke and that mice deficient in autophagy mechanisms resisted smoke-induced shortening of cilia and smoke-induced impairment of mucociliary clearance. Cigarette smoke exposure also increased the turnover of cilia proteins by autophagy, a process mediated by cytosolic deacetylase HDAC6 (123). In this model, administration of tubastatin A, an inhibitor of HDAC6, or 4-phenyl butyric acid, a chemical chaperone, prior to cigarette smoke exposure protected mice from cigarette smoke–induced reduction in mucociliary clearance (123, 125). Similarly, blockage of autophagy enhances primary cilia growth and cilia-associated signaling during normal nutritional conditions (126), and autophagic degradation of a ciliopathy protein, OFD1 (oral-facial-digital syndrome 1), at centriolar satellites promotes primary cilia biogenesis (127).

Bronchiectasis

Bronchiectasis is characterized by a localized, irreversible dilation of segments of the bronchial tree in association with loss of airway smooth muscle and elastic fibers (128). The most common cause is infection, but it is also associated with inhalation of toxic gases, aspiration of stomach

contents, and drug use (128). Interestingly, other than bronchiectasis associated with PCD, the incidence of cilia ultrastructural defects is not significantly different in idiopathic bronchiectasis than in normal controls (129). Cilia orientation in individuals with idiopathic bronchiectasis is typically not different than in controls (129, 130). Conflicting data exist regarding whether cilia beat frequency in idiopathic bronchiectasis is reduced compared with cilia beat frequency in normal controls (129, 131). The addition of sputum from patients with bronchiectasis to nasal epithelial fragments suspended in tissue culture medium results in reduced cilia beat frequency, but cilia beat frequency improves after treatment with antibiotics (132). Defective signaling of sensory proteins in motile cilia may lead to ciliopathy and to the development of bronchiectasis (133).

Asthma

Mucociliary clearance is impaired in asthma (134). Autopsy specimens from cases of fatal asthma show loss of cilia, shedding of the bronchial epithelium, and failure of clearance of mucus with bronchial plugging (134, 135). Many functional studies in asthma subjects have documented impaired mucociliary clearance (136–139), and abnormal clearance of secretions is clinically apparent to asthma patients (136).

Although alterations in the characteristics of mucus play a role, changes in cilia structure and function also contribute to reduced mucociliary clearance in asthma. The shedding of ciliated epithelium observed during autopsy (134, 135) is also observed in cells obtained via biopsy and in cells shed from the airway epithelium and recovered by bronchoalveolar lavage (140). Electron microscopy of epithelial biopsies in both children and adults with asthma shows damage to ciliated cells, with vacuolization of the endoplasmic reticulum and mitochondria, loss of cilia, and abnormal cilia structure (141, 142). In contrast, ultrastructural examination of nasal biopsies from patients with aspirin-exacerbated respiratory disease (characterized by aspirin sensitivity, nasal polyposis, and asthma) shows no cilia abnormalities (143). Cilia beat frequency is reduced in moderate and severe asthma compared with cilia beat frequency in controls (144), and the cilia beat direction is disorganized (142). Subjects with moderate and severe asthma have more dyskinetic and immotile cilia than do controls, whereas cilia length is unchanged in asthmatics (144). Related to these abnormalities in function is the finding that subjects with severe asthma have more cilia disorientation, cilia depletion, and microtubule defects than do both normal controls and subjects with mild asthma (144). Sputum from asthma patients has an inhibitory effect on cilia beating in bronchial epithelial explants (145). A number of mediators implicated in asthma, including prostaglandins, bradykinin, prostacyclin, and leukotriene D4, increase cilia beat frequency (56, 146), although varying effects on cilia beat frequency have been reported for histamine and leukotriene C4 (56, 146, 147).

The Th2-type cytokine IL-13 is found at increased levels in the airways of asthmatics and is a key mediator of the epithelial abnormalities of asthma (148). In culture models of mucociliary differentiation, IL-13 promotes goblet cell differentiation and reduced numbers of ciliated cells (149). IL-13 is associated with modulation of expression and localization of ezrin, which anchors basal bodies to the apical cytoskeleton, and IL-13 exposure interferes with the apical localization of ezrin, leading to reduced numbers of basal bodies (16, 149, 150). The addition of IL-13 to differentiated airway epithelium in culture reduces both the number of ciliated cells and the number of cilia per cell (16). In cultured airway epithelium, IL-13 also decreases and eventually eliminates cilia beat frequency (149). These effects appear to be mediated by an IL-13-induced decrease in expression of *FOXJ1*, via a decrease in *FOXJ1* promoter activity, and of ezrin (16). Using a candidate gene approach, Kovacic et al. (151) found that variants in *KIF3A*, which codes for a member of the kinesin family of microtubule motors critical to intraflagellar transport, were

significantly associated with asthma. Kim and colleagues (152) found an association in a Korean population between *KIF3A* polymorphisms and aspirin-associated respiratory disease.

