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PRIMARY CILIARY DYSKINESIA

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Abstract
Primary ciliary dyskinesia (PCD) is caused by ultrastructural ciliary defects that lead to abnormal ciliary beating and, subsequently, mucociliary dysfunction. PCD presents clinically with bronchiectasis, sinusitis, and, in up to 50% of cases, situs inversus. The ultrastructural defects of cilia are diverse but include in many cases outer and/or inner dynein arms. Recent advances have shown that ciliary defects in the embryonic node during development are responsible for the random right–left axis determination in these patients. Genetic approaches have elucidated at least some of the heterogeneous molecular defects underlying PCD. This article summarizes the current knowledge about this disease with respect to clinical manifestations, laboratory diagnosis and pathogenesis, situs inversus, genetics, and therapeutic considerations.

A report of a patient with the seemingly disparate symptoms of bronchiectasis and situs inversus 100 years ago is likely the first account of primary ciliary dyskinesia (PCD). Kartagener refined the description of the syndrome to include chronic sinusitis. However, only approximately 30 years ago, Afzelius and co-workers identified absent axonemal dynein arms in motile cilia with the ‘9 + 2’ microtubular arrangement of the airway epithelium and in sperm flagella as the cellular defect leading to what had come to be known as Kartagener’s syndrome or immotile cilia syndrome. Recent studies have demonstrated considerable heterogeneity of dynein arm morphology at the ultrastructural level among patients with this syndrome. Moreover, half of patients with clinical symptoms and ciliary ultrastructural defects do not exhibit situs inversus. Thus, the term PCD is currently used to describe individuals with congenital abnormalities of cilia and flagella and the clinical symptoms of bronchiectasis and chronic sinusitis. Kartagener’s syndrome, which in addition to bronchiectasis and sinusitis includes situs inversus, is thus considered a subset of PCD.

The overall incidence of PCD is 1 in 20 000, with enrichment in certain populations. PCD is usually an autosomal recessive disorder, but unusual cases of PCD with apparent dominant or X-linked inheritance pattern have also been reported.

Clinical Manifestations
Many clinical features of PCD reflect abnormal ciliary beating leading to impaired mucociliary clearance. Symptoms of mucociliary dysfunction in the nose, sinuses, and middle ear are recurrent or persistent rhinitis, sinusitis, and otitis media. Chronic productive cough is the major symptom of mucociliary dysfunction in the lower airways, and this chronic bronchitis can lead to bronchiectasis. Neonatal respiratory problems, situs inversus, and male infertility, common in PCD, are most likely linked to ciliary or flagellar dysfunction.

Chronic nasal congestion is common and often present from early infancy, with little or no seasonal variation. Almost all PCD patients have chronic sinusitis, radiographically demonstrated by mucosal thickening, cloudiness, and/or opacification of all paranasal sinuses. Nasal polyps occur in approximately one-third of patients and may be apparent in early childhood. Almost all patients have chronic otitis media that is much more prominent in early childhood. At the time of diagnosis, most patients

have either chronic tympanic membrane perforations or have had multiple tympanostomies with insertion of ventilation tubes. Many have conductive hearing loss.

Chronic productive cough is an almost universal feature of PCD. The cough is most apparent in the early morning but may occur at night or in association with exercise. In many children, exercise tolerance is normal but becomes impaired with advancing obstructive airway disease. Findings on chest examination are variable, with localized crackles that may or may not clear following forceful cough. Wheezing is relatively uncommon.

Chronic airway infection ultimately results in bronchiectasis. Cultures of sputum or bronchoscopic aspirates may yield *Hemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Streptococcus pneumoniae*. Patients with long-standing disease may have chronic infection with *Pseudomonas aeruginosa*. Pulmonary function tests may be normal early but typically demonstrate mild to moderate obstruction that becomes more severe in adulthood. Bronchodilator responsiveness is variable. Longitudinal analyses of children with PCD suggest that lung function may remain stable over relatively long periods of time.

