HHS Public Access

Author manuscript

Pediatr Pulmonol. Author manuscript; available in PMC 2021 December 27.

Published in final edited form as:

Pediatr Pulmonol. 2020 July ; 55(7): 1828–1837. doi:10.1002/ppul.24809.

Childhood rare lung disease in the 21st century: "-omics" technology advances accelerating discovery

Timothy J. Vece, MD[1], **Jennifer A. Wambach, MD**[2], **James S. Hagood, MD**[1]

[1]Division of Pediatric Pulmonology, Program for Rare and Interstitial Lung Disease, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[2]Division of Newborn Medicine, Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

Abstract

Childhood rare lung diseases comprise a large number of heterogeneous respiratory disorders that are individually rare but are collectively associated with substantial morbidity, mortality, and healthcare resource utilization. Although the genetic mechanisms for several of these disorders have been elucidated, the pathogenesis mechanisms for others remain poorly understood and treatment options remain limited. Childhood rare lung diseases are enriched for genetic etiologies; identification of the disease mechanisms underlying these rare disorders can inform the biology of normal human lung development and has implications for the treatment of more common respiratory diseases in children and adults. Advances in "-omics" technology, such as genomic sequencing, clinical phenotyping, biomarker discovery, genome editing, in vitro and model organism disease modeling, single-cell analyses, cellular imaging, and high-throughput drug screening have enabled significant progress for diagnosis and treatment of rare childhood lung diseases. The most striking example of this progress has been realized for patients with cystic fibrosis for whom effective, personalized therapies based on *CFTR* genotype are now available. In this chapter, we focus on recent technology advances in childhood rare lung diseases, acknowledge persistent challenges, and identify promising new technologies that will impact not only biological discovery, but also improve diagnosis, therapies, and survival for children with these rare disorders.

Keywords

emerging technologies; genomic diagnosis; rare lung disease

1 | INTRODUCTION

There have been dramatic advances in pediatric lung disease in recent years that have significantly impacted patient care. Significant progress has been made in our understanding of the underlying mechanisms of cystic fibrosis, and we now have targeted, precision

Correspondence: James S. Hagood, MD, Division of Pediatric Pulmonology, Program for Rare and Interstitial Lung Disease, University of North Carolina at Chapel Hill, 202 Mary Ellen Jones CB7578, 116 Manning Drive, Chapel Hill, NC 27599-7578. jhagood@unc.edu.

therapy available for up to 90% of cystic fibrosis patients that leads to significant clinical improvement.[1–3] There are also a number of adult interstitial lung diseases for which improved understanding of the underlying mechanisms of disease have led to precision medicine, such as the use of sirolimus for lymphangioleiomyomatosis.[4] The clinical improvement in lymphangioleiomyomatosis led to the use of sirolimus for other lymphatic anomalies including pediatric lymphatic anomalies, with some significant clinical improvements.[5,6]

Advances have been slower in children's interstitial lung disease (chILD), likely due to the heterogeneous and rare nature of the disorders. Nevertheless, there have been important advances in our understanding of disease mechanisms and genotype-phenotype interactions in chILD. The addition of the suffix "-omics" to a molecular term (eg, genomics, proteomics) indicates a global, comprehensive analysis of a set of molecules (eg, sequencing the entire genome), and many of the technologies we discuss here are based in or rely on this type of approach (discussed further in Section 4.1 below). This review will focus on some of the newer technologies and recent advances in chILD diagnosis and treatment, and will offer potential avenues for further advances in these rare, pediatric lung diseases.

2 | ADVANCES IN DIAGNOSIS AND CLINICAL PHENOTYPING

A number of testing modalities have been used for the diagnosis of chILD disorders.[7–9] A detailed clinical history is always required, with special attention to possible familial disease, as a number of chILD disorders are inherited. Chest computed tomography (CT) is among the earliest tests performed in most patients with suspected chILD, and is required to demonstrate the diffuse parenchymal changes frequently seen in these disorders.[9] Chest CT is considered by many experienced clinicians to be diagnostic in some forms of chILD, such as neuroendocrine hyperplasia of infancy (NEHI) and bronchiolitis obliterans, with a compatible clinical history.[10–13] Laboratory testing can be useful to evaluate for infectious processes, and to aid in the diagnosis of specific disorders such as antineutrophil cytoplasmic antibody (ANCA) vasculitis.[9,14,15] While some disorders can be diagnosed without lung biopsy, such as NEHI or genetic disorders of surfactant metabolism, many chILD disorders require lung biopsy for definitive diagnosis. Idiopathic pulmonary capillaritis is indistinguishable from idiopathic pulmonary hemosiderosis clinically, therefore a lung biopsy is critical to distinguish between these causes of pulmonary hemorrhage, as treatment differs for these two disorders.[16]

While traditional diagnostic testing is required for initial characterization of chILD disorders, there are issues that necessitate newer testing modalities. Although a detailed history is critical, it rarely provides a definitive diagnosis. Chest CT exposes the patient to radiation, and findings are most often nonspecific. Even in disorders for which CT can be diagnostic, such as NEHI, there can be variability in chest CT findings, such as in atypical NEHI.[17,18] Lung biopsies may suggest diagnoses, but are often not diagnostic per se, such as occurs with a nonspecific interstitial pneumonia (NSIP) pattern, which in older children can be seen in surfactant disorders, as well as a number of connective disease disorders with pulmonary involvement.[19,20] It is important to remember, therefore, that in many cases even a well-defined histopathologic pattern on lung biopsy is not necessarily diagnostic. Recent

advances in genomic testing, biomarker discovery, and expanded gene panels have been crucial for extending our understanding of chILD disorders.

2.1 | Advances in genetic disorders of surfactant metabolism

Genetic disorders of surfactant metabolism were among the first chILD disorders with an identified genetic basis.[21–23] Currently recognized disorders in this category are due to pathogenic sequence variations in one of seven genes, with variable age of onset, inheritance patterns, and extrapulmonary findings (Table 1). While some of the genetic causes of surfactant dysfunction have been known for over 20 years, recent advances, particularly in genotype-phenotype relationships, have significantly improved our understanding of the natural history of these disorders.

In subjects with biallelic variants in *ABCA3*, genotype can be predictive of presentation and outcome. Individuals with biallelic null (nonsense or frameshift) variants in *ABCA3* present with respiratory failure at birth and die within the first year of life without lung transplantation, whereas those with either one or two missense, in-frame insertion/deletions, or splicing variants have variable presentation and clinical courses (mortality of 70% and 60% at 1 year of life, respectively).[24] These genotype-phenotype correlations have facilitated family discussions regarding early referral for lung transplant or withdrawal of life support as appropriate courses of action for infants with biallelic loss-of-function variants in *ABCA3*.[24]

There have also been significant advances in surfactant protein (SP)-C–related lung disease. The understanding that deleterious variants in the region of *SFTPC* coding for the terminal BRICHOS domain mediate different mechanisms of cellular dysfunction vs those found outside that region has been a critical insight.[25] The BRICHOS domain is important to maintain the strongly hydrophobic early surfactant from aggregating before being incorporated into lamellar bodies. BRICHOS variants, which are the most common variants identified in symptomatic individuals, cause aggregation in the endoplasmic reticulum (ER) and Golgi apparatus, and may inhibit autophagy, an important mechanism for degradation and recycling of cellular components that is critical to cell health. In contrast, non-BRICHOS variants cause activation of the unfolded protein response and ER stress. The development of a SP-C disease mouse model demonstrated significantly increased ER stress with resultant fibrosis offering clues to the pathogenesis of human disease.[26] As more research is needed, these findings may have important implications for therapeutic interventions: two of the available treatments for SP-C–related lung disease, azithromycin and hydroxychloroquine, reduce inflammation and inhibit autophagy, respectively, and thus may have differing efficacy based on genotype.

Recent insights have also improved our understanding of genotype-phenotype correlations for sequence variants of *NKX2.1*, which encodes the thyroid transcription factor 1. Classically, patients with variants in *NKX2.1* manifested brain-thyroid-lung disease with chorea, hypothyroidism, and chronic lung disease.[27] Recent studies have identified phenotypic variability with some individuals having neurologic, pulmonary, and thyroid disease and others with findings limited to one organ system.[28] Patients can have variable pulmonary findings ranging from severe, neonatal respiratory disease consistent with

surfactant dysfunction to recurrent pulmonary infections, as SPs A and D are involved in innate immunity. In fact, individuals can have multiple histopathologic findings within the same lung biopsy.[28]

A newly understood cause of pulmonary alveolar proteinosis (PAP), one of the classic presentations of surfactant-related disorders, has recently been reported. Individuals with variants in methionyl-tRNA synthetase (*MARS*) were found to manifest PAP in lung biopsies.[29] This discovery was made using whole exome sequencing (WES) from a number of affected individuals within a geographically constrained cohort. Variants in *MARS* also cause hepatic disease, which is an important diagnostic clue for clinicians evaluating patients with PAP. *MARS* belongs to a group of genes that encode for aminoacyl transfer RNA synthetases, which are critical in forming individual transfer RNA (tRNA) for translating messenger RNA (mRNA) and protein translation. Variants in these genes cause widely variable diseases, some of which also have a propensity for lung disease.[30] In adults, autoantibodies to tRNA synthetase are associated with dermato/polymyositis with subsequent high risk for interstitial lung disease.[31] Studying how variants in, as well as autoantibodies to, these tRNA synthetases result in lung disease could lead to an improved understanding of the mechanisms of lung disease in both disorders of surfactant metabolism and immune dysregulation, as well as potential novel treatment approaches.

2.2 | Newly described disorders found with genetic testing

Advances in genetic testing have increased the number and scope of chILD diseases, especially for chILD disorders related to immune dysfunction, which account for the majority of chILD diagnoses in children over 2 years of age. Advances in rapid, massively parallel genome sequencing have permitted rapid and affordable WES, leading to identification of multiple new disorders.

One of these newly described diseases is the first known genetic cause of pulmonary hemorrhage. Pathogenic variants identified in coatomer associated protein alpha (*COPA*) were found in five un-related families with familial pulmonary hemorrhage and arthritis.[32] Since the original publication, multiple other reports have expanded both the phenotype of COPA syndrome and the underlying mechanisms of disease pathogenesis.[33–36] Currently, it is hypothesized that increased ER stress in COPA syndrome leads to skewing the immune system to autoimmunity and the resultant symptoms, possibly through increased type 1 interferon pathway activation.[36]

Two other recently described chILD disorders both involve dysregulated immune stimulation and lymphoproliferation. Variants in *LRBA* and *STAT3* gain of function variants both lead to unregulated expansion of immune cells.[37,38] In the lungs, this leads to evidence of lymphoproliferation, with nests of immune cells in the distal airways and interstitium, as well as autoimmune lung disease. These recently described disorders demonstrate the utility of exome sequencing to improve diagnosis for children with rare lung diseases.

Importantly, while the individual disorders are too rare for controlled clinical trials, improved understanding of the disease mechanisms may inform more specific therapies. Recently, effective treatment of COPA syndrome patients with ruxolitinib or baricitinib

to inhibit type 1 interferon pathways has been reported.[39,40] Patients with *LRBA* variants treated with abatacept, an agonist of cytotoxic T lymphocyte antigen-4, the downstream protein that is deficient in *LRBA* variants, showed significant improvement in chest CT findings and lung function testing.[37] These examples offer a paradigm for improved and targeted therapy in chILD.

2.3 | Genetic testing choices in chILD

As clinical genomic testing advances rapidly, determining the most efficient and accurate genomic tests for chILD disorders is imperative. Genetic panels, which can include multiple genes associated with a clinical syndrome, are commonly ordered. The molecular diagnosis of cystic fibrosis is usually made with panels of common cystic fibrosis transmembrane regulator (*CFTR*) variants as part of newborn screening in most states.[41] Gene panels are also frequently ordered for primary ciliary dyskinesia (PCD), and in fact recent guidelines on PCD diagnosis recommend genetic testing before invasive testing.[42,43] WES, which involves sequencing all exons in the human genome using rapid, massively parallel sequencing, is widely available and may decrease time to chILD diagnosis as has been recently reported for molecular diagnosis of inborn errors of metabolism.[44] WES has also been used for testing for neurologic conditions, and a molecular diagnosis was found in up to 40% of patients (including some with two molecular diagnoses) and has been shown to be a cost-effective, diagnostic method.[45] Finally, whole genome sequencing (WGS), which has previously been too expensive, difficult to analyze, or available only as part of research protocols, is becoming more accessible for clinical diagnosis. Rapid WGS for disorders in the neonatal intensive care unit has been shown to be effective[46,47] and may decrease time to diagnosis.

There are important considerations with all three approaches to genetic testing for chILD. Although genetic panels may be individually less expensive and easier to interpret than WES or WGS, candidate gene panels may miss potential diagnoses if the gene is not included on the panel, or if deletion or duplication of a gene is present rather than a sequence variant. Additionally, with an ever-expanding list of genes associated with chILD, most clinically available panels are not comprehensive and there is tremendous variability among vendors, which can confuse clinicians and patients. WES is often more expensive than candidate gene panels (although the cost has been decreasing rapidly), and there is potential for incidental genomic findings (eg, variants in cancer predisposing genes),[48] which may be stressful for patients and families. Also, some exons may be missed with WES. WGS is the most expensive option and requires significant bioinformatics expertize to interpret results.

2.4 | Biomarkers in chILD

In addition to genetic testing, identification of disease-associated biomarkers may aid in the diagnostic evaluation of chILD. A recent study by Deterding et al[49] showed unique protein signatures in NEHI and disorders of surfactant metabolism. Other studies have focused on chest CT findings as a biomarker for NEHI.[17] Additionally, there are a number of blood biomarkers in adult and pediatric interstitial lung diseases, including KL-6, MUC5B, and the metalloproteinases that could provide important insights into pediatric diseases.[50,51] All of

these biomarkers require further study, however, they offer potential advances in diagnosis and disease monitoring in chILD.

3 | NEW APPROACHES TO IDENTIFY MOLECULAR MECHANISMS AND THERAPEUTIC TARGETS

The interpretation of clinical significance for rare, often private genomic variants identified by WES or WGS remains a significant challenge for the application of genomic data to diagnosis, pathogenesis, and treatment, that is, "personalized" or "precision" medicine. The goal of personalized medicine is to tailor therapies for individual patients based on their genomic variants. Monogenic disorders result from alteration of a single gene, are inherited or spontaneously arise in autosomal dominant, autosomal recessive or X-linked dominant/recessive patterns (eg, Mendelian disorders), and have emerged as potential targets for individualized therapeutic interventions. Although individually rare, over 100 monogenic respiratory disorders have been identified,[2] and the most well characterized is cystic fibrosis resulting from recessive variants in the *CFTR* gene. Treatment for individuals with cystic fibrosis has been transformed by the application of sequencing technologies, model systems for functional characterization of *CFTR* variants, and high-throughput drug screening.[1,3,52] While over 1700 individual *CFTR* variants have been identified, *CFTR* variants are grouped by pathogenesis mechanisms including absence of mature protein (nonsense, frameshift variants), disruption of intracellular trafficking to the epithelial cell surface, and impaired chloride transport (https://www.cftr2.org).[53] Functional characterization of *CFTR* variants and high-throughput screening of small-molecule compounds have led to personalized therapies (eg, VX-770, VX-661, VX-809, VX-445) based on *CFTR* genotype,[1–3,54–57] with combination drugs that can now treat approximately 90% of CF patients, and can serve as a paradigm for understanding and treating other monogenic lung diseases.

3.1 | Interpretation of genomic variation

Considerable phenotypic variability is observed among individuals with monogenic respiratory disorders, likely related to the specific types of variants[24,58–60] but also by genetic, epigenetic, developmental, and/or environmental modifiers. The majority of variants identified among individuals with monogenic respiratory diseases are extremely rare (minor allele frequency <0.005) or private.[24,61] Null (nonsense or frameshift) variants have more predictable effects on protein function, whereas the effects of missense variants, which account for majority of identified variants, are less predictable (silent or pathogenic) and these variants are often classified as "variants of uncertain significance" or VUS.[24,48] Additional variants include insertion/deletions ("indels") which can be in-frame or disrupt the reading frame, noncoding variants which can alter RNA splicing or regulation of gene expression, and copy number variants (deletions or duplications). Strategies to aid in the interpretation of these genomic variants include familial segregation of variants with phenotype, in silico pathogenicity prediction algorithms, data sharing, and functional assays.[62] In familial segregation studies, specific relatives (affected or unaffected) are evaluated to determine whether the VUS identified in the proband segregates with the disease phenotype. In silico bioinformatic prediction algorithms (ie, using computational modeling to predict whether a variant affects molecular function) can be extremely useful

for large-scale screening of variant predicted pathogenicity,[63] but typically focus on coding variation, frequently conflict in their interpretation, and currently provide in-sufficient evidence for clinical application.[64–67] Data shared from large-scale genome and exome sequencing projects (~140,000 adults) and chromosomal microarrays (~35,000 individuals) have been organized into web-based, easily-accessed databases which provide variant frequencies (https://gnomad.broadinstitute.org, https://decipher.sanger.ac.uk).[68,69] On the individual gene level, Gene-Matcher is a web-based tool designed to connect clinicians and investigators with interests in the same gene to increase understanding of rare diseases and identify novel candidate genes (https://genematcher.org).[70] Functional studies can be powerful tools to assess variant pathogenicity, but are time and resource intensive and not scalable for application to the hundreds to thousands of variants identified with exome and genome sequencing. Functional assays studies typically use transfection or viral transduction strategies to overexpress variant constructs in immortalized cell lines, which may not accurately model the biologic impact of the variant on in vivo gene or protein function, resulting in misclassification of variant pathogenicity.[48,71,72]

3.2 | Advances in computational and functional methods

Despite these significant challenges, methods for computational and functional assessment of genomic variants continue to advance. REVEL (Rare Exome Variant Ensemble Learner) is an in silico ensemble method that combines results from 18 different individual prediction algorithms into a single score.[73] CADD (Combined Annotation Dependent Depletion) integrates diverse genomic features (neighboring sequence context, gene models, evolutionary constraint, epigenetic results, functional predictions) into a single score for both coding and noncoding variants.[64,67] Both REVEL and CADD can be applied to large-scale genomic data for variant annotation and prioritization for data sharing and functional studies. Improvements in efficiency and scalability of functional studies have been enabled by multiplexed techniques. Multiplexed mutagenesis combines single-stranded DNA templates, endonuclease nicking and exonuclease degradation, isothermal assembly, and amplification for cloning to generate multiple variant constructs in a single reaction.[74] Saturational genome editing leverages CRISPR/Cas9 RNA-guided cleavage (see below), multiplex homology-directed repair and donor templates to generate all possible variants within specific gene regions in the native gene context.[75] Multiplexed assays of variant effect (MAVEs) can simultaneously measure the functional effects of all possible variants within protein coding, intronic, or transcriptional regulatory regions using fluorescent reporters, enzymatic activity, protein interactions or other molecular or cellular phenotypes.[71,76] Multiplexed assays share a similar framework of variant synthesis, introduction of variants into a model system, selection for the phenotype of interest, and post-selection library sequencing to correlate variants with phenotype.[71] In addition to considerations of reproducibility, scalability, and cost, ideal multiplexed assays have large, measurable differences between pathogenic and benign variants. Several different multiplexed assays may be required for specific regions of the gene or protein.[77,78] Coordinated efforts to produce large-scale functional data for specific genes (eg, medically actionable genes such as *BRCA1*) will provide a genotype-phenotype atlas to assist with interpretation of genomic variants.[79] For rare lung diseases, multiplexed technologies will permit functional characterization of all possible variants within a specific gene, potentially

before any of them are identified in a symptomatic patient. This approach may be especially relevant for missense, in-frame indels and splicing variants in genes associated with neonatal respiratory failure (eg, *ABCA3, SFTPB, SFTPC*), in that genotype-phenotype correlations are variable, with some patients improving with chronic ventilation and some patients progressing to lung transplantation.[24,80–83]

3.3 | High-throughput small-molecule screening

The development of personalized therapies for patients with cystic fibrosis, including correction of intracellular trafficking ("correctors") and improvement of chloride channel activity ("potentiators"), have been achieved through high-throughput drug screening campaigns.[84,85] Small-molecule compounds are chemically stable, bioavailable, and have become the predominant therapy for correction of human monogenic diseases.[86] Therapeutic strategies for small-molecule correction of monogenic diseases include inhibition of target activity, activation of abnormal proteins (eg, correction of misfolding or mistrafficking,[54,87] impaired channel activity[2]), and restoration of normal levels of protein expression.[88] A wide spectrum of biochemical and cell-based assays can be adapted, miniaturized, and optimized in microplate format for high-throughput screening.[89–92] Multiple small-molecule libraries are commercially available for screening including smaller libraries of FDA-approved compounds which have safety and tolerability data (eg, Pharmakon library of ~1800 compounds), as well as larger libraries of up to ~130,000 compounds. Assay performance is typically monitored by using Z' factors[93] for assay readouts from the mutant proteins compared to wild-type or the mutant protein with or without a corrector.[63] Candidate compounds identified by high-throughput screens require significant medicinal chemistry evaluation and prioritization to optimize potency, selectivity, and safety, and reduce off-target effects.[88] In addition to variant-specific treatments for cystic fibrosis, high-throughput drug screening has been used to identify small molecules to ameliorate the accumulation of misfolded proteins resulting from variants in *SERPINA1* which encodes α-1-antitrypsin,[94–96] and to inhibit myofibroblast differentiation for treatment of pulmonary fibrosis.[97]

4 | EXPERIMENTAL MODELS AND EMERGING RESEARCH TOOLS

The approaches described above to unlock disease mechanisms and uncover therapeutic targets have been revolutionized in the past few decades by powerful emerging genomic tools, experimental model systems, and computational and bioinformatic advances.

4.1 | The role of sequencing and the -omics revolution

A critical technology underlying much of contemporary biomedicine, and many of the approaches described in this review, is rapid and accurate nucleic acid sequencing, and so-called "massively parallel" sequencing, which has experienced a dizzying acceleration of speed combined with a plummeting of cost, revealing genomic information at a previously incomprehensible rate.[98] This has ushered in the era of multiple "-omics," aimed initially at uncovering entire genomes (the entire DNA sequence of an organism), but the technology has also facilitated rapid analysis of messenger RNA (transcriptomics), microRNA and other noncoding RNAs, as well as analysis of DNA modifications such as cytosine methylation,

and histone modifications (epigenomics). Simultaneously, advances in mass spectroscopy and other technologies have allowed similarly rapid analysis of proteins (proteomics), lipids (lipidomics), and other metabolites (metabolomics). Rapid transcriptomics combined with microfluidics and/or ligation of specific adapters ("molecular barcoding") has now made it possible to analyze the transcriptional profile of thousands of single cells simultaneously (single-cell RNA sequencing; scRNA-seq), allowing the analysis of cellular heterogeneity at an unprecedented scale. This has already facilitated discovery of previously un-appreciated pulmonary cell types, such as the airway ionocyte, which expresses high levels of CFTR.[99,100] These technologies have ushered in a new era of cell biology, and funding efforts have been launched to develop comprehensive revised maps and atlases of tissues at the cellular and molecular level. The LungMAP (https://lungmap.net/) has provided a wealth of multi-omic data on the developing lung in mice and humans, with a focus on the alveolar parenchyma.[101,102] Combining massively parallel sequencing, molecular barcoding, and advanced microscopy approaches makes it possible to map high-content molecular data back to intact tissues,[103] further refining molecular mapping and atlas efforts, exemplified by the current second phase of the LungMAP as well as the Human Biomolecular Atlas Program (HuBMAP[104]) and Human Cell Atlas (https://www.humancellatlas.org/). A recent review captures how these emerging technologies have shaped our current understanding of lung development and regeneration after injury.[105] To date the application of these technological advances to human disease is only beginning, but landmark efforts to "map" rare lung diseases are beginning to emerge.[106]

4.2 | The importance and potential of model organisms

Model organisms help define the roles of genes in vivo. Work in yeast, fruit flies, worms and mice, for example, have uncovered a tremendous range of knowledge about fundamental biological mechanisms such as cell division and function, circadian rhythms, DNA repair and developmental biology. Fundamental studies of protein translation in yeast cells, for example, can uncover therapeutic approaches to correct CFTR function in cystic fibrosis,[107] arguably among the most dramatic therapeutic advances in current biomedicine. Critical technological advances include the ability to "knock out" or "knock in" genes in organisms, revealing effects in development and in laboratory models of disease[108]; use of reporters such as fluorescent protein genes for lineage tracing[109]; and conditional activation or inactivation of genes. Such advances have made it now possible to turn on or turn off expression of a particular gene in a particular group of cells in a particular tissue at precise time points, which is an incredible technological feat that is now taken as a matter of course in lung research.

4.3 | Gene editing

Another technological great leap forward has been enabled by rapid and precise genome editing technology. Using CRISPRs (clusters of regularly interspaced short palindromic repeats) together with the Cas9 enzyme, an RNA-guided endonuclease, cuts can be made in precise locations in the DNA sequence, facilitating the introduction of specific altered sequences. This allows, for example, the creation of a transgenic animal harboring a gene variant associated with disease in a group of patients, rapidly generating very precise models for studying disease pathogenesis and for testing novel therapies. CRISPR technology can

also be applied to in vitro screening approaches to interrogate a broad range of biological questions, and can accelerate drug discovery and development.[110] Perhaps the most exciting application of genome editing is the ability to correct a genetic defect in progenitor cells and transplant them back into patients, which has revitalized interest in gene therapy.[111]

4.4 | In vitro models

Mouse models have ushered in an era of highly granular understanding of lung biology and disease, but retain important limitations. Microscopic structure, cellular composition, and gene expression differ between mice and humans. Importantly, many therapeutic strategies that work in mice fail in humans. On the other hand, access to human lung tissue is limited and the single time point of tissue collection precludes longitudinal studies. Most available tissues are from explanted lungs at transplantation, which are at end stage with alterations that have progressed over years. Recent refinements in ex vivo and in vitro models have made it feasible to recapitulate key cellular interactions and pathophysiologic events over short- to medium-term timescales.

Lung "organoids," of varying cellular composition, have been used to model numerous aspects of lung and airways disease in vitro (reviewed in Barkauskas et al[112]). Recent studies using coculture of adult epithelial and mesenchymal cells recapitulate distal lung structure, function and response to profibrotic insults, and have yielded novel insights into the two-way communication between these cell types during homeostasis and evolution of fibrosis.[113,114] Organoids offer tremendous promise as tools to study complex cellular interactions and developmental and disease pathways using human tissue with relevance to particular diseases and individuals, but they are subject to some important limitations (reviewed in Lehmann et al[115]). They lack body axis, immune system, nerves, and vascular components. However with regard to cellular phenotypes and cellular interactions, they offer multiple advantages vs traditional two-dimensional (2D) tissue culture.

4.5 | Stem cells and regenerative medicine

The scientific breakthrough of the recent past with possibly the highest potential impact for rare diseases is the discovery that mature somatic cells can be reprogrammed to a pluripotent stem cell state with the addition of four transcription factors.[116] Induced pluripotent stem cells (iPSCs) can be derived from patients' tissues, allowing the possibility of recapitulating the effects of an individual's genotype on cellular functions in vitro. Many laboratories have expended tremendous effort into differentiating iPSCs into organ-specific cell types, including lung epithelial cells.[117] iPSC l-derived 3D organoids with genetic variants associated with Hermansky-Pudlak syndrome-associated interstitial pneumonia (HPSIP) were recently shown to recapitulate disease pathophysiology, with fibrotic features (contraction, extracellular matrix deposition, profibrotic gene expression) seen in organoids with gene variants associated with HPSIP.[118] The study of stem and progenitor cells, whether induced or isolated from bone marrow or other tissues, combined with technological advances in culture substrates and conditions, has ushered in an era of "regenerative medicine," making possible the therapeutic use of stem cells or their products to treat a wide variety of diseases, in addition to creating more accurate research models.

5 | DISCUSSION AND FUTURE PROSPECTS

How do these advances shape the future for children living with rare and interstitial lung diseases, as well as those of their caregivers and clinicians? It is possible using today's technology, even with an extremely rare clinical phenotype, to rapidly identify a potentially deleterious gene variant, predict its effects in silico, quickly (within months) generate an animal model, assess alterations at a cellular and molecular level, determine pathophysiologic mechanisms, and test therapeutic interventions. It is also possible without knowing the genetic basis of a disease to create iPSC-derived cells and organoids with which to study cellular pathophysiology and test therapeutic interventions using libraries of available compounds. Pediatric pulmonologists and others caring for children with complex respiratory disease in the past few decades have witnessed life-changing advances in care for diseases that in the past were certain lethal diagnoses, including cystic fibrosis and spinal muscular atrophy. The tools are already in place to develop similarly revolutionary approaches to almost any genetic disease.

What are the barriers? Rare diseases have complex phenotypes, and no individual clinician or clinical center is likely to have enough affected patients to advance knowledge regarding natural history, pathophysiology, and treatment effectiveness. National and even international registries permit aggregation of information from larger numbers of affected individuals. New knowledge and technological advances are being generated at a pace that is impossible even for academic specialists to keep up with, much less busy clinicians. Advocacy groups such as the Cystic Fibrosis Foundation (CFF) and PCD Foundation monitor research advances relevant to their constituencies and make the information available through newsletters and electronic communications. Groups supporting more rare disorders lack the personnel and resources for such efforts, as they are often composed of caregivers struggling to support individuals with complex care needs. Small organizations may need to join forces with other disease groups, or take advantage of the resources available through larger organizations such as the American Thoracic Society. Research is very expensive, particularly when it involves emerging technologies, so clinicians, researchers and advocacy groups need to work together to raise awareness of research needs and encourage funders, whether they be government agencies, corporations, philanthropic foundations or individual donors, to invest in rare disease research.

We are poised to see dramatic improvements in the care of chILD and other rare lung diseases, but the "next generation" of clinicians and researchers need to be optimally prepared to generate and deploy the novel diagnostics and therapies of the future. Specialists in training should study genomic science, data science, and bioinformatics so that they can access, understand, and leverage the massive and complex data currently being generated. We need more physician scientists with targeted training to bridge the gaps between "bedside, bench, and server." Even those who will not be engaged in direct discovery should be conversant enough with emerging tools and discoveries to be able to offer their patients full access to truly personalized care. Ideally we should facilitate communication among families, clinicians, cutting edge researchers, and funders, to spur collaboration and remove barriers to discovery and transformative therapy. The future is now.

ACKNOWLEDGMENTS

National Institutes of Health U01 HL134745 (JAW) and Children's Discovery Institute of St Louis Children's Hospital Foundation (JAW).

REFERENCES

1. Van Goor F, Hadida S, Grootenhuis PD, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. Proc Natl Acad Sci U S A. 2009;106:18825–18830. [PubMed: 19846789]
2. Accurso FJ, Rowe SM, Clancy JP, et al. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med. 2010;363:1991–2003. [PubMed: 21083385]
3. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med. 2011;365:1663–1672. [PubMed: 22047557]
4. Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angio-myolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N Engl J Med. 2008;358:140–151. [PubMed: 18184959]
5. Ozeki M, Nozawa A, Yasue S, et al. The impact of sirolimus therapy on lesion size, clinical symptoms, and quality of life of patients with lymphatic anomalies. Orphanet J Rare Dis. 2019;14:141. [PubMed: 31196128]
6. Ricci KW, Hammill AM, Mobberley-Schuman P, et al. Efficacy of systemic sirolimus in the treatment of generalized lymphatic anomaly and Gorham-Stout disease. Pediatr Blood Cancer. 2019;66:e27614. [PubMed: 30672136]
7. Deutsch GH, Young LR, Deterding RR, et al. Diffuse lung disease in young children: application of a novel classification scheme. Am J Respir Crit Care Med. 2007;176:1120–1128. [PubMed: 17885266]
8. Fan LL, Dishop MK, Galambos C, et al. Diffuse lung disease in biopsied children 2 to 18 years of age. Application of the chILD Classification Scheme. Ann Am Thorac Soc. 2015;12:1498–1505. [PubMed: 26291470]
9. Kurland G, Deterding RR, Hagood JS, et al. An official American Thoracic Society clinical practice guideline: classification, evaluation, and management of childhood interstitial lung disease in infancy. Am J Respir Crit Care Med. 2013;188:376–394. [PubMed: 23905526]
10. Brody AS, Crotty EJ. Neuroendocrine cell hyperplasia of infancy (NEHI). Pediatr Radiol. 2006;36:1328. [PubMed: 16957891]
11. Brody AS, Guillerman RP, Hay TC, et al. Neuroendocrine cell hyperplasia of infancy: diagnosis with high-resolution CT. Am J Roentgenol. 2010;194:238–244. [PubMed: 20028928]
12. Moonnumakal SP, Fan LL. Bronchiolitis obliterans in children. Curr Opin Pediatr. 2008;20:272–278. [PubMed: 18475095]
13. Guillerman RP. Imaging of childhood interstitial lung disease. Pediatr Allergy Immunol Pulmonol. 2010;23:43–68. [PubMed: 22332031]
14. Vece TJ, Fan LL. Interstitial lung disease in children older than 2 years. Pediatr Allergy Immunol Pulmonol. 2010;23:33–41. [PubMed: 22332030]
15. Deterding RR. Infants and young children with children's interstitial lung disease. Pediatr Allergy Immunol Pulmonol. 2010;23:25–31. [PubMed: 22332029]
16. Fullmer JJ, Langston C, Dishop MK, Fan LL. Pulmonary capillaritis in children: a review of eight cases with comparison to other alveolar hemorrhage syndromes. J Pediatr. 2005;146:376–381. [PubMed: 15756223]
17. Spielberg DR, Brody AS, Baker ML, Woods JC, Towe CT. Ground-glass burden as a biomarker in neuroendocrine cell hyperplasia of infancy. Pediatr Pulmonol. 2019;54:822–827. [PubMed: 30843378]
18. Rauch D, Wetzke M, Reu S, et al. Persistent tachypnea of infancy. Usual and aberrant. Am J Respir Crit Care Med. 2016;193:438–447. [PubMed: 26474448]
19. Chibbar R, Shih F, Baga M, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia with mutation in surfactant protein C in familial pulmonary fibrosis. Mod Pathol. 2004;17:973–980. [PubMed: 15133475]

20. Antoniou KM, Margaritopoulos G, Economidou F, Siafakas NM. Pivotal clinical dilemmas in collagen vascular diseases associated with interstitial lung involvement. Eur Respir J. 2009;33:882–896. [PubMed: 19336591]
21. Nogee LM, de Mello DE, Dehner LP, Colten HR. Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. N Engl J Med. 1993;328:406–410. [PubMed: 8421459]
22. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N Engl J Med. 2001;344:573–579. [PubMed: 11207353]
23. Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med. 2004;350:1296–1303. [PubMed: 15044640]
24. Wambach JA, Casey AM, Fishman MP, et al. Genotype-phenotype correlations for infants and children with ABCA3 deficiency. Am J Respir Crit Care Med. 2014;189:1538–1543. [PubMed: 24871971]
25. Peca D, Boldrini R, Johannson J, et al. Clinical and ultrastructural spectrum of diffuse lung disease associated with surfactant protein C mutations. Eur J Hum Genet. 2015;23:1033–1041. [PubMed: 25782673]
26. Katzen J, Wagner BD, Venosa A., et al. An SFTPC BRICHOS mutant links epithelial ER stress and spontaneous lung fibrosis. JCI Insight. 2019;4(6):pii: 126125. [PubMed: 30721158]
27. Carre A, Szinnai G, Castanet M, et al. Five new TTF1/NKX2.1 mutations in brain-lung-thyroid syndrome: rescue by PAX8 synergism in one case. Hum Mol Genet. 2009;18:2266–2276. [PubMed: 19336474]
28. Hamvas A, Deterding RR, Wert SE, et al. Heterogeneous pulmonary phenotypes associated with mutations in the thyroid transcription factor gene NKX2–1. Chest. 2013;144:794–804. [PubMed: 23430038]
29. Hadchouel A, Wieland T, Griese M, et al. Biallelic mutations of methionyl-tRNA synthetase cause a specific type of pulmonary alveolar proteinosis prevalent on reunion island. Am J Hum Genet. 2015;96:826–831. [PubMed: 25913036]
30. Fuchs SA, Schene IF, Kok G, et al. Aminoacyl-tRNA synthetase deficiencies in search of common themes. Genet Med. 2019;21: 319–330. [PubMed: 29875423]
31. Takei R, Yamano Y, Kataoka K, et al. Predictive factors for the re-currence of anti-aminoacyl-tRNA synthetase antibody-associated interstitial lung disease. Respir Investig. 2020;58:83–90.
32. Watkin LB, Jessen B, Wiszniewski W, et al. COPA mutations impair ER-Golgi transport and cause hereditary autoimmune-mediated lung disease and arthritis. Nat Genet. 2015;47:654–660. [PubMed: 25894502]
33. Vece TJ, Watkin LB, Nicholas S, et al. Copa syndrome: a novel autosomal dominant immune dysregulatory disease. J Clin Immunol. 2016;36:377–387. [PubMed: 27048656]
34. Taveira-DaSilva AM, Markello TC, Kleiner DE, et al. Expanding the phenotype of COPA syndrome: a kindred with typical and atypical features. J Med Genet. 2019;56:778–782. [PubMed: 30385646]
35. Tsui JL, Estrada OA, Deng Z, et al. Analysis of pulmonary features and treatment approaches in the COPA syndrome. ERJ Open Res. 2018;4(2), 10.1183/23120541.00017-2018
36. Volpi S, Tsui J, Mariani M, et al. Type I interferon pathway activation in COPA syndrome. Clin Immunol. 2018;187:33–36. [PubMed: 29030294]
37. Lo B, Zhang K, Lu W, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science. 2015;349:436–440. [PubMed: 26206937]
38. Milner JD, Vogel TP, Forbes L, et al. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Blood. 2015;125:591–599. [PubMed: 25359994]
39. Fremond ML, Legendre M, Fayon M, et al. Use of ruxolitinib in COPA syndrome manifesting as life-threatening alveolar haemorrhage. Thorax. 2019;75(1):92–95. [PubMed: 31666386]
40. Krutzke S, Rietschel C, Horneff G. Baricitinib in therapy of COPA syndrome in a 15-year-old girl. Eur J Rheumatol. 2019;7(Suppl 1):S78.

41. Rosenfeld M, Sontag MK, Ren CL. Cystic fibrosis diagnosis and newborn screening. Pediatr Clin North Am. 2016;63:599–615. [PubMed: 27469178]
42. Shapiro AJ, Davis SD, Polineni D, et al. Diagnosis of primary ciliary dyskinesia. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;197:e24–e39. [PubMed: 29905515]
43. Paff T, Kooi IE, Moutaouakil Y, et al. Diagnostic yield of a targeted gene panel in primary ciliary dyskinesia patients. Hum Mutat. 2018; 39:653–665. [PubMed: 29363216]
44. Stray-Pedersen A, Sorte HS, Samarakoon P, et al. Primary immunodeficiency diseases: genomic approaches delineate heterogeneous Mendelian disorders. J Allergy Clin Immunol. 2017;139:232–245. [PubMed: 27577878]
45. Cordoba M, Rodriguez-Quiroga SA, Vega PA, et al. Whole exome sequencing in neurogenetic odysseys: an effective, cost- and time-saving diagnostic approach. PLOS One. 2018;13:e0191228. [PubMed: 29389947]
46. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med. 2015;3:377–387. [PubMed: 25937001]
47. Petrikin JE, Cakici JA, Clark MM, et al. The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. NPJ Genom Med. 2018;3:6. [PubMed: 29449963]
48. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424. [PubMed: 25741868]
49. Deterding RR, Wagner BD, Harris JK, DeBoer EM. Pulmonary aptamer signatures in children's interstitial and diffuse lung diseases. Am J Respir Crit Care Med. 2019;200(12):1496–1504. [PubMed: 31409098]
50. Doan ML, Elidemir O, Dishop MK, et al. Serum KL-6 differentiates neuroendocrine cell hyperplasia of infancy from the inborn errors of surfactant metabolism. Thorax. 2009;64:677–681. [PubMed: 19237389]
51. Nathan N, Corvol H, Amselem S, Clement A. Biomarkers in interstitial lung diseases. Paediatr Respir Rev. 2015;16:219–224. [PubMed: 26027849]
52. Emond MJ, Louie T, Emerson J, et al. Exome sequencing of extreme phenotypes identifies DCTN4 as a modifier of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. Nat Genet. 2012;44:886–889. [PubMed: 22772370]
53. Boyle MP, De Boeck K. A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. Lancet Respir Med. 2013;1: 158–163. [PubMed: 24429096]
54. Clancy JP, Rowe SM, Accurso FJ, et al. Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. Thorax. 2012;67:12–18. [PubMed: 21825083]
55. Kym PR, Wang X, Pizzonero M, Van der Plas SE. Recent progress in the discovery and development of small-molecule modulators of CFTR. Prog Med Chem. 2018;57:235–76. [PubMed: 29680149]
56. Han ST, Rab A, Pellicore MJ, et al. Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. JCI Insight. 2018;3(14), 10.1172/jci.insight.121159
57. Wang X, Liu B, Searle X, et al. Discovery of 4-[(2R,4R)-4-({[1-(2,2-difluoro-1,3-benzodioxol-5-yl)cyclopropyl]carbonyl}amino)-7-(difluoromethoxy)-3,4-dihydro-2H-chromen-2-yl]benzoic acid (ABBV/GLPG-2222), a potent cystic fibrosis transmembrane conductance regulator (CFTR) corrector for the treatment of cystic fibrosis. J Med Chem. 2018;61:1436–49. [PubMed: 29251932]
58. Davis SD, Ferkol TW, Rosenfeld M, et al. Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. Am J Respir Crit Care Med. 2015;191:316–324. [PubMed: 25493340]
59. Knowles MR, Drumm M. The influence of genetics on cystic fibrosis phenotypes. Cold Spring Harb Perspect Med. 2012;2:a009548. [PubMed: 23209180]

60. Kroner C, Wittmann T, Reu S, et al. Lung disease caused by ABCA3 mutations. Thorax. 2017;72:213–220. [PubMed: 27516224]
61. Sen P, Yang Y, Navarro C, et al. Novel FOXF1 mutations in sporadic and familial cases of alveolar capillary dysplasia with misaligned pulmonary veins imply a role for its DNA binding domain. Hum Mutat. 2013;34:801–811. [PubMed: 23505205]
62. Manolio TA, Fowler DM, Starita LM, et al. Bedside back to bench: building bridges between basic and clinical genomic research. Cell. 2017;169:6–12. [PubMed: 28340351]
63. Kinting S, Hoppner S, Schindlbeck U, et al. Functional rescue of misfolding ABCA3 mutations by small molecular correctors. Hum Mol Genet. 2018;27:943–953. [PubMed: 29325094]
64. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46:310–315. [PubMed: 24487276]
65. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. 2001;11:863–874. [PubMed: 11337480]
66. Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet. 2001;10:591–597. [PubMed: 11230178]
67. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2019;47:D886–D94. [PubMed: 30371827]
68. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. bioRxiv. 2020. 10.1101/531210
69. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: database of chromosomal imbalance and phenotype in humans using ensemble resources. Am J Hum Genet. 2009;84:524–533. [PubMed: 19344873]
70. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36:928–930. [PubMed: 26220891]
71. Starita LM, Ahituv N, Dunham MJ, et al. Variant interpretation: functional assays to the rescue. Am J Hum Genet. 2017;101: 315–325. [PubMed: 28886340]
72. Gibson TJ, Seiler M, Veitia RA. The transience of transient over-expression. Nat Methods. 2013;10:715–721. [PubMed: 23900254]
73. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. Am J Hum Genet. 99, 2016:877–885. [PubMed: 27666373]
74. Wrenbeck EE, Klesmith JR, Stapleton JA, Adeniran A, Tyo KE, Whitehead TA. Plasmid-based one-pot saturation mutagenesis. Nat Methods. 2016;13:928–930. [PubMed: 27723752]
75. Findlay GM, Daza RM, Martin B, et al. Accurate classification of BRCA1 variants with saturation genome editing. Nature. 2018;562: 217–222. [PubMed: 30209399]
76. Gasperini M, Starita L, Shendure J. The power of multiplexed functional analysis of genetic variants. Nat Protoc. 2016;11:1782–1787. [PubMed: 27583640]
77. Majithia AR, Tsuda B, Agostini M, et al. Prospective functional classification of all possible missense variants in PPARG. Nat Genet. 2016;48:1570–1575. [PubMed: 27749844]
78. Starita LM, Young DL, Islam M, et al. Massively parallel functional analysis of BRCA1 RING domain variants. Genetics. 2015;200: 413–422. [PubMed: 25823446]
79. Weile J, Roth FP. Multiplexed assays of variant effects contribute to a growing genotype-phenotype atlas. Hum Genet. 2018;137: 665–678. [PubMed: 30073413]
80. Eldridge WB, Zhang Q, Faro A, et al. Outcomes of lung transplantation for infants and children with genetic disorders of surfactant metabolism. J Pediatr. 2017;184:157–164. e2. [PubMed: 28215425]
81. Wambach JA, Wegner DJ, Depass K, et al. Single ABCA3 mutations increase risk for neonatal respiratory distress syndrome. Pediatrics. 2012;130:e1575–e1582. [PubMed: 23166334]

82. Liptzin DR, Patel T, Deterding RR. Chronic ventilation in infants with surfactant protein C mutations: an alternative to lung transplantation. Am J Respir Crit Care Med. 2015;191:1338–1340. [PubMed: 26029841]
83. Lopez-Andreu JA, Hidalgo-Santos AD, Fuentes-Castello MA, et al. Delayed presentation and prolonged survival of a child with surfactant protein B deficiency. J Pediatr. 2017;190:268–270. e1. [PubMed: 28888561]
84. Verkman AS, Galietta LJ. Chloride channels as drug targets. Nat Rev Drug Discov. 2009;8:153–171. [PubMed: 19153558]
85. Verkman AS, Lukacs GL, Galietta LJ. CFTR chloride channel drug discovery—inhibitors as antidiarrheals and activators for therapy of cystic fibrosis. Curr Pharm Des. 2006;12:2235–2247. [PubMed: 16787252]
86. Mullard A FDA drug approvals. Nat Rev Drug Discov. 2016;2017(16): 73–76.
87. Pedemonte N, Lukacs GL, Du K, et al. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest. 2005;115:2564–2571. [PubMed: 16127463]
88. Markossian S, Ang KK, Wilson CG, Arkin MR. Small-molecule screening for genetic diseases. Annu Rev Genomics Hum Genet. 2018;19:263–288. [PubMed: 29799800]
89. Ilagan MX, Kopan R. Monitoring notch activation in cultured mammalian cells: transcriptional reporter assays. Methods Mol Biol. 2014; 1187:143–154. [PubMed: 25053487]
90. Ilagan MX, Lim S, Fulbright M, Piwnica-Worms D, Kopan R. Real-time imaging of notch activation with a luciferase complementation-based reporter. Sci Signal. 2011;4:rs7. [PubMed: 21775282]
91. Le Bourdonnec B, Windh RT, Ajello CW, et al. Potent, orally bioavailable delta opioid receptor agonists for the treatment of pain: discovery of N,N-diethyl-4-(5-hydroxyspiro[chromene-2,4'-piperidine]-4-yl)benzamide (ADL5859). J Med Chem. 2008;51: 5893–5896. [PubMed: 18788723]
92. Le Bourdonnec B, Windh RT, Leister LK, et al. Spirocyclic delta opioid receptor agonists for the treatment of pain: discovery of N,N-diethyl-3-hydroxy-4-(spiro[chromene-2,4'-piperidine]-4-yl) benzamide (ADL5747). J Med Chem. 2009;52:5685–5702. [PubMed: 19694468]
93. Zhang JH, Chung TD, Oldenburg KR. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen. 1999;4:67–73. [PubMed: 10838414]
94. Cummings EE, O'Reilly LP, King DE, et al. Deficient and null variants of SERPINA1 are proteotoxic in a *Caenorhabditis elegans* model of alpha1-antitrypsin deficiency. PLOS One. 2015;10:e0141542. [PubMed: 26512890]
95. Hidvegi T, Stolz DB, Alcorn JF, et al. Enhancing autophagy with drugs or lung-directed gene therapy reverses the pathological effects of respiratory epithelial cell proteinopathy. J Biol Chem. 2015;290: 29742–29757. [PubMed: 26494620]
96. Wang Y, Cobanoglu MC, Li J, et al. An analog of glibenclamide selectively enhances autophagic degradation of misfolded alpha1-antitrypsin Z. PLOS One. 2019;14:e0209748. [PubMed: 30673724]
97. Sieber P, Schafer A, Lieberherr R, et al. Novel high-throughput myofibroblast assays identify agonists with therapeutic potential in pulmonary fibrosis that act via EP2 and EP4 receptors. PLOS One. 2018;13:e0207872. [PubMed: 30485339]
98. Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics. 2016;107:1–8. [PubMed: 26554401]
99. Plasschaert LW, Zilionis R, Choo-Wing R, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature. 2018;560:377–381. [PubMed: 30069046]
100. Montoro DT, Haber AL, Biton M, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. Nature. 2018;560:319–324. [PubMed: 30069044]
101. Ardini-Poleske ME, Clark RF, Ansong C, et al. LungMAP: the molecular atlas of lung development program. Am J Physiol Lung Cell Mol Physiol. 2017;313:L733–L740. [PubMed: 28798251]

102. Ding J, Ahangari F, Espinoza CR, et al. Integrating multiomics longitudinal data to reconstruct networks underlying lung development. Am J Physiol Lung Cell Mol Physiol. 2019;317:L556–L568. [PubMed: 31432713]
103. Pichon X, Lagha M, Mueller F, Bertrand E. A growing toolbox to image gene expression in single cells: sensitive approaches for demanding challenges. Mol Cell. 2018;71:468–480. [PubMed: 30075145]
104. Hu BC. The human body at cellular resolution: the NIH Human Biomolecular Atlas Program. Nature. 2019;574:187–192. [PubMed: 31597973]
105. Whitsett JA, Kalin TV, Xu Y, Kalinichenko VV. Building and regenerating the lung cell by cell. Physiol Rev. 2019;99:513–54. [PubMed: 30427276]
106. McDonough JE, Ahangari F, Li Q, et al. Transcriptional regulatory model of fibrosis progression in the human lung. JCI Insight. 2019; 4(22), 10.1172/jci.insight.131597
107. Veit G, Oliver K, Apaja PM, et al. Ribosomal stalk protein silencing partially corrects the ΔF508-CFTR functional expression defect. PLOS Biol. 2016;14:e1002462. [PubMed: 27168400]
108. Baron RM, Choi AJ, Owen CA, Choi AM. Genetically manipulated mouse models of lung disease: potential and pitfalls. Am J Physiol Lung Cell Mol Physiol. 2012;302:L485–L497. [PubMed: 22198907]
109. Tata PR, Rajagopal J. Plasticity in the lung: making and breaking cell identity. Development. 2017;144:755–766. [PubMed: 28246210]
110. le Sage C, Lawo S, Cross BCS. CRISPR: a screener's guide. SLAS Discov. 2020;25(3):233–240. [PubMed: 31658850]
111. Kc M, Steer CJ. A new era of gene editing for the treatment of human diseases. Swiss Med Wkly. 2019;149:w20021. [PubMed: 30685869]
112. Barkauskas CE, Chung MI, Fioret B, Gao X, Katsura H, Hogan BL. Lung organoids: current uses and future promise. Development. 2017;144:986–997. [PubMed: 28292845]
113. Tan Q, Choi KM, Sicard D, Tschumperlin DJ. Human airway organoid engineering as a step toward lung regeneration and disease modeling. Biomaterials. 2017;113:118–132. [PubMed: 27815996]
114. Tan Q, Ma XY, Liu W, et al. Nascent lung organoids reveal epithelium- and bone morphogenetic protein-mediated suppression of fibroblast activation. Am J Respir Cell Mol Biol. 2019;61:607–19. [PubMed: 31050552]
115. Lehmann R, Lee CM, Shugart EC, et al. Human organoids: a new dimension in cell biology. Mol Biol Cell. 2019;30:1129–1137. [PubMed: 31034354]
116. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–676. [PubMed: 16904174]
117. Jacob A, Morley M, Hawkins F, et al. Differentiation of human pluripotent stem cells into functional lung alveolar epithelial cells. Cell Stem Cell. 2017;21:472–488. e10. [PubMed: 28965766]
118. Strikoudis A, Cieslak A, Loffredo L, et al. Modeling of fibrotic lung disease using 3D organoids derived from human pluripotent stem cells. Cell Rep. 2019;27:3709–3723. e5. [PubMed: 31216486]

TABLE 1

Disorders of surfactant metabolism

Protein	Gene	Inheritance pattern	Age of onset	Extrapulmonary symptoms
Surfactant protein B	*SFTPB*	AR	Neonatal	None
Surfactant protein C	*SFTPC*	AD	Variable	None
ABCA3	*ABCA3*	AR	Biphasic	None
TTF-1	*NKX2.1*	AD	Variable	Hypothyroidism, hypotonia, chorea
GM-CSF receptors α and β	*CSF2RA, CSF2RB*	AR	Infancy	None
Methionyl-tRNA synthetase	*MARS*	AR	Unknown	Liver dysfunction

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.