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Restoring Ciliary Function: Gene Therapeutics for Primary Ciliary Dyskinesia

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Primary ciliary dyskinesia (PCD) is a genetic disease characterized by defects in motile cilia, which play an important role in several organ systems. Lung disease is a hallmark of PCD, given the essential role of cilia in airway surface defense. Diagnosis of PCD is complicated due to its reliance on complex tests that are not utilized by every clinic and also its phenotypic overlap with several other respiratory diseases. Nonetheless, PCD is increasingly being recognized as more common than once thought. The disease is genetically complex, with several genes reported to be associated with PCD. There is no cure for PCD, but gene therapy remains a promising therapeutic strategy. In this review, we provide an overview of the clinical symptoms, diagnosis, genetics, and current treatment regimens for PCD. We also describe PCD model systems and discuss the therapeutic potential of different gene therapeutics for targeting the intended cellular target, the ciliated cells of the airway.

Keywords: AAV, cilia, lung, gene therapy, viral vectors

INTRODUCTION

CILIA ARE HAIR-LIKE organelles that protrude outward from epithelial and other cell types.¹ In mammals, ciliated epithelial cells line the surface of the upper and lower airways, forming one of the most important components of effective airway defense. The rhythmic, coordinated beating of cilia aids in propelling mucus and trapped foreign particles from the airways and lungs, a process known as mucociliary clearance (MCC).²

Motile cilia are also important in other organs, such as the brain³ and the reproductive system.^{4,5} One type of motile cilia, nodal cilia, is involved in the formation of the left-right body axis during embryonic development.⁶ Primary ciliary dyskinesia (PCD), historically referred to as immotile cilia syndrome,⁷ is a genetic disease characterized by the dysfunction of motile cilia.

Most of the morbidity observed in PCD is associated with the lungs and upper airway.⁸ People with PCD exhibit a wide range of symptoms, including chronic cough,

lung infections, otitis media, and chronic rhinosinusitis. Bronchiectasis usually develops in childhood,⁸ and some individuals with PCD eventually require lung transplantation.^{9,10}

Due to defects in (1) the sperm flagella, (2) the efferent duct in the testes, and (3) the fallopian tubes, males^{11,12} and females¹³ with PCD can be infertile. Since the genes that affect ciliary movement also affect the function of nodal cilia in the developing embryo, ~50% of people with PCD present with organ laterality defects, such as *situs inversus* and heterotaxy.¹⁴ In most cases, PCD has not been reported to decrease overall life expectancy,^{10,15} although comprehensive studies designed to substantiate this statistic are lacking.

PCD was originally described in 1933 by Kartagener reporting on individuals with a combination of chronic sinusitis, bronchiectasis, and *situs inversus*; this became known as Kartagener's Syndrome. Decades later, and due to technological advances, the presence of immotile cilia

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and defective ciliary structure were observed in people with these conditions.^{7,16}

Eventually, it was demonstrated that in some individuals, the disease could be present even in the absence of ciliary ultrastructural abnormalities or complete lack of ciliary movement, and as such, the disease was renamed “primary ciliary dyskinesia.”^{17–19} Over 50 genes have been associated with PCD, most of which express proteins that localize to motile cilia.²⁰

Once thought of as a rare genetic disease with a global incidence of about 1 in 20,000 individuals,²¹ in recent years it has become clear that PCD is often mis- or underdiagnosed.²² A recent report using genetic databases revised the global incidence of PCD to ~1:7,500.²³ The appreciation of the higher incidence of PCD, and the fact that current therapies do not treat the underlying cause of the disease, has highlighted the potential of gene therapy for a cure.

This review summarizes the clinical aspects, molecular mechanisms, diagnostic methods, genetics, current treatments for PCD, as well as cell and animal models of the disease. The prospect of gene therapy strategies for PCD is then discussed, with a focus on the ciliated epithelial cell as the target cell type.

CLINICAL SYMPTOMS

The onset of symptoms associated with PCD presents in term infants as neonatal respiratory distress syndrome, the result of inadequate MCC of airway mucus secretions.²⁴ Indeed, impaired MCC is the hallmark feature of PCD, and it results in life-long chronic wet cough and upper airway disease.²⁴

People with PCD usually experience frequent exacerbations of airway disease due to a constant cycle of inflammatory and infective events that are severe enough to require short-term hospitalization for management.²⁵ This cycle is often unresolved and becomes chronic, releasing into the airways damaging neutrophilic and bacterial products (e.g., neutrophil elastase and *Pseudomonas aeruginosa*-derived pyocyanin²⁶). By adulthood, most people with PCD have developed irreversible lung disease and bronchiectasis.^{24,27}

Since ciliated cells line the upper airways and the Eustachian tubes, people with PCD often present with otitis media and rhinosinusitis. These symptoms can result in (1) a reduction, although variable, in smell;^{24,28,29} (2) persistent sinus infections; and (3) conductive hearing loss,³⁰ although not all these symptoms are present in all people with PCD.

A concerted effort in PCD research has led to an improved understanding of genotype-phenotype relationships.^{31,32} For example, people with *DNAH11* mutations were reported to experience more frequent episodes of rhinosinusitis, whereas people with central complex defects in the ciliary axoneme, as detected by electron microscopy (EM), were less likely to report otological dysfunction.^{32,33}

Lung function in people with PCD declines with age, and worse lung function has been associated with mutations in the *CCNO*, *MCIDAS*, *CCDC39*, and *CCDC40* genes.^{32,34–36} Severely affected people with PCD require lung transplantation or lobectomy. By comparison, PCD resulting in milder lung disease (e.g., missense mutations in *CCDC103* or some *DNAH11* variants) may not be diagnosed until the sixth or even seventh decade of life.^{35,37,38}

Due to the presence of motile cilia in the efferent duct and fallopian tubes, as well as structural similarities between the flagella (sperm tail) and motile cilia, some people with PCD are infertile.^{11,12} Infertility in PCD remains poorly described, especially in females. In the embryonic node, ciliary motility plays a crucial role in the formation of left/right symmetry, and dysfunction of nodal cilia can lead to a full or partial reversal of the organs (*situs inversus/heterotaxy*).¹⁴ This results in a small number of people with PCD who present with complex congenital heart disease.^{39,40} Genotyping has revealed PCD as part of a wider spectrum of syndromes known as motile ciliopathies.¹⁴ Individuals affected by rarer multiple motile ciliopathies caused by mutations in genes such as *OFDI*, *RPGR*, and *FOXJ1* can experience additional conditions, such as developmental delay, hydrocephalus, microcephaly, lissencephaly, cystic kidneys, retinitis pigmentosa, and/or other syndromic features that are associated with motile or primary ciliary dysfunction.^{41–43}