Most patients have a history of transient respiratory problems in the first days of life, with tachypnea, cough, increased secretions, and hypoxemia. The etiology for these respiratory problems is often unexplained or attributed to aspiration pneumonitis or neonatal pneumonia. The possibility of PCD is rarely considered except in cases with situs inversus, persistent atelectasis, persistent pneumonia, or a family history of PCD. The high occurrence of transient neonatal respiratory distress in PCD patients suggests that ciliary activity may be important for clearing fluid during the transition from a fluid-filled fetal lung to an air-filled neonatal lung.

Situs inversus occurs in up to 50% of patients with PCD. Typically, all viscera in the chest and abdomen are transposed (situs inversus totalis). Male infertility is common and attributable to impaired sperm motility since ultrastructural and functional ciliary defects are mirrored in sperm flagella. However, some patients with PCD may have absent dynein arms in their cilia but normal dynein arms in sperm with normal motility. The occurrence of ciliary defects in cells lining the fallopian tubes has led to speculation that infertility and ectopic pregnancies could be increased in women with PCD, but this area has not been examined systematically.

Hydrocephalus or dilated ventricles have been reported in a few patients with PCD. Ultrastructural and functional defects in ventricular ependymal cilia provide a theoretical basis for this association, a hypothesis supported by hydrocephalus observed in knockout mouse models.

### Laboratory Diagnosis and Pathogenesis

Although a variety of heterogeneous ciliary ultrastructural lesions have been described among patients with PCD, a few studies have suggested that clinical symptoms suggestive of PCD are not always accompanied by evidence of an ultrastructural ciliary defect. This could be due to specimen processing or as yet uncharacterized or poorly understood ciliary defects not amenable to conventional electron microscopic inspection. Despite such reports, electron microscopic documentation of ciliary defects remains the standard for diagnosing PCD. In a recent study, 43% of patients exhibited abnormalities (absence or dysmorphology) of the outer dynein arm, 29% of the inner dynein arm, and 24% of both arms. The remaining 4% exhibited anomalies of the central microtubular pairs, radial spokes, or nexin links. A valid diagnosis of PCD thus may be indicated by the absence of both dynein arms, by the absence of either the inner or the outer dynein arm, or by consistent evidence of dynein arm dysmorphology (Figure 1). PCD in association with an absence of radial spokes or the transposition of an outer...
peripheral microtubular doublet to occupy the locus of a central microtubular doublet yielding an 8 + 2 axonemal configuration has also been described.

In the normal cilium, the bending motility of the axoneme mediated by ATPase-driven microtubular sliding is a highly ordered event. In contrast, the disorganization of the PCD axoneme yields a beat pattern that ranges from complete immotility to a vigorous motility that may appear virtually normal to an untrained observer. Thus, assessment of motility to aid the laboratory diagnosis and pathogenesis of PCD has been problematic, although it has been used successfully in specialized centers. Efforts to simply evaluate ciliary clearance by assessing the time required for a patient to taste a drop of saccharin placed on the nasal turbinate have fallen into disrepute. To date, the only definitive relationship between ciliary motility and ultrastructural integrity in PCD has been the evidence that cilia with no axonemal dynein arms reveal no motility. Other phenotypes seem to exhibit some, albeit dysfunctional, motion. Ultrastructural evidence has shown that the central microtubular pairs of cilia serve as vectors indicating the direction of beat (airway cilia with absent central pair beat in a circular pattern), and that in PCD the beating direction of adjacent cilia is disoriented relative to one another. Although this feature may help to identify patients with PCD, it also has been observed, at least focally, among individuals with transient upper respiratory infections and thus should not be considered an index lesion.