Acute and Chronic Infection

Both infectious microorganisms and the immune/inflammatory response to infection can alter airway cilia function, leading to impaired mucociliary clearance and retained secretions (153). Recurrent bronchitis is associated with loss of ciliated cells in children (154). A number of microorganisms impair cilia function by mechanisms including reducing cilia beat frequency, disrupting cilia coordination, and inducing cilia dyskinesia (155, 156). Some bacterial pathogens—including *Actinobacillus pleuropneumoniae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Mycoplasma hyopneumoniae*, and *Bordetella* species—specifically target ciliated cells for adherence (155). Epithelial samples from chronically infected patients with bronchiectasis show a disruption of cilia orientation associated with decreased mucociliary clearance, although cilia ultrastructure and cilia beat frequency remain normal (157). Lung samples from patients infected with respiratory syncytial virus show epithelial damage and loss of cilia associated with decreased expression of FOXJ1, findings replicated in a mouse model (156).

In addition to the effect of the microorganism, the host response to infection may contribute to deficient mucociliary clearance. Human neutrophil elastase causes epithelial disruption and, at high concentrations, reduces cilia beat frequency (153). Reactive oxygen species generated by polymorphonuclear leukocytes, especially hydrogen peroxide, decrease cilia beat frequency (158).

Interstitial Lung Disease

Limited data are available on cilia structure and function in interstitial lung disease. Mucociliary clearance is normal in subjects with asbestosis, sarcoidosis, and pulmonary fibrosis (159, 160). Transcriptional profiling of lung tissue samples from 119 subjects with idiopathic pulmonary fibrosis (IPF) categorized these subjects into two distinct groups that were defined by expression of cilia-related genes, a finding validated in an independent cohort of 111 IPF patients (161). Interestingly, patients with high expression of these cilia-related genes had more microscopic honeycombing, but not fibroblastic foci, and had higher expression of MUC5B and MMP7.

Lung Transplantation

Infection plays an important role in early death after lung transplant and may also contribute to the development of bronchiolitis obliterans syndrome, a leading cause of later deaths posttransplant (162). Mucociliary clearance is impaired early after lung transplant (163, 164). Studies of cilia beat frequency in adults have had mixed findings; some studies showed reduced cilia beat frequency in transplanted bronchi (165), whereas others did not (163, 166, 167). In a study in children post–lung transplant for CF as well as for nonsuppurative lung disease, significant ultrastructural abnormalities in the epithelium, including loss of ciliated cells, ciliated cells with loss of cilia, and cilia disorientation, were observed 7 to 12 months posttransplant distal to the anastomosis (168).

Bone Marrow Transplant

Bronchiolitis obliterans is a common pulmonary complication following stem cell transplantation and an important cause of death following transplant (169). In a study of nasal cilia morphology and function in 36 Chinese patients after allogeneic stem cell transplant, cilia beat frequency was lower in the transplant patients than in controls, with a greater reduction in cilia beat frequency

in patients with bronchiolitis obliterans (170). Assessment of 19 Chinese patients both before and after stem cell transplant showed reduced cilia beat frequency both before and after transplant compared with cilia beat frequency of age- and sex-matched controls but showed no correlation between cilia abnormalities and pulmonary dysfunction (171).

Mechanical Ventilation

The use of invasive mechanical ventilation for the treatment of respiratory failure may induce airway epithelial injury and cilia dysfunction. In acutely intubated patients, reduced mucociliary clearance was associated with duration of mechanical ventilation, with smoking status, and with isolation of pathogenic bacteria in the tracheobronchial tree (172). Airway epithelial injury, including cilia loss, was found in experimental animals undergoing various modes of mechanical ventilation, with some differences in pattern of injury depending on ventilator mode (173, 174). In contrast to the case for invasive mechanical ventilation, in a group of patients using nasal continuous positive airway pressure for obstructive sleep apnea, no decrement in nasal clearance was observed (175).

Shock or Sepsis

Little is known about cilia function in patients with sepsis or shock. Absence of cilia motility has been reported postmortem in patients who died of sepsis or multiorgan system failure (176).