CILIA STRUCTURE AND FUNCTION

Cilia are small hair-like structures that project from epithelial cells and are crucial for an effective MCC system² in the respiratory tract. The structure of the cilium consists of nine outer doublet microtubules that surround a central pair of singlet microtubules. Radial spokes connect the outer microtubule doublets to the central pair, and the nexin-dynein regulatory complex links the adjacent microtubule doublets together. On each of the outer microtubule doublets, there are outer (ODA) and inner dynein arms (IDA) that are repeated throughout the cilium.²⁰

These dynein arms stimulate the movement of the cilium, which through adenosine triphosphate hydrolysis causes force on the microtubules, thus allowing the cilium to bend.^{44,45} Motile cilia and the overlaying airway surface liquid (ASL) are considered the first line of defense of the airway. The mucus layer of the ASL is produced by goblet cells and submucosal glands.²

Inhaled foreign particles/pathogens are deposited and trapped in this mucus layer, and the cilia beat in unison to coordinate MCC and clear these out of the airways. Indeed, effective MCC prevents pathogenic bacteria, viruses, and other noxious agents from depositing in the airways, which, in turn, helps prevent respiratory infections.²

In a healthy airway, the ciliated epithelial cells are lined with motile cilia that are $\sim 7 \mu\text{m}$ long in the upper airway and $\sim 4 \mu\text{m}$ long in the small airways⁴⁶ and beat at ~ 7 to $\sim 16 \text{ Hz}$.⁴⁷ The typical beating pattern of cilia initiates with a forward effective stroke that reaches into the periciliary liquid layer and expels the mucus layer followed by a recovery stroke where the cilia return to their original position. In PCD, the motile cilia are either completely static, stiff, slow, restricted, display a rotary motion, or are absent/reduced in number,^{48,49} all of which contribute to an ineffective MCC.

DIAGNOSTIC METHODS

Securing or excluding a diagnosis of PCD is complex. The first barrier to getting a diagnosis is recognition of the clinical signs and symptoms by medical professionals. A survey conducted by Behan et al.⁵⁰ suggested that it is common for individuals to visit a health care professional with clinical signs or symptoms of PCD more than 40 times before they are referred for diagnostic testing.⁵⁰

For people with PCD, the average age of diagnosis in Europe is thought to be 5 years, but it is likely that many of the adults diagnosed with bronchiectasis and labeled as “idiopathic,” in fact, do have unrecognized PCD as the underlying cause of disease.²²

Established diagnostic guidelines for PCD exist in both Europe and North America.⁵¹ These guidelines recommend one of two methods for confirmation of a diagnosis of PCD: (1) identification of a recognized ultrastructural defect by EM or (2) identification of pathogenic mutations in one of the PCD genes.^{52,53} An international consensus statement describes the three different ultrastructural defects used to confirm a diagnosis of PCD by EM: (1) outer dynein arm absence, (2) outer and IDA absence, or (3) microtubular disorganization and inner arm defect.⁵⁴

Additional structural defects (*i.e.*, central complex defects, mislocalization of basal bodies, or dynein arm defects affecting only a minority of cilia) can aid a clinical diagnosis when supported by adjunct tests.⁵⁴ These adjunct tests include (1) measurement of nasal nitric oxide (nNO), (2) a characteristic ciliary beat pattern as visualized by high-speed video microscopy, or (3) confirmation, by immunofluorescence, of loss of protein, including, but not limited to, DNAH5, GAS8, DNAH11, or SPEF2.^{55–58} Standardized guidelines exist for the measurement of nNO, whereas high-speed video microscopy and immunofluorescence are considered subjective.^{59,60}

Gene discovery in PCD has revealed multiple genotypes with normal nNO and/or cilia ultrastructure as assessed by EM.^{57,60–65} Of note, almost 30% of known cases of PCD do not have a specific gene identified despite extensive genotyping. Variants of unknown significance are also common, necessitating additional pathology-based

testing. Since sensitivity or specificity is imperfect for all diagnostic tests, a multi-test approach is ordinarily required to confirm or to exclude a diagnosis.⁵²

GENETICS

The first PCD-associated gene to be described was *DNAI1*, which encodes for an intermediate chain in the outer dynein arm.⁶⁶ Absence of DNAI1 leads to ciliostasis, and EM analysis revealed that mutations in *DNAI1* result in the loss of the outer dynein arms.⁶⁶ Following the characterization of *DNAI1*, another PCD-causing gene, *DNAH5*, was reported.⁶⁷

DNAH5 is the large heavy chain of the outer dynein arm consisting of 4,626 amino acids (aa), and to date over 500 PCD-causing variants in the *DNAH5* gene have been confirmed.⁶⁸ For several years, the *DNAH5* gene was thought to be the most common genetic cause of PCD.⁶⁹ However, another gene, *DNAH11* (a dynein heavy chain located on the outer dynein arm), was recently reported as the most common genetic cause of PCD.²³

People with pathogenic variants in *DNAH11* are more likely to remain undiagnosed due to the normal ultrastructural findings from EM assessment of the axoneme, coupled with a milder clinical manifestation of the disease.^{32,62,63} Despite being the most commonly affected genes, *DNAH5* and *DNAH11* are considered challenging targets for gene therapy due to their large size. The third and fourth most commonly mutated genes in PCD are *CCDC39* and *CCDC40*, respectively,²³ which are crucial to microtubular stabilization.^{70,71}

High-speed video assessment of ciliated epithelial cells from people with *CCDC39* and *CCDC40* mutations showed rigid dyskinetic and ineffective movement of cilia, and EM revealed the loss of IDA and microtubular disorganization.^{70,71} People with PCD who have either *CCDC39* or *CCDC40* mutations are more likely to have severe lung disease when compared with people with mutations in either *DNAH5* or *DNAH11* genes.^{31,32}

CCDC39 and *CCDC40* are also much smaller (941 and 1,142 AA, respectively) and therefore are easier to target using a replacement strategy with gene therapy vectors. The prevalence of *CCDC39* and *CCDC40* mutations, along with their more severe disease phenotype renders these viable targets. Genetic distribution of PCD varies from country to country, with some founder effects and more common mutations identified in different countries.