**Situs Inversus**

The reasons for situs inversus totalis in up to half of the patients with PCD remained uncertain until recently (and continues to be controversial). The first clear indication that situs inversus was related to abnormal ciliary function was the cloning of an axonemal dynein heavy-chain gene, left–right dynein, found to be mutated in a strain of mice with a 50% incidence of situs inversus. This murine gene was expressed in the embryonic node at embryonic day 7.5 – the location and time of right–left axis determination. Cilia were found in the embryonic node at this developmental time as well, but ultrastructural analysis revealed them to be of the ‘9 + 0’, usually nonmotile, variety. Thus, it came as a surprise when it was shown that embryonic node cilia are in fact motile, despite their ‘9 + 0’ configuration. With an unusual circular motion, their motility was different from that of ‘9 + 2’ cilia, but their beating was critical for correct left–right axis determination. Immotile embryonic node cilia were associated with a 50% chance of developing situs inversus. The flow across the embryonic node created by motile cilia seems to be sensed by nonmotile, flow-sensing cilia, localized in the periphery of the embryonic node. The bending of these cilia elicits a calcium signal on only one side of the node. This signal could cause the appropriate, unilateral expression of genes that ultimately determine the correct left–right axis. Abnormalities in motile or nonmotile cilia of the embryonic node will lead to random axis determination and thus to situs inversus in 50% of cases. Several mouse models of random left–right axis determination are used for further investigation of these issues, but their detailed description is beyond the scope of this article.

Ciliary defects can also affect primary or nonmotile cilia (with the ‘9 + 0’ microtubule arrangement). Since many cell types in the body express nonmotile cilia, PCD can also be associated with disorders that reflect nonmotile ciliary dysfunction other than situs inversus, such as cystic kidney disorder and retinitis pigmentosa.

**Genetics**

Genetic approaches to investigate the heterogeneous molecular defects underlying PCD have focused on identification of disease-causing genes using genetic linkage analysis (positional cloning) and candidate gene analysis. Although a genetic linkage of PCD families to the HLA locus on chromosome 6 was identified, candidate gene analysis in this region has not revealed the causative gene. Linkage analysis using inbred families and homozygosity mapping has identified four PCD loci: DNAH5 on chromosome 5p15, CILD2 on 19q, and additional loci on 16p12 and 15q13–15. Selection of candidate genes for mutational analysis has also proven successful, with identification of mutations in DNAI1 on chromosome 9p13–p21 and DNAH11 on 7p15. However, genetic heterogeneity can even be found within groups of families with the same ultrastructural defect.

Mutations in genes encoding two different axonemal outer dynein arm components (DNAI1 and DNAH5) have been shown to cause PCD in patients lacking outer dynein arms. Mutations in the DNAI1 and DNAH5 genes are proposed to account for approximately 24% of PCD cases overall and presumably for a larger percentage of the subgroup of PCD cases associated with outer dynein arm deficiencies.

Six DNAI1 mutations have been reported, and these account for mutations in 6 of 47 PCD families screened so far. One mutation, a T insertion predicted to cause a splice-site mutation (219 + 3insT), occurs more frequently. The relative frequency of this allele may indicate a mutation hotspot in the DNAI1 gene or a population founder effect in PCD.
Ten DNAH5 mutations have been reported. Of 25 PCD families compatible for linkage to the DNAH5 gene, mutations were found in 8. These are mostly premature truncation mutations predicting loss of motor- and microtubule-binding sites from the protein. Two missense mutations also occur, both located in functionally conserved amino acids. No apparent phenotype differences occur between patients with combinations of truncation or missense mutations. However, a patient homozygous for a splice-site mutation (IVS74-1G>4C) has been reported with a partial outer dynein arm deficiency and 54% shortened outer dynein arms, contrasting with patients homozygous for two truncation mutations who displayed the complete absence of outer dynein arms, suggesting a genotype–phenotype correlation.

Defects in another axonemal heavy-chain dynein, DNAH11, were identified in a patient with Kartagener’s syndrome and normal cilia ultrastructure. DNAH11 is the human homolog of the mouse left–right dynein gene. DNAH11 mutations are therefore associated with situs inversus and possibly a minority of PCD