# Future Directions in Early Cystic Fibrosis Lung Disease Research

## An NHLBI Workshop Report

**Bonnie W. Ramsey[1,2], Susan Banks-Schlegel[3], Frank J. Accurso[4], Richard C. Boucher[5], Garry R. Cutting[6], John F. Engelhardt[7], William B. Guggino[6], Christopher L. Karp[8], Michael R. Knowles[5], Jay K. Kolls[9], John J. LiPuma[10], Susan Lynch[11], Paul B. McCray, Jr.[12], Ronald C. Rubenstein[13], Pradeep K. Singh[2], Eric Sorscher[14], and Michael Welsh[15]**

[1]Seattle Children's Research Institute and [2]University of Washington School of Medicine, Seattle, Washington; [3]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; [4]University of Colorado School of Medicine, Denver, Colorado; [5]University of North Carolina School of Medicine, Chapel Hill, North Carolina; [6]Johns Hopkins School of Medicine, Baltimore, Maryland; [7]University of Iowa School of Medicine, Iowa City, Iowa; [8]Division of Molecular Immunology, Cincinnati Children's Hospital Research Foundation, and the University of Cincinnati College of Medicine, Cincinnati, Ohio; [9]Louisiana State University School of Medicine at New Orleans, New Orleans, Louisiana; [10]University of Michigan School of Public Health, Ann Arbor, Michigan; [11]University of California at San Francisco School of Medicine, San Francisco, California; [12]University of Iowa Children's Hospital, Iowa City, Iowa; [13]Children's Hospital of Philadelphia and Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; [14]University of Alabama at Birmingham School of Medicine, Birmingham, Alabama; and [15]Howard Hughes Medical Institute, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa

**Since the 1989 discovery that mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene cause cystic fibrosis (CF), there has been substantial progress toward understanding the molecular basis for CF lung disease, leading to the discovery and development of new therapeutic approaches. However, the earliest impact of the loss of CFTR function on airway physiology and structure and its relationship to initial infection and inflammation are poorly understood. Universal newborn screening for CF in the United States represents an unprecedented opportunity for investigating CF clinical manifestations very early in life. Recently developed animal models with pulmonary phenotypic manifestations also provide a window into the early consequences of this genetic disorder. For these reasons, the National Heart, Lung, and Blood Institute (NHLBI) convened a working group of extramural experts, entitled "Future Research Directions in Early CF Lung Disease" on September 21–22, 2010, to identify future research directions of great promise in CF. The priority areas identified included (*1*) exploring pathogenic mechanisms of early CF lung disease; (*2*) leveraging newborn screening to elucidate the natural history of early lung disease; (*3*) developing a spectrum of biomarkers of early lung disease that reflects CF pathophysiology, clinical outcome, and response to treatment; (*4*) exploring the role of genetics/genomics (e.g., modifier genes, gene–environmental interactions, and epigenetics) in early CF pathogenesis; (*5*) defining early microbiological events in CF lung disease; and (*6*) elucidating the initial airway inflammatory, remodeling, and repair mechanisms in CF lung disease.**

**Keywords:** cystic fibrosis; airway disease; innate immunity; microbiology; genetics

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

Emerging evidence suggests that lung disease begins very early in life in cystic fibrosis (CF), although it is initially "silent" without overt signs and symptoms of a progressive disease process. The nature of CF lung abnormalities in the first years of life remains poorly understood, and the possibility of preventing or delaying the onset of disease through early intervention has scarcely been explored. A new frontier in CF, made possible by the early diagnosis of CF with newborn screening, is to understand and characterize presymptomatic lung disease in infants and young children, leading to early interventions to mitigate disease progression at stages when therapeutic intervention or prevention may be most effective.

#### What This Study Adds to the Field

This review provides a summary of recommendations from an NHLBI workshop convened to review the progress and direction of CF research and to identify and prioritize the research opportunities that hold the most promise to provide new mechanistic insights into the genesis and evolution of early CF lung disease.

Cystic fibrosis (CF) is a life-shortening autosomal recessive disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (1, 2). CFTR is an anion channel that influences the composition and quantity of liquid on the surface of epithelia. Significant advances have increased understanding of CFTR structure and function and how mutations disrupt function (2). Survival and quality of life have improved, through better management of nutrition and respiratory infections, rather than through interventions that target the basic defect. Promising therapies directed to correcting dysfunctional CFTR (3, 4) are being tested in older children and adults with CF, but their effectiveness in infants and young children remains unexplored.

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*Author Contributions*: All authors contributed equally to this article.