For example, the most common PCD causative gene in the Netherlands is *ODAD1*.⁷² Internationally, biallelic mutations in the *DNAH11*, *DNAH5*, *CCDC39*, *CCDC40*, and *DNAI1* genes account for $\sim 50\%$ PCD cases.²³ However, there are reports of mutations in at least another 50 genes that cause PCD.²⁰ A current list is maintained by the NIH Clinical Genome Resource (<https://clinicalgenome>

.org/), which uses an evidence-based scoring system to describe 88 motile ciliopathy gene-disease associations.⁷³

However, the strength of the evidence for the involvement of each one of these genes in causing PCD is variable. Broadly speaking, PCD-causing genes fall into one

of several categories²⁰; these, along with the gene names, are shown in Table 1. A targeted gene panel can be used to test for PCD, but with improved cost efficiencies, whole exome or whole genome sequencing are now also feasible. The mode of inheritance of PCD is autosomal recessive,

Table 1. Primary ciliary dyskinesia genes and categories

| Category | Gene Name | Inheritance Pattern | Ciliary Ultrastructure Defects | |
|---|---|---|---|--|
| Dynein arm structural proteins | <i>DNAH5</i> | | ODA | |
| | <i>DNAH9</i> | | Partial ODA (peripheral axoneme) | |
| Heavy chains | <i>DNAH11</i> | | Near normal | |
| | <i>DNAI1</i> | | | |
| Light chains | <i>DNAI2</i> | | ODA | |
| | <i>ODAD1 (CCDC114)</i> | | Partial ODA (peripheral axoneme) | |
| Docking complex | <i>ODAD2 (ARMC4)</i> | Autosomal recessive | Partial ODA (peripheral axoneme) | |
| | <i>ODAD3 (CCDC151)</i> | | | |
| | <i>ODAD4 (TTC25)</i> | | ODA | |
| | <i>ODAD5 (CLXN)</i> | | | |
| Dynein assembly factors | <i>DNAAF1 (LRRC50)</i> | X-linked | | |
| | <i>DNAAF2 (KTU)</i> | | | |
| | <i>DNAAF3</i> | | | |
| | <i>DNAAF4 (DYX1C1)</i> | | | |
| | <i>DNAAF5 (HEATR2)</i> | | | |
| | <i>DNAAF6 (PIH1D3)</i> | | | |
| | <i>DNAAF7 (ZMYND10)</i> | | | ODA and IDA |
| | <i>DNAAF11 (LRRC6)</i> | | | |
| | <i>DNAAF12 (LRRC56)</i> | | | |
| | <i>DNAAF13 (SPAG1)</i> | | | |
| Microtubular stabilization | <i>DNAAF16 (C21orf59)</i> | | IDA (respiratory); ODA and IDA sperm | |
| | <i>DNAAF17 (CFAP300)</i> | | | |
| | <i>TTC12</i> | | | |
| | <i>CCDC39</i> | | Microtubular disorganization and IDA | |
| Radial spoke head/central complex components | <i>CCDC40</i> | | | |
| | <i>RSPH1</i> | Autosomal recessive | | |
| | <i>RSPH9</i> | | | |
| | <i>RSPH4a</i> | | | |
| | <i>RSPH3</i> | | CC defects | |
| | <i>STK36</i> | | | |
| | <i>HYDIN</i> | | | CC defects/near normal |
| <i>SPEF2</i> | | | CC defects | |
| Nexin-dynein regulatory complex components | <i>DNAJB13</i> | | | |
| | <i>DRC1</i> | | | |
| | <i>DRC2</i> | | Microtubular disorganization/near normal | |
| Regulators of ciliogenesis | <i>DRC4</i> | | | |
| | <i>MCIDAS</i> | | Absent or reduced cilia | |
| | <i>CCNO</i> | | | |
| | <i>TP73</i> | Autosomal recessive | Mislocalized basal bodies and reduced cilia length | |
| Other genes associated with motile ciliary function | <i>FOXJ1</i> | Autosomal dominant | Mislocalized basal bodies and reduced cilia length | |
| | <i>CCDC103</i> | Autosomal recessive | ODA and IDA | |
| | <i>GAS2L2</i> | | Cilia orientation defect/near normal | |
| | <i>OFD1</i> | X-linked | Occasional elongated cilia with protein accumulations in the tips | |
| | <i>RPGR</i> | | Cilia orientation defect/near normal | |
| | Other genes associated with motile ciliary function | <i>NEK10</i> | Autosomal recessive | Near normal |
| <i>CEP164</i> | | Long cilia/microtubular disorganization | | |
| <i>TUBB4B</i> | | Autosomal dominant | | Short, sparse cilia with disorganized axonemes and protein accumulations in the tips |

CC, central complex; IDA, inner dynein arm; ODA, outer dynein arm.

except for (1) the two X-linked forms *OFD1* and *DNAAF6* (*PIH1D3*), and (2) the autosomal dominant forms (*FOXJ1*, *TUBB4B*).

CURRENT TREATMENTS

There is no cure for PCD. Treatment options are limited, and do not correct the fundamental defects in ciliary function. Currently, PCD lung disease is managed by a regimen of physiotherapy, which helps clear secretions from the airways that would typically be handled by the mucociliary escalator.⁷⁴ Respiratory physiotherapists teach age-appropriate personalized airway clearance techniques, including adjuncts such as breathing exercises and vests, and flutter devices.

Most therapeutic options for PCD lung disease use evidence borrowed from treatment regimens for cystic fibrosis (CF) lung disease and/or bronchiectasis. Exacerbations are ordinarily targeted with prolonged courses of antibiotics.²⁵ However, pathogens isolated in sputum typically change with the course of the disease, and as expected with time, antibiotic therapies become less effective.

The most frequently isolated pathogens from the airways of children and young adults with PCD are *Haemophilus influenzae* and *Staphylococcus aureus*.⁷⁵ Adults with PCD are more likely to be colonized with *Pseudomonas aeruginosa*, and as in CF, this correlates to lung function decline and subsequent increased morbidity and mortality.³⁸ For people with PCD, the eradication regimen for *Pseudomonas* is very similar to that used in CF and bronchiectasis, where nebulized antibiotics are typically used.⁷⁶

Two clinical trials of PCD-targeted medications have been conducted.^{77,78} The first trial assessed hypertonic saline in 22 people with PCD, and although it did not reach significance in its primary endpoint of change in quality of life (QoL) as measured by the St George's Respiratory Questionnaire (SGRQ) total score, there was a significant improvement in health perception measured by quality of life bronchiectasis (QoL-B).⁷⁸

The second trial was pan-European and evaluated the impact of azithromycin treatment on exacerbations.⁷⁷ The trial met its primary endpoint of reducing the time to next exacerbation, providing good proof-of-concept that PCD-specific trials can be conducted.⁴⁷ Establishing a clinical trial network and a shared core outcome set for conducting PCD-specific clinical trials should facilitate future trials.^{47,79} Trials using both re-purposed and new therapeutics are needed to generate an essential evidence base for treating PCD.

