

## Movement

### The Emerging Genetics of Primary Ciliary Dyskinesia

With every breath, the human lung is exposed to inhaled pathogens. Complex, local defenses have evolved to protect the airway, including the mucociliary escalator, which mechanically eliminates bacteria that deposit at the epithelial surface. To achieve effective mucociliary clearance, the conducting airways closely coordinate airway surface fluid volume, fluid composition, and ciliary function (1). Indeed, motor cilia covering the respiratory epithelium are critical for efficient clearance of the lower respiratory tract, moving fluids, mucus, and inhaled foreign materials vectorially from the distal airways.

In primary ciliary dyskinesia (PCD), ineffective ciliary function leads to persistent infection at the respiratory epithelial surface. Occurring with an estimated incidence of 1 in 15,000 to 30,000 births, PCD results in the retention of inhaled particles, including bacteria, in the lung, paranasal sinuses, and middle ear, due to impaired mucociliary clearance (2). Unfortunately, the diagnosis can be challenging, and it may be particularly difficult to distinguish primary ciliary disease from the secondary changes after a viral infection. The saccharine test and its variants have been used, but they can only exclude PCD, not make the diagnosis (3). Nasal nitric oxide concentrations are extremely low in PCD (4), but are nonspecific. Their value to PCD may be as a screening test. Examination of ciliary beat frequency and pattern from a nasal brush biopsy, followed by ultrastructural analysis with transmission electron microscopy, is often diagnostic (3, 4). However, epithelial injury caused by infection or inflammation can reduce the number of cilia available for analysis. Furthermore, ultrastructural defects need to be rigorously defined and require special expertise to identify abnormalities. Changes must be differentiated from acquired forms due to the different environmental and infectious agents that cause secondary ciliary changes. Moreover, not all cases of PCD have associated ultrastructural abnormalities. Thus, the diagnostic limitations of this condition underscore the need for better testing.

Further diagnostic tests for PCD have been proposed. Airway epithelial cells grown in primary culture at an air-liquid interface differentiate (5), allowing the epithelium to recover with restored ciliary morphology. Secondary changes disappear, and PCD can be diagnosed based on abnormalities of ciliary beat frequency and ciliary ultrastructure. High-resolution immunofluorescence imaging has been used to demonstrate the mislocalization of dynein arm proteins in some patients with PCD (6). Finally, and now increasingly, genetic testing is becoming closer to reality.

As predicted from the complexity of ciliary structure and function, PCD is genetically heterogeneous. In most instances, the disease is expressed in an autosomal recessive pattern (7, 8). Our understanding of the link between ultrastructural changes of cilia and the underlying genetic defect of PCD has lagged, which is, in part, related to the fact that mutations in any of the 250 proteins that constitute a cilium could potentially cause the disease (9).

The complex genetics of PCD is unfolding, largely because we better understand how primitive microorganisms move. The ciliary axoneme is highly conserved across species, and considerable homology exists between structural elements of protozoan flagella, such as those found in *Chlamydomonas reinhardtii*, and the mammalian cilium. This similarity has provided important insights into human homologs important for normal ciliary function. It has also allowed predictions of those structures in a respiratory cilium that, if mutated, could be causative for PCD. Human homologs of *Chlamydomonas* outer dynein arms have been identified as candidate proteins. One of the first genes tested encodes the human intermediate dynein (DNAI1) of the outer dynein arm that has high sequence identity with the *Chlamydomonas* gene, IC78. Several loss-of-function DNAI1 mutations have been found in patients who have specific outer dynein arm defects (10, 11).

In this issue of the *Journal* (pp. 120–126), Hornef and colleagues (12) identify multiple variants within another candidate gene, the heavy-chain dynein (DNAH5), which has been previously shown to result in ultrastructural changes and loss of function in patients who have classic PCD (13). What is noteworthy is the high frequency of DNAH5 mutations found, accounting for gene defects in almost half of the subjects tested who had outer dynein arm defects and possibly almost 30% of all patients with PCD. Without more detailed knowledge about prevalence, and in particular mild mutations, this figure can only be considered a guide. The article provides some of the strongest supporting evidence that genetic testing could be potentially feasible as a diagnostic test for PCD. The identification of disease-causing mutations and genetic testing should provide a clearer understanding of the clinical spectrum of respiratory tract involvement in this disease. It is reasonable to assume that PCD exists as a continuum; milder phenotypes must exist, which would be manifested by more subtle (if any) ultrastructural defects and modest ciliary dysfunction. Thus, genetic defects leading to abnormal ciliary function are underappreciated, and may be more commonly involved in chronic pulmonary disease found in infants and children.