Correspondence and requests for reprints should be addressed to Bonnie W. Ramsey, M.D., CFFT Therapeutics Development Network Coordinating Center, 2001 Eighth Avenue, Mailstop CW8-5B, Seattle, WA 98121. E-mail: bonnie.ramsey@seattlechildrens.org

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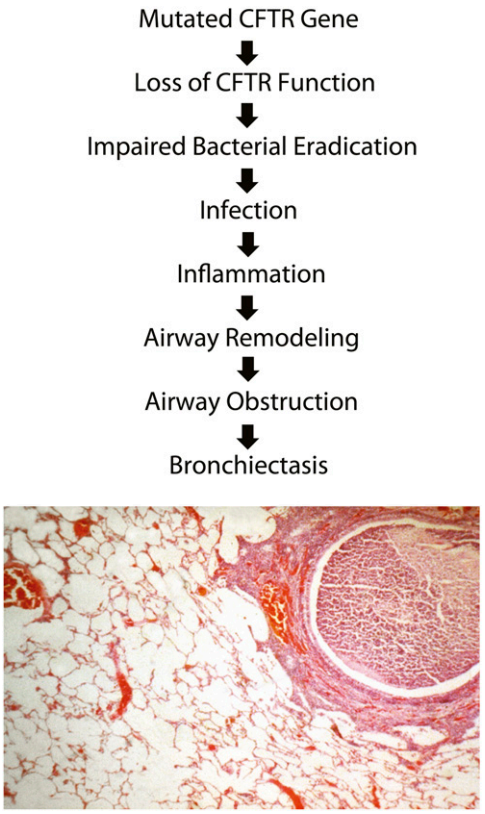


***Figure 1.*** The steps hypothesized to be relevant to the progression from dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) to initial infection and inflammation and eventual development of airway structural damage are described. At each step, genetic and environmental modifiers can operate to alter outcome. The micrograph displays a bronchial airway from an individual with CF with the hallmark pathological findings including airway obstruction with mucus and inflammatory cells and peribronchial inflammatory response.

Because lung disease causes most CF morbidity and mortality, much research has focused on understanding the pathophysiological cascade of events progressing from CFTR mutations to irreversible lung damage (Figure 1). Impaired eradication of bacteria early in life induces a predominantly neutrophilic inflammatory response that injures the lung (5, 6). Airway remodeling and airway obstruction ensue (7). The temporal sequence from loss of CFTR function to initial bacterial airway infection, and the impact of environmental factors such as viruses on this process, are not clear.

Since 2010, newborn screening for CF has been universal in the United States and many other countries (8), providing an unprecedented opportunity to longitudinally monitor disease progression (6, 9) and evaluate emerging therapeutic approaches from infancy (3, 4, 10). New CF animal models (11–14) provide a pulmonary phenotype for studying the origins of disease. In addition, researchers can employ improved and emerging technologies to measure airway physiology, structure, and disease progression (10, 15–22). These factors position the field to address questions about the initiating events of CF lung disease. With several promising therapies on the horizon, it is imperative that the pathogenesis of early lung disease be elucidated so that future interventions can prevent and not simply treat CF lung disease. For this reason, the National Heart, Lung, and Blood Institute (NHLBI, Bethesda, MD) convened a workshop on September 21–22, 2010 to review the progress of CF research and to prioritize research opportunities (Table 1) that promise to provide mechanistic insight into the genesis and evolution of early CF lung disease.

## PRIORITY 1: EXPLORE PATHOGENIC MECHANISMS OF EARLY CF LUNG DISEASE

CF lung disease is a multistep process. Immediately after birth, CF lungs may have a host defense defect that impairs bacterial eradication (14) and induces inflammation and airway remodeling. At some point, airways become chronically infected, and the cycle of infection, inflammation, and remodeling accelerates to obstruct and destroy airways. Loss of CFTR could also affect multiple individual processes along the pathway, leading toward structural damage. Elucidating discrete responsible mechanisms is also made difficult by the spatial and temporal heterogeneity of the disease (22).

The field is now positioned to begin elucidating the initial defects that trigger lung disease and define the directional connections among the many reported defects. Three animal models (11–13, 23) are available to study mechanisms—pigs, ferrets, and mice; each can generate *in vitro* models for dissecting molecular mechanisms and comparison with humans. Differences and similarities among CF models may inform investigators about mechanisms in early disease. Potential research opportunities include the following:

- Elucidate the defect(s) that impairs host defense in the newborn lung.
- Identify the sequence of events that causes progression of airway disease and learn how loss of CFTR alters the process.
- Investigate cross-species comparisons to clarify early pathological events.
- Define mechanisms underlying the heterogeneity of CF lung disease.

## PRIORITY 2: LEVERAGE NEWBORN SCREENING TO ELUCIDATE THE NATURAL HISTORY AND CLINICAL MANIFESTATIONS OF EARLY CF LUNG DISEASE

Study of presymptomatic CF infants identified by newborn screening will allow characterization of the earliest manifestations of the disease, including the relationship between lung infection and inflammation (6, 7, 9, 22), stratification by mutation subclass (2, 24), follow-up throughout the life span (6, 25), and intervention before irreversible disease develops (4, 10, 15, 24, 25). Technologies to elucidate features of CF physiology (airway surface liquid generation, mucus clearance, etc.) (16, 17) and disease biomarkers (9, 18–20) are in place. New CFTR modulators (4, 10, 24) and interventions that alter fluid and electrolyte transport (3, 4, 10, 16, 24, 26) will serve as valuable tools for better understanding early CF lung disease and establishing validated biomarkers and clinical end points in young, presymptomatic patients. Creating clinical sample repositories

**TABLE 1. RESEARCH PRIORITIES FOR EARLY CF LUNG DISEASE**

1. Explore pathogenic mechanisms of early CF lung disease
2. Leverage newborn screening to elucidate the natural history and clinical manifestations of early CF lung disease
3. Develop biomarkers of early lung disease that reflect CF pathophysiology, clinical outcome, and response to treatment
4. Identify the role of genetics, genomics, and epigenetics in early CF disease pathogenesis
5. Define early microbiological events in CF lung disease and how they lead to chronic infection
6. Elucidate the initial airway inflammatory responses, innate and adaptive immune responses, and mechanisms of repair/remodeling in early CF lung disease

*Definition of abbreviation*: CF = cystic fibrosis.

from subjects with early CF will enable future research. Potential research opportunities include the following:

- Develop data and specimen (e.g., DNA, plasma) repositories beginning in the newborn period to expedite research.
- Elucidate the natural history and clinical manifestations of early CF lung disease.
- Develop new and improved technologies to monitor disease progression and outcome.
- Determine the mechanisms responsible for pulmonary exacerbation in early CF.

## PRIORITY 3: DEVELOP BIOMARKERS OF EARLY LUNG DISEASE THAT REFLECT CF PATHOPHYSIOLOGY, CLINICAL OUTCOME, AND RESPONSE TO TREATMENT

Characterization of lung disease in very young patients is challenging (15, 22, 25) because CF lung disease likely starts in the small airways (22), where early changes are physiologically and structurally difficult to measure . Early lung disease in CF is heterogeneous, with various degrees of air trapping and atelectasis (9, 21, 22, 27). Although imaging studies cannot visualize airways less than 1 mm in cross-section, the presence of structural changes in small airways can be visualized as trapped air even before any measurable changes in air flow (9, 21, 22). The use of bronchoalveolar lavage (BAL) has provided significant insight into early infection and inflammatory responses (5, 6, 21). Studies have assessed peripheral airway physiology in infants and preschool children by lung function testing, using the raised volume rapid thoracic compression (RVRTC) technique and the lung clearance index, which represent promising techniques that may become useful measures of small airway obstruction (6, 9, 15, 21, 28). Although promising, many of these procedures expose infants to ionizing radiation (computed tomography, CT) (29) or require sedation (BAL, CT, RVRTC), increasing cost/risk and reducing usefulness for therapeutic trials. Thus, there is a critical need for minimally invasive measures of early lung disease that reflect early CF pathophysiology, clinical outcomes, and responses to treatments. There is also a need to develop biomarkers that can discriminate levels of CFTR function and reflect its impact on ion transport and airway function (26). Potential research opportunities include the following:

- Establish reliable clinical or physiological end points for evaluating the efficacy/safety of novel therapies.
- Identify new and improved biomarkers of disease onset, progression, and severity that reflect clinical outcome or response to treatment.
- Elucidate the connections between structural changes and physiological abnormalities, using new or improved technologies adapted for use in infants and young children.

## PRIORITY 4: EXPLORE THE ROLE OF GENETICS, GENOMICS, AND EPIGENETICS IN EARLY CF LUNG DISEASE PATHOGENESIS

The relationship between CFTR genotype and pulmonary disease severity does not show a close correlation (30, 31). Several factors account for this complexity. First, many distinct CFTR mutations exist. Second, different mutations disrupt CFTR to variable extents. Third, transcriptional regulation of CFTR gene expression is spatially and temporally controlled and may vary as disease progresses. Fourth, several modifier genes and genomic regions influence lung disease severity (31, 32). Fifth, microRNAs (33) may play a significant role in disease modification, but have been little investigated in CF. Sixth, infection, inflammation, and other factors might produce epigenetic changes including histone acetylation, gene methylation, and other alterations (34, 35). Seventh, environmental factors account almost equally to genetic factors for variation in lung disease; unique exposures contribute the majority of the effect (36).

Several advances provide an opportunity to probe this complexity and decipher how these factors influence CF lung disease. These include next-generation sequencing technologies, genome-wide methods for assessing epigenetic modifications (35), and methodologies for understanding gene networks. The reservoir of naturally occurring mutations in CF centers and databases provides an important resource for study (2, 37), although efforts would benefit from expanding detailed genotypic and phenotypic resources and banked samples. Recently developed pig (14) and ferret (11) CF models and newer murine models (12, 13) provide powerful new opportunities for evaluating mechanisms of disease and therapies. Potential research opportunities include the following:

- Expand knowledge of genetic heterogeneity and diversity of CFTR mutations linked to functional and phenotypic consequences.
- Identify modifier genes.
- Use genetic studies to gain mechanistic insights into CF biology.
- Identify gene–gene and gene–environmental interactions in early lung pathogenesis.
- Define epigenetic signatures in early life that predict disease status later in life.
- Identify regions of the genome that regulate CFTR transcription and other relevant genes and determine their influence on disease severity.
- Define microRNAs and small interfering RNAs influencing development and function of airway cells in early CF lung disease.

## PRIORITY 5: DEFINE MICROBIOLOGICAL EVENTS IN EARLY CF LUNG DISEASE AND HOW THEY LEAD TO CHRONIC INFECTION

Bacterial airway infection and the resulting inflammation cause lung function decline (2, 6, 38). Antiinfective therapy has improved survival, but treatment remains inadequate: bacterial lineages persist despite countless antibiotic courses; antibiotics only reduce bacterial load, which rebounds off therapy (39); and antibiotic responses wane over time (39, 40). New ideas about CF microbiology (Figure 2) have come from the use of non–culture-based detection techniques, studies on microbial interactions, progress in understanding pathogen evolution, and studies on biofilms (15, 41, 42). Better understanding CF microbiology could improve outcomes in chronically infected patients.

Defining how CFTR mutations cause infection susceptibility and identifying bacteria causing infection remain critical tasks. Research has focused on *Pseudomonas aeruginosa* (*Pa*) (38, 43) because *Pa* acquisition is associated with mortality and lung function decline (44, 45). However, non–culture-based techniques on upper airway samples have revealed a multitude of bacterial species (42). Identifying which species are actually present in CF lungs, and determining which contribute to disease, remain major questions. Other major challenges include identifying factors that promote the transition from early transient to

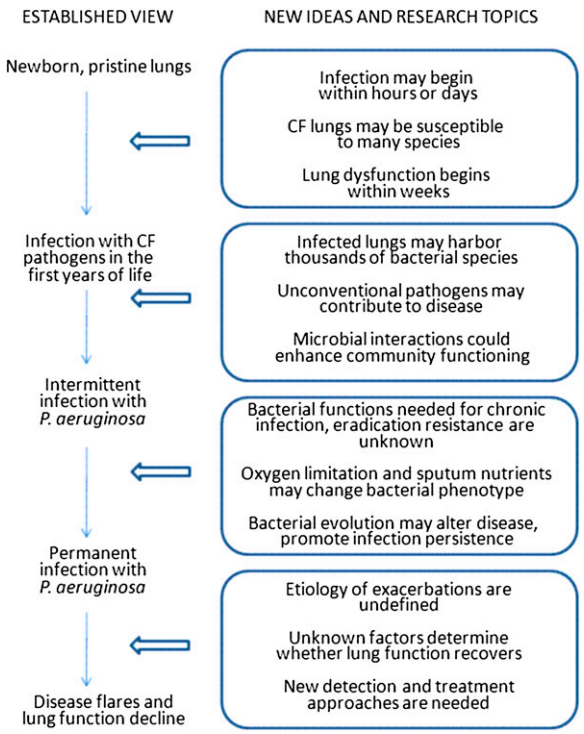


*Figure 2.* New ideas about each of the stages of cystic fibrosis infection suggest future research directions.

chronic endobronchial infection, resistant to eradication. Identifying the nature and diversity of bacterial genetic adaptations, pivotal host–bacteria interactions, and microbiological changes that occur during pulmonary exacerbation (46) could suggest therapeutic strategies. Key research directions include the following:

- Define how CFTR mutations cause susceptibility to infection, reservoirs of infecting bacteria, timing of infection onset, and organisms involved.
- Investigate microbial diversity in the early CF lung and identify organisms causing disease.
- Characterize key bacterial and host factors pivotal to the transition from acute to chronic infection.
- Develop new agents with activity against bacteria in chronic infections, or that enhance the activity of existing drugs.
- Identify mechanisms and biomarkers of exacerbations and develop new treatment approaches that improve patient management and outcomes.

## PRIORITY 6: ELUCIDATE THE INITIAL AIRWAY INFLAMMATORY RESPONSE, INNATE AND ADAPTIVE IMMUNE RESPONSES, AND MECHANISMS OF REPAIR/REMODELING IN EARLY CF LUNG DISEASE

Current understanding of how the CF airway changes over the first year(s) of life including composition of the microbiome, innate immunity, injury and repair remains rudimentary, but it is likely that inflammatory responses are central to the development of early airway disease (6, 7, 47). Recent advances in lung biology, including delineation of molecular programs underlying lung development and responses to injury and remodeling (48), recognition of the critical role of airway epithelial cells in inducing and regulating innate and adaptive immunity in the lung (49), and recognition of the fact that resolution of inflammation in the lung is an active, regulated process (50), provide novel tools for understanding early pathogenesis in the CF airway. Improved knowledge of inflammatory processes has obvious implications, both for insight into disease pathogenesis and for development of novel antiinflammatory therapeutics. Potential research opportunities include the following:

- Identify the primary pathogenetic locus (or loci) that underlies the dysregulated inflammatory milieu of the CF airway.
- Define molecular and cellular mechanisms responsible for developmental, inflammatory, innate and adaptive immunity, repair, and remodeling abnormalities of the early CF airways.
- Develop validated biomarkers for assessing dysregulated airway inflammation, immunity, injury, and remodeling in early CF.

## CONCLUSIONS

Emerging evidence suggests that CF lung disease begins in infancy and is initially "silent" without overt signs or symptoms. By the time symptoms appear, the disease process is established. Thus, understanding how loss of CFTR predisposes airways to infection and initiates the cascade of inflammation, remodeling, and airway obstruction is critical for developing new therapeutic and preventive strategies. New CF animal models and technologies for disease characterization are in place and can provide insights to inform future human studies of primary prevention and/or mitigation of CF lung disease in infancy and early childhood. The priority areas that this workshop identified provide a roadmap for better understanding the earliest stages of CF lung disease and preventing or delaying its onset.

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***Participants***: Co-Chairs: Michael Welsh, M.D., University of Iowa, Iowa City, IA; Bonnie Ramsey, M.D., Seattle Children's Research Institute, Seattle, WA and University of Washington School of Medicine, Seattle, WA; Members- Group Leaders: Group 1, Eric Sorscher, M.D., University of Alabama, Birmingham, AL; Group 2, John Engelhardt, Ph.D., University of Iowa, Iowa City, IA; Group 3, Pradeep Singh, M.D., University of Washington, Seattle, WA; Group 4, Christopher Karp, M.D., Children's Hospital Medical Center, Cincinnati, OH; Group 5, Garry Cutting, M.D., Johns Hopkins University, Baltimore, MD; Group 6, Richard Boucher, M.D., University of North Carolina, Chapel Hill, NC; Group Members: Frank Accurso, M.D., University of Colorado School of Medicine, Denver, CO (Groups 1, 4, and 6); William Guggino, Ph.D., Johns Hopkins University, Baltimore, MD (Groups 2 and 6); Michael Knowles, M.D., University of North Carolina, Chapel Hill, NC (Group 5); Jay Kolls, M.D., Louisiana State University Health Science Center, New Orleans, LA (Groups 4 and 5); John Lipuma, M.D., University of Michigan, Ann Arbor, MI (Group 3); Susan Lynch, Ph.D., University of California at San Francisco School of Medicine, San Francisco, CA (Group 3); Paul McCray, M.D., University of Iowa, Iowa City, IA (Groups 4 and 5); Ronald Rubenstein, M.D., Ph.D., Children's Hospital of Philadelphia, and Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA (Groups 1, 2, and 6); NHLBI Staff: Susan Banks-Schlegel, Ph.D., Division of Lung Diseases, Bethesda, MD; James P. Kiley, Ph.D., Division of Lung Diseases, Bethesda, MD; Cystic Fibrosis Foundation Staff/Consultants: Drucy Borowitz, M.D., State University of New York at Buffalo, NY; Christopher Penland, Ph.D., Cystic Fibrosis Foundation, Bethesda, MD; Steven Rowe, M.D., MSPH, University of Alabama, Birmingham, AL.

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