Effect of Drugs

Various drugs affect airway cilia function. Activation of axonemal cAMP-dependent protein kinase A increases cilia beat frequency by phosphorylation of dynein light chains (35). β-Agonists, including salmeterol and salbutamol, raise intracellular cAMP levels and thereby increase cilia beat frequency (177). Consistent with this observation, inhalation of salbutamol increases mucociliary clearance in normals and in those with chronic bronchitis (178). Similarly, methylxanthines (e.g., theophylline, aminophylline) inhibit phosphodiesterase and increase cAMP, leading to cilia stimulation (179). The phosphodiesterase 4 inhibitor roflumilast increases cilia beat frequency in vitro and reverses the decrease in cilia beat frequency observed with treatment with cigarette smoke extract (180). The cholinergic agents acetylcholine and pilocarpine increase cilia beat frequency (181), and conversely, anticholinergic drugs reduce cilia beat frequency (182). Topical application of corticosteroids to airway epithelium in culture results in a small increase in cilia beat frequency (181). Prolonged inhaled steroid treatment in asthmatics reverses epithelial damage seen in the same subjects prior to steroid treatment, including damage in areas of epithelium that showed loss of cilia (183). Inhaled beclomethasone in patients with COPD does not affect mucociliary clearance (184). Sisson and colleagues (185) found that alcohol rapidly stimulates cilia beating through both nitric oxide– and cAMP-dependent mechanisms and suggested that this pathway is downregulated by chronic alcohol exposure, leading to chronic cilia dysfunction.

Aspirin decreases mucociliary clearance, although whether this effect is due to changes in water transport and airway secretions, or to changes in cilia beating and coordination, is not clear (186). Volatile anesthetics depress cilia beat frequency in cultured rat tracheal cells; halothane and isoflurane have a greater effect than sevoflurane (187). *N*-Acetylcysteine reduces cilia beat frequency in cultured nasal epithelium, but no effect was seen in nasal epithelium of CF patients taking the drug (188).

Lung Cancer

Because of the role of primary cilia in cell cycle regulation, primary cilia may be important in tumorigenesis, and genes important in cilia formation and function exhibit dysregulated expression in multiple tumor types (189). Few data exist, however, on airway cilia structure and function in the setting of lung cancer, although the histological progression from normal histology to dysplasia to lung cancer is characterized by changes such as loss of cilia (190). Less commonly, lung cancer variants with ciliated epithelium are reported (191–194). Loss of cilia function does not appear to predispose to lung cancer, and lung cancer in PCD is rare (195). Mucociliary clearance is slower in individuals with lung cancer than in those with chronic bronchitis and no cancer, matched for smoking history (196). Expression of ciliated cell genes is markedly decreased in a basal cell–high lung adenocarcinoma subset, which is characterized by increased expression of airway basal cell–related genes and is associated with smoking status, with higher frequency of *KRAS* mutations, and with a particularly aggressive clinical phenotype (197).

HIV

Most data on cilia function in the setting of HIV infection are focused on nasal cilia. Nasal mucociliary clearance was compared between HIV-positive individuals and HIV-negative controls; no subjects had active nasal symptoms or sinusitis at the time of the study. Findings included reduced mucociliary clearance in HIV-positive individuals, with a trend toward worsening mucociliary clearance with progression of HIV infection to AIDS as well as a trend toward worsened mucociliary clearance in those subjects with a history of recurrent sinus infections (198). A subsequent study corroborated altered mucociliary clearance and documented abnormalities in cilia axonemal structure in HIV-positive individuals, but the majority of the individuals studied had respiratory infections at the time of evaluation, which may have impacted the results because acute infection reversibly affects cilia structure and function (199). HIV-positive individuals have reduced nasal nitric oxide levels compared with those of controls, which may correlate with reduced cilia function (200). Guaifenesin treatment in HIV-positive individuals may reduce nasal symptoms but is not associated with improvements in mucociliary clearance (201).

FUTURE DIRECTIONS

Although the critical role of airway ciliated cells and cilia in human health is not in question, there are many areas in which new insights will help us understand the role of ciliated cells and cilia in the pathogenesis of human disease. First, although much is known about the control of ciliated cell differentiation and about the structure and maintenance of cilia in many model systems and in the murine lung, molecular and biological studies of ciliated cells and cilia in human airways are limited. These studies would be greatly accelerated by the development of methods to isolate pure populations of airway ciliated cells in health and disease. Second, although a great deal of progress has been made on the identification of genetic variants associated with PCD, little attention has focused on genetic variation in the context of what are thought of as the acquired disorders of airway cilia dysfunction. In this regard, there may be mild variants in the genes controlling airway cilia structure and function that contribute to the increased susceptibility to disease associated with environmental stresses, particularly cigarette smoking. Finally, most of the data regarding airway cilia–related abnormalities in acquired lung disorders are descriptive, and the underlying mechanisms are not clearly defined. Despite the knowledge of abnormal mucociliary clearance

in many acquired lung disorders, new classes of pharmaceutical agents to reverse or prevent abnormal mucociliary clearance cannot be developed until the dysfunctional biology responsible for mucociliary dysfunction can be understood.

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