Having a genetic test will be helpful in patients with a puzzling upper or lower airway disease, and antenatal diagnosis could be offered to affected families. There may be other implications. First, knowledge of the genes involved, and their protein products, will allow us to understand the basic biology of these complex structures. Second, it is not unreasonable to speculate that a PCD phenotype with normal ciliary genes may be produced by mutations in genes encoding proteins responsible for ciliary assembly, or which interact with cilia. There is a precedent for this; a cystic fibrosis phenotype has been described with a normal cystic fibrosis transmembrane regulator (CFTR) gene sequence (14), presumably due to a problem with one of the many proteins required for post-translational processing of CFTR, or with which the mature protein interacts at the cell surface. Identification and study of these patients may help us understand the intracellular processing of ciliary proteins. Finally, we may have a key to understand fundamental developmental processes. It has been elegantly shown that laterality is determined by nodal cilia (15), hence the finding of mirror image arrangement in

nearly half of patients with PCD. PCD has also been shown to be associated with complex congenital heart disease, in particular with disorders of laterality (16). Perhaps more basic knowledge will provide insights into congenital cardiac defects.

In summary, PCD is clinically and genetically heterogeneous. Candidate genes important for ciliary function have been identified by studies of primitive protozoans and could potentially be associated with PCD. The genetic basis of PCD and current limitations in its diagnosis argues for a more comprehensive effort for defining disease-causing defects, which is a goal of the National Institutes of Health-sponsored Genetic Disorders of Mucociliary Clearance Consortium, a clinical research network based in the United States established to improve diagnostic testing and treatment of rare airway diseases, including PCD. Such efforts will ultimately improve our diagnostic abilities and, it is hoped, therapeutic endeavors for this disease.

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## Treatment of Empyema in Children From Hippocrates' Time to the Present, and Back Again

The importance of draining a pleural space infection was known to Hippocrates, and the importance of avoiding complications by definitive treatment at an early stage may have been inferred. As he said with characteristic brevity, "When the empyema is treated either by cautery or incision, if pure and white pus flow from the wound, the patients recover, but if mixed with blood, slimy and fetid, they die" (1).

The treatment of empyema remains controversial, particularly in children. Therapeutic options include systemic antibiotics alone; thoracentesis; tube thoracostomy, with or without instillation of fibrinolytic agents; and more invasive techniques such as thoroscopic surgery, mini-thoracotomy, and standard thoracotomy with decortication (removal of the fibrinous "peel" from the lungs). How is one to choose among these options and why is this area so controversial? First, experience in adults cannot be extrapolated to children. In contrast with adults, most children who develop empyema are previously healthy. Second, prognostic factors that can help guide invasiveness of therapy in adults, such as pleural fluid lactate dehydrogenase (LDH), glucose levels, and pleural fluid pH, are not as useful in children (2, 3). In addition, outcomes criteria vary widely between studies.

The American College of Chest Physicians published evidence-based guidelines for the management of parapneumonic effusions in adults in 2000 (4). Patients were grouped into four risk categories on the basis of pleural space anatomy, pleural fluid bacteriology, and chemistry, and drainage was recommended for the two highest risk groups. Furthermore, patients in these higher categories had better outcomes (less mortality and failure of primary interventions) when treated by fibrinolytics or surgical intervention than patients treated with drainage alone. These findings are of questionable relevance in pediatrics because risk categories are not so easily defined in children and mortality is fortunately extremely rare. In addition, a subsequent meta-analysis (5) and Cochrane review (6) both failed to support the routine use of fibrinolytic therapy.

Conflicting data also complicate decision making in children. One randomized controlled trial found a modest decrease in length of stay (7.4 vs. 9.5 d) in children treated with intrapleural urokinase compared with those treated with saline (7). In contrast, another prospective trial did not find a substantial benefit of fibrinolysis (8). Several studies in children favor a primary operative approach over chest tube alone or chest tube plus