There are no guidelines for the treatment of upper airway symptoms. Treatment for rhinosinusitis often involves topical nasal steroids to reduce inflammation, oral and topical antibiotics to treat infections, and nasal rinsing to remove secretions. For hearing loss linked to otitis media, the use of ventilation tubes is controversial, as

permanent discharge can be caused.^{33,80,81} Other non-respiratory symptoms, such as retinitis pigmentosa, infertility, and hydrocephalus, are typically treated when appropriate, giving rise to a multidisciplinary treatment regimen designed to improve QoL. Genetic counseling, dietary advice, and psychological support are also important for improved outcomes for the successful life-long management of PCD.

PRE-CLINICAL MODELS

The testing of gene therapeutics requires the utilization of both *in vitro* and *in vivo* model systems that recapitulate aspects of PCD. Next, we focus on cell (*in vitro*) and animal models of PCD.

IN VITRO MODELS

Cell culture models are an important diagnostic tool for PCD and represent useful models for the development and assessment of candidate therapeutics. Commonly used *in vitro* models involve culturing differentiated cells or using tissues harvested or biopsied from the airway epithelium of people with PCD.

Airway epithelial cells grown on permeable supports at air-liquid interface (ALI) develop a mucociliated morphology, thus providing a model for the study of airway epithelial lineages, differentiation, and function; this system is considered the gold standard in culturing cells isolated from the respiratory epithelium. ALI cultures have proven to be useful for the analysis of the ciliary phenotype of people with PCD, particularly when the health of the cells harvested from brush biopsies is poor. Despite minor ciliary phenotype changes when grown at ALI, the overall PCD diagnoses remained unaffected.⁸² The proliferative capacity of airway basal epithelial cells is limited; however, recent studies showed that the proliferative potential of basal cells can be expanded by transducing cells with the mouse polycomb complex protein (*BMI-1*) and human telomerase reverse transcriptase (*hTERT*)⁸³ or via SMAD inhibition.⁸⁴

In addition, co-culture of basal cells with mouse embryonic fibroblast feeder cells in a media containing Rho-associated kinase inhibitor allows for long-term culture of functional basal cells, with multipotent differentiation capacity.^{85,86} These improvements allow for long-term expansion of basal cells from patients while maintaining their differentiation capacity, thus generating large numbers of ciliated cells for analyses.

Recent advances in 3D culture technology have led to the establishment of explant spheroids derived from PCD nasal brush biopsies,⁸⁷ as well as airway organoids, which can be derived via directed differentiation of human/induced pluripotent stem cells (h/iPSC⁸⁸⁻⁹⁰) or adult tissue-resident stem cells (ASC⁹¹).

Stem cell-derived organoids grown in 3D phenocopy reflect the structural and functional aspects of the *in vivo* respiratory epithelium, and can differentiate into ciliated cells in the presence of appropriate molecular cues, such as the concurrent inhibition of Notch and activation of the bone morphogenetic protein signaling pathways.⁹²

Ciliary defects were maintained in airway organoids derived from nasal inferior turbinate brush samples obtained from several people with PCD; these organoids were amenable to CRISPR-Cas9-mediated prime editing and repair of the PCD gene-specific mutation.⁹¹ Hawkins et al. established a protocol to derive basal cells by directed differentiation of hiPSCs, followed by culture of the derived basal cells (iBC) at ALI to generate mucociliated cells.⁸⁶

This protocol was subsequently used to generate iBCs and ciliated cells from patient iPSCs harboring the *DNAH5* mutation, with absent ciliary motility in the mutant cells.⁸⁶ Although h/iPSC-derived cells and organoids share essential features with their endogenous counterparts, differentiation protocols are time-consuming and may result in organoids with variable proportion of cell types.

In addition, the source of the cells can influence the differentiation status of the organoids: h/iPSC-derived organoids represent a more developmental state of cells than the mature state observed in organoids derived from ASCs.⁹³ Nevertheless, *in vitro* airway culture models derived from human tissues provide a multifunctional platform that facilitates (1) the investigation of the pathogenicity of PCD gene mutations, (2) the study of disease mechanism(s), (3) the performance of therapeutic screens, and (4) the evaluation of gene therapeutics.

MAMMALIAN ANIMAL MODELS

Several mouse models with mutations in PCD genes have been described. Thus far, homozygosity in mice appears to be required for a disease phenotype. Interestingly, many of these mouse models exhibit more severe symptoms than those found in people with PCD, owing to a greater prevalence in laterality and other organ developmental defects that negatively impact survival in mice.

Mdnah5^{-/-} mice, which were generated by insertional mutagenesis causing a frameshift in the *Dnah5* gene,⁹⁴ present with respiratory distress, and show evidence of spontaneous respiratory infections, both of which are hallmarks of PCD.⁹⁴ *Situs* abnormalities, either complete or partial reversal of organs, were noted in *Mdnah5*^{-/-} mice.⁹⁴ Analysis of cilia from the brain and trachea of this mouse model showed immotility, and subsequent ultrastructural analysis revealed an absent ODA. *Mdnah5*^{-/-} mice developed hydrocephalus within 5 days of birth, and showed growth retardation and abnormal gait; these animals did not survive beyond 3 weeks of age.⁹⁴ Another *Dnah5* mutant model, the *Dnah5*^{del593} mouse, results from an in-frame deletion of 593 aa.⁹⁵

Laterality defects were prominent in this mouse model, with ~35% of homozygous mice presenting with *situs inversus totalis* and ~40% of homozygous mice presenting with heterotaxy and congenital heart defects, leading to postnatal mortality between 2 and 4 weeks of age.⁹⁵ A third *Dnah5* model, the *Dakshi Dnah5* (*b2b016Clo*) mouse, displays a milder overall disease phenotype than the *Mdnah5*^{-/-} mouse.⁹⁶ The *Dakshi Dnah5* homozygote mice have organ defects, including *situs inversus* and congenital heart disease, and in some instances, duplex/duplicated kidneys.⁹⁶ Analysis of the ciliary activity in the trachea of the *Dakshi Dnah5* mouse showed preservation of ciliary movement, although ciliary beat frequency (CBF) and MCC were markedly reduced by 64% and 65%, respectively.⁹⁶ Some homozygous *Dakshi Dna5h* pups developed hydrocephalus, but homozygous *DakshiDna5h* pups without overt hydrocephaly survived into adulthood.⁹⁶ In the same report, the authors also evaluated the disease phenotype of the *Wdr69*^{-/-} mouse. Although the *Wdr69* gene has not been formally characterized, mutations in this gene have been predicted to cause ciliary defects in mammals.^{96–98} Indeed, *Wdr69*^{-/-} mice exhibited *situs solitus*, *situs inversus*, and/or heterotaxy with complex congenital heart defects.⁹⁶ In addition, some *Wdr69*^{-/-} mice also developed hydrocephalus, which was found to be associated with early mortality (by 2–3 weeks of age).⁹⁶ Interestingly, analysis of ciliary movement in tracheas harvested from *Wdr69*^{-/-} mice showed preserved ciliary motion with no significant decrease in CBF. However, ciliary motion was found to be dyskinetic, and MCC was reduced.⁹⁶

Other PCD mouse models display a milder disease phenotype. The *Dnahc11iv* mouse model arose from a spontaneous mutation in the *Dnahc11* gene, the mouse homolog of *DNAH11*.⁹⁹ These mice have immotile cilia, *situs* defects, rhinitis, and sinusitis, but interestingly do not present with significant fertility issues despite an observed decrease in sperm motility,⁹⁹ which is in stark contrast to what is seen in people with PCD.¹³

Although there is an age-related progression of sinus disease in *Dnahc11iv* mice, they do not present with lung pathology, and have been reported to have a normal lifespan.⁹⁹ A conditional knockout (KO) mouse model of *Dnaic1*, the mouse homolog of *DNAI1*, has also been generated.¹⁰⁰ When these *Dnaic1*^{flox/flox}/*CreER1* mice were treated with tamoxifen at 8–12 weeks of age, there was a significant reduction in nasal MCC within 1 month of treatment, with negligible MCC observed in the nasopharynx after 3 months. However, MCC was observed in the trachea for up to 6 months, although the clearance rate was reduced.¹⁰⁰ Tamoxifen-treated *Dnaic1*^{flox/flox}/*CreER1* mice developed severe rhinosinusitis, but interestingly, these mice did not present with lower airway disease at any of the time points examined (6–11 months), and their life expectancy was not impacted.¹⁰⁰

The PCD-causing genes *CCDC39* and *CCDC40* are associated with higher risk of a severe disease phenotype in people.^{31,32} However, studies on rodent models for these genes to date describe a much more severe phenotype than is seen in humans. *Ccdc39*^{-/-} mice (line *b2b1304Clo*) were generated as part of a large-scale mouse mutagenesis screen using ethylnitrosourea (ENU),⁹⁵ and have an A to T mutation at position 2, leading to a null genotype.⁹⁶ *Ccdc39*^{-/-} mice present with *situs inversus* and heterotaxy, the latter of which causes congenital heart defects and perinatal death.⁹⁶ Those *Ccdc39*^{-/-} mice that do not present with heterotaxy develop severe hydrocephalus and die within 1 week of birth.⁹⁶ Analysis of cilia from the trachea of *Ccdc39*^{-/-} mice revealed that they were mostly immotile, with only a few cilia showing a slow, dyskinetic movement, which in combination result in a vastly reduced CBF and MCC.⁹⁶

The *Ccdc39*^{prh/prh} mouse model, resulting from a splice site mutation in *Ccdc39*, presents with severe disease that is characterized by hydrocephaly, *situs* abnormalities, and impaired brain development, with homozygous mice succumbing by 3 weeks of age.¹⁰¹ A rat version of the *Ccdc39*^{prh/prh} mouse was also generated,¹⁰² and gave rise to animals that developed a severe hydrocephaly by postnatal day 11, progressing to subarachnoid hemorrhage in the brain accompanied by macrophage and neutrophil invasion.¹⁰² Gradual growth delays were observed, and the affected rats did not survive past 30 days.

For *CCDC40*, there is only one available mouse model, *Ccdc40*^{lnks},¹⁰³ that was generated by an ENU screen that created a V792X mutation. The homozygous *Ccdc40*^{lnks} pups exhibit various organ laterality defects and hydrocephaly, and they do not survive past weaning.¹⁰³

Central to the clinical translation of gene therapeutics is the availability of an animal model that recapitulates the disease phenotype observed in people with PCD. A naturally occurring large animal model of PCD would be incredibly useful for the preclinical evaluation of therapeutics. Interestingly, PCD has been reported to spontaneously occur in dogs, and it has been described in at least 19 different breeds.^{104–109} Specifically, in the Old English Sheepdog, a stop mutation (p.Arg96X) in the *CCDC39* gene has been reported.⁷¹ Affected dogs develop respiratory symptoms, such as nasal discharge and cough, shortly after birth.¹¹⁰ Other symptoms were consistent with those found in people with PCD, including bronchiectasis, bronchopneumonia, and *situs inversus*.¹¹⁰ In addition, in male homozygous dogs, sperm were reduced in number, had lower motility, and had flagellar structural abnormalities.¹¹⁰ In the Alaskan Malamute dog, a frameshift mutation in *NME5* encoding the protein NME/NM23 family member 5 also results in PCD.¹¹¹ Affected dogs presented with nasal discharge and chronic cough, with chronic inflammation, rhinitis,

bronchitis, and bronchiectasis being also reported.¹¹¹ Numerous ultrastructural ciliary defects were noted, including abnormal number of microtubules and shortened or absent ODA and IDA.¹¹¹ However, to date, the function of NME5 in mammals is not known, and there is only one study linking this gene to PCD in humans.¹¹²

GENE THERAPY FOR PCD

Gene therapy has the potential for an enormous therapeutic impact on PCD lung disease. This section describes the vector systems that are commonly used for lung gene transfer, with a special emphasis on targeting the ciliated cells of the airway. We also summarize the PCD gene therapy studies and report the findings to date.

ADENO-ASSOCIATED VIRAL VECTORS

In recent years, gene therapeutics based on adeno-associated virus (AAV) have demonstrated success in the clinic.¹¹³ Advantages for the use of AAV vectors include their relatively low immunogenicity, a wide tissue tropism, and a demonstrated long-term safety profile.^{114–116}

The recombinant virus' cellular tropism and immunological properties can be altered by utilizing different AAV capsid serotypes, thus optimizing the vector for different organ systems.¹¹⁷ Numerous naturally occurring AAV capsids have been isolated from different animal species,^{118–124} greatly enriching the vector "toolkit" available for clinical development.

In general, AAV serotypes 1, 5, and 6 have been shown to effectively target cells of the lung epithelium when compared with other AAV serotypes.^{125–129} Site-directed modifications to airway-tropic AAV capsids, that is, AAV6, improved transduction efficiency in cellular models of human airway, as well as in rodent airway *in vivo*.¹³⁰ DNA shuffling of AAV serotypes, followed by directed evolution, produced a new AAV capsid, AAV2.5T, that was shown to be more efficient in targeting cells of the lung epithelium than AAV2, 5, or 9.¹³¹

Several AAV capsids have been shown to target ciliated airway epithelial cells, the primary target for PCD gene therapy. AAV2 is a poor transducer of the airway epithelium¹³²; whereas co-treatment of AAV2 with proteasome inhibitors, which promote AAV capsid processing and trafficking to the nucleus, was demonstrated to increase transduction of ciliated cells *in vitro*.¹³³

AAV5 effectively transduces ciliated cells in the mouse nose¹³⁴ and lung^{134,135} *in vivo*. AAV6 has also been shown to transduce ciliated cells in human airway epithelial (HAE) cultures,¹³⁶ and a modified AAV6 capsid, AAV6.2, was shown to transduce ciliated cells in both HAE cultures and *in vivo* in mouse lung.^{130,135} AAV8 and 9 have also been reported to transduce ciliated cells of the murine airway *in vivo*.^{134,137}

Finally, AAV1 has been demonstrated to have a favorable transduction profile in HAE cells *in vitro* compared with AAV2 and 5,¹²⁵ and importantly for clinical translation, *in vivo* inhalation studies evaluating AAV1 in nonhuman primates demonstrated transduction of the ciliated epithelium in the trachea and bronchus.¹³⁸

Despite the obvious advantages of AAV vectors as gene therapeutics, some of their properties limit their application to PCD. AAV cannot accommodate large transgenes (>5.3 kb)¹³⁹ without a significant impact on vector manufacturability, which presents a logistical issue for the transfer of the large PCD genes *DNAH1* (13.6 kb) and *DNAH5* (13.9 kb).

In addition, the generation of AAV capsid-specific neutralizing antibodies following the first AAV vector dosing in lung has been reported to significantly impact the effectiveness of subsequent vector dosing.¹⁴⁰ It is encouraging, however, that immunosuppression has been reported to facilitate effective redosing of AAV in ferret lung.¹⁴¹

A recent addition to the AAV vector toolkit is the utilization of capsids from other parvoviruses to form chimeric recombinant vectors.^{142,143} Human bocavirus 1 (HBoV1) naturally infects the human airway,¹⁴⁴ and HBoV1 vectors have been used successfully to generate chimeras with AAV vectors.^{142,143} The HBoV1 capsid conferred tropism to ciliated epithelial cells more efficiently than other human bocavirus capsids as demonstrated by flow cytometry analysis of transduced HAE cells *in vitro*.¹⁴² Another advantage to the use of HBoV/AAV chimeric vectors is their increased packaging capacity (5.5–6 kb) compared with that of standard AAV vectors,^{142,143} which allows for the incorporation of larger genes and inclusion, if warranted, of large regulatory elements into the gene expression cassette.

LENTIVIRAL VECTORS

Lentiviral-based gene therapeutics engineered from the well-studied human immunodeficiency virus type 1 (HIV-1) have been demonstrated to have a favorable transduction and safety profile in the airway *in vivo*.¹⁴⁵ Lentiviral vectors (LVV) are ideal for inherited lung diseases requiring long-term therapeutic gene expression to correct the disease pathophysiology.¹⁴⁵

An important consideration for the clinical development of LVV as a therapeutic for diseases, such as PCD, is its packaging capacity,¹⁴⁶ which allows for the insertion of large genes. Further, the inclusion of elements such as a cell-specific promoter, that is, *FOXJ1*¹⁴⁷ or the use of envelope glycoproteins (GPs) from airborne viruses that are naturally tropic for ciliated cells, that is, the spike envelope of the severe acute respiratory syndrome-associated coronavirus¹⁴⁸ or the Sendai virus envelope proteins F and HN,¹⁴⁹ are molecular strategies that have been already

demonstrated *in vitro* and *in vivo* to improve the preferential targeting and positive transduction of ciliated cells.¹⁴⁸

Another advantage of LVV is its ability to integrate stably into the host genome.¹⁵⁰ As such, should the stem cells of the airway be positively transduced, it is expected that the diseased lung would be repopulated with gene-corrected cells, thus reducing the need for LVV redosing that would otherwise be necessary to maintain the therapeutic benefit.

Nonetheless, should redosing of the LVV be required, it has been reported that preexisting GP-specific binding and/or neutralizing antibodies are not detrimental to effective redosing¹⁵¹ and that with time, the LVV-specific immune responses wane to levels that do not significantly impair redosing.¹⁵²

Ultimately, although LVV transduction of progenitor cells may be ideal, it is not known whether expression of the ciliary axoneme proteins in cells other than ciliated cells may adversely impact the health of the transduced cell and ultimately negatively impact the health status of the lung epithelium. As such, molecular strategies aimed at targeting and/or restricting expression of ciliary genes in the ciliated cell are preferred. In this instance, we expect that LVV redosing will be necessary to maintain a steady state of the required population of gene-corrected ciliated cells to confer correction of the disease pathology.

NON-VIRAL VECTORS

Compared with viral vectors, non-viral vectors have several advantages, including increased packaging capacity and low immunogenicity. In particular, lipid nanoparticles (LNPs) have been demonstrated to have a favorable safety profile.^{153–155} Some of the components of LNPs are naturally found in human lung surfactant fluid,^{156,157} contributing to their low immunogenicity.

LNPs have been formulated to carry nucleic acids, such as mRNA, and are typically composed of phospholipids, neutral or cationic lipids, polyethylene glycol, and cholesterol.¹⁵⁸ The ratio of each of these components can be adjusted to improve *in vivo* delivery to the lung.¹⁵⁹ In addition to nucleic acids, LNPs have been formulated to deliver small-molecule drugs, thus increasing their versatility in the clinic.¹⁶⁰

Some obstacles to effective lung-directed non-viral gene therapy for the lung are similar to those of viral vectors, including the challenge of overcoming the physical barrier (including MCC) and phagocytosis of the vector particles by resident immune cells such as alveolar macrophages.¹⁶⁰ Formulation considerations are also important, as they will impact where the particles will be deposited after aerosolization.¹⁶⁰ One consideration for PCD is that, if mRNA is the payload, redosing will be necessary, and strategies to achieve this will have to be designed, given the short half-life of RNA.

Although the overall efficiency of non-viral-based gene transfer remains an issue, and delivery via inhalation will require additional optimization to improve therapeutic effectiveness,¹⁶¹ LNP delivery for PCD remains an active area of pre-clinical development, as discussed later.

GENE EDITING AND OTHER GENE TARGETING APPROACHES

Over the past decade, gene editing approaches, particularly using the CRISPR/Cas9 system, have gained popularity as a potential therapy for correction of defective genes.¹⁶² Extensive work on these technologies has allowed for the development of new and improved editing enzymes with higher and more specific activity.¹⁶²

In addition, two related technologies, base editing and prime editing, have emerged as strategies to directly correct single nucleotide mutations without the need for endogenous cellular repair mechanisms, which may not be as precise.¹⁶² These gene editing approaches are advantageous when dealing with large genes such as *DNAH11* and *DNAH5*. In this instance, the mutation in the gene can simply be edited rather than relying on the replacement of a large DNA strand, which creates logistical issues for delivery. Another iteration of the CRISPR/Cas9 system, PASTE, allows for the insertion of large genes into specific sites without reliance on DNA repair pathways while reducing off-target events.¹⁶³

Many delivery mechanisms for gene editing machinery have been explored, including both viral and non-viral vector systems.¹⁶² Antisense oligonucleotides (ASOs) are another alternative to traditional gene therapies. Indeed, several ASO strategies for correcting both nonsense and splicing mutations in CF have been developed and are now in clinical trials,¹⁶⁴ suggesting that these strategies can be employed to correct mutations in other lung diseases such as PCD. The strategies mentioned earlier are important new advances in “personalized” gene therapies that can be tailored to the gene mutation in the affected individual.

PCD GENE THERAPY STUDIES

Despite the obvious potential of gene therapy for PCD, a limited number of studies have been conducted (Table 2); some of these studies have utilized LVV. Chhin et al.¹⁶⁵ used a vesicular stomatitis virus glycoprotein pseudotyped LVV, based on simian immunodeficiency virus, to transduce *DNAI1* patient-derived cells *in vitro*, which resulted in the improvement of CBF as well as the re-establishment of ODA content in the ciliary axoneme.¹⁶⁵

In a separate study, Ostrowski et al. used an influenza hemagglutinin (HA)-pseudotyped LVV to deliver *Dnaic1* to the mouse lung.¹⁶⁶ In ALI cultures of *Dnaic1* mouse airway epithelia transduced apically with the LVV, an increase in airway surface area covered by cilia was observed.¹⁶⁶ Interestingly, intranasal delivery of an HA-pseudotyped LVV encoding a reporter (β -galactosidase) transgene to PCD mice was ineffective at transducing the cells of the nasal epithelium,¹⁶⁶ which in this mouse model is the site of disease pathology.¹⁰⁰ The authors speculated that the disease pathology present in the nasal epithelium inhibited effective LVV-mediated transduction.¹⁶⁶ Lai et al. evaluated the utility of HA-pseudotyped FIV and HIV LVVs expressing transcription activator-like effector nucleases to treat *DNAH11* mutant epithelial spheroids derived from PCD patient cells.¹⁶⁷ Their approach resulted in site-specific recombination and effectively restored ciliary beating in about 30% of ciliated cells.¹⁶⁷

In recent years, there has been significant interest in the development of a gene therapeutic for PCD, and several companies have initiated gene therapy programs for PCD using non-viral vectors. Translate Bio (acquired by Sanofi; www.sanofi.com) used LNPs to deliver mRNA to the lung and demonstrated that delivery of *DNAI1* mRNA to CD-1 wild-type mouse lung, by either intratracheal instillation or nebulization, resulted in detectable expression of *DNAI1* by both western blot and immunohistochemistry.¹⁶⁸ In addition, treatment with LNP-*DNAI1* *in vitro* in a PCD model resulted in an increase in CBF.¹⁶⁸ Likewise, ReCode

Table 2. Primary ciliary dyskinesia gene therapy studies

| Authors/Company | Year | Gene | Model | Approach/Vector | Key Findings | Refs. |
|---------------------|------|----------------------------|---|--|---|---------|
| Chhin et al. | 2009 | <i>Dnaic1</i> <i>DNAI1</i> | Patient HAE cells | VSV-G pseudotyped LVV (SIV) | Improvement in CBF; increase in ODA content in axoneme | 165 |
| Ostrowski et al. | 2014 | <i>Dnaic1</i> <i>DNAI1</i> | Mouse ALI cultures; conditional KO mouse | HA-pseudotyped LVV | Increase in number of ciliated cells <i>in vitro</i> ; poor transduction of PCD mouse airways | 166 |
| Lai et al. | 2016 | <i>DNAH11</i> | Patient-derived spheroids of epithelial cells | HA-pseudotyped LVV (expressing TALENs) | Site-specific recombination and normalization of ciliary beating (33%) and pattern (29%) in cells | 167 |
| Munye et al. | 2016 | <i>DNAH5</i> | Airway cells <i>in vitro</i> ; mice (lung) | Liposome-minicircle DNA | Expression from minicircle DNA was higher than from plasmid DNA <i>in vivo</i> | 173 |
| ETHRIS | 2019 | <i>CCDC40</i> | Patient HAE cells | LNP-mRNA | Restoration of <i>CCDC40</i> expression, improved MCC | 172 |
| Translate Bio | 2021 | <i>DNAI1</i> | Mice (lung) | LNP-mRNA | <i>DNAI1</i> expression detected in mouse lung | 168 |
| ReCode Therapeutics | 2022 | <i>DNAI1</i> | KO HAE cells, mouse airway cells <i>in vitro</i> , and NHP (lung) | LNP-mRNA | Improved ciliary activity and rescue of function <i>in vitro</i> , LNP-mRNA was well tolerated in NHP | 169,170 |

ALI, air-liquid interface; CBF, ciliary beat frequency; HA, hemagglutinin; HAE, human airway epithelial; KO, knockout; LVV, LV vectors; MCC, mucociliary clearance; NHP, nonhuman primate; ODA, outer dynein arm; PCD, primary ciliary dyskinesia; SIV, simian immunodeficiency virus; TALEN, transcription activator-like effector nuclease; VSV-G, vesicular stomatitis virus glycoprotein.

Table 3. Gene therapy approaches for primary ciliary dyskinesia

| Vector/Strategy | Advantages | Disadvantages |
|-------------------|---|--|
| AAV | Wide tissue tropism Low immunogenicity High titer production | Small packaging capacity Non-integrating: requires redosing |
| Lentivirus | Large packaging capacity Integration leads to long-term expression | Risk of insertional mutagenesis Production issues for some pseudotypes |
| Non-viral vectors | Capable of carrying various large payloads Low immunogenicity | Relatively inefficient <i>in vivo</i> Requires redosing |
| Gene editing | Direct targeting of genomic mutation | Requires an efficient vector delivery system Risk of off-target effects |
| ASOs | Advantageous for splicing mutations | Requires an efficient vector delivery system Requires redosing |

AAV, adeno-associated virus; ASO, antisense oligonucleotide.

Therapeutics (<https://recodetx.com/pipeline/>) is developing an LNP-mRNA for people with PCD *DNAI1* mutations, and reported that *in vitro* delivery of LNP-DNAI1 to *DNAI1*-knockdown HAE cells improved ciliary activity, and also rescued ciliary activity in *Dnaic1* KO mouse airway cells.¹⁶⁹ Of note, these LNPs were also well tolerated when delivered to the lungs of non-human primates.¹⁷⁰ ReCode announced in March 2023 that the dosing of a cohort of healthy people with the LNP-DNAI1 product was initiated.¹⁷¹ ETHRIS has also developed an LNP-mRNA gene therapeutic, ETH42, for people with *CCDC40* mutations (<https://www.ethris.com/pipeline>). Early reports indicate that the restoration of *CCDC40* expression in cells from affected people improved MCC.¹⁷²

In addition to the non-viral work by the companies cited earlier, Munye et al.¹⁷³ utilized liposome-delivered minicircle DNA to demonstrate the expression of *DNAH5* following the transfection of airway cells *in vitro*. Interestingly, when the liposome-delivered minicircle DNA was administered *in vivo* it was less immunogenic, and resulted in more durable *DNAH5* expression, when compared with that of plasmid DNA. However, *DNAH5* expression from minicircle DNA significantly decreased 14 days after dosing.¹⁷³

CONCLUSION

Gene therapy holds enormous potential for the treatment of PCD. Different gene therapy approaches for PCD discussed in this review are summarized in Table 3. Since PCD is primarily an autosomal recessive disease, in most cases gene replacement is an appropriate therapeutic strategy. The lack of disease in heterozygote people suggests that correction of PCD can be achieved with $\leq 50\%$ of normal PCD gene expression. Viral vectors based on AAV and LVV have been developed to effectively target ciliated cells of the upper and lower airways.

In addition, several non-viral strategies, currently under development, may overcome some disadvantages associated with using viral vectors. Proof-of-concept of gene

therapy for PCD has been demonstrated in several model systems.^{165–167,172,173} These studies serve as evidence that ciliated cells with immotile or defective cilia can, indeed, be corrected.

However, despite its obvious potential, numerous challenges to effective gene therapy for PCD remain. Although the ciliated cells of the airway can be readily accessed via the inhaled route, the overall airway surface is large, and effective physical delivery of gene therapeutics to all lung areas remains a technical challenge.

The development of new delivery devices that maximize vector deposition in the airway, while at the same time minimizing vector loss, is essential. In addition, the airway possesses numerous physical and biological barriers, such as the mucus layer, MCC, antimicrobial agents, and immune cells, all of which are expected to inhibit, to some degree, the uptake of the gene therapeutic by ciliated epithelial cells.

Although effective vector-mediated targeting of ciliated cells has been observed, it remains to be seen whether vector-mediated gene transfer to ciliated cells can be improved. Strategies such as LVV pseudotyping or directed evolution of AAV vectors may aid in the development of vectors that target ciliated epithelial cells more efficiently.

Another important consideration for PCD gene therapy is that ciliated epithelial cells have a limited half-life *in vivo*,¹⁷⁴ thus requiring vector redosing to maintain the therapeutic benefit. In this instance, targeting the basal stem cells of the lung with an integrating vector such as an LVV may be required. A potential risk of this approach, and indeed of any approach where broad transduction is achieved, is the overexpression of ciliary proteins in non-ciliated cells.

It remains unknown whether overexpression of ciliary proteins in target or non-target cells can lead to cytotoxicity; future studies will be required to address this issue. Strategies to restrict expression of the therapeutic protein to ciliated cells by use of a cell-specific promoter (such as FOXJ1,¹⁴⁷) can be employed to mitigate the potential risk of overexpression-associated toxicity in non-target cells.

Another key challenge for the clinical development of a PCD gene therapeutic is that the disease is caused by mutations in more than 50 genes,²⁰ with no one gene accounting for over 50% of cases.²³ As such, the gene therapeutic must be tailored to each gene if the underlying genetic cause is to be targeted.

Further, the two most common PCD genes, *DNAH11* and *DNAH5*, are over 13 kb in size, which presents an additional challenge for a gene replacement strategy due to the packaging capacity of the currently available gene transfer vectors. For people with mutations in these genes, alternative molecular strategies will be required, such as gene editing or the engineering of vector therapeutics that can accommodate very large nucleic acid payloads.

Lastly, a major unknown in PCD is the number of ciliated cells that need to be corrected for a clinical benefit to be attained. To date, only one report addresses this question, and

estimates that restoring *DNAI1* to 20% of normal levels is sufficient to restore detectable MCC.¹⁶⁶ It is unknown how this percentage translates to the required number of gene-corrected ciliated cells. Further research into the impact of gene dosage of ciliated cells and its effect on physiologically relevant levels of ciliary beat and MCC is warranted to understand the threshold of gene expression required for partial or full restoration of lung function in people with PCDD.

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N.W.K. and M.P.L. conceived and designed the outline of the review. All authors contributed to the review, read and approved the final manuscript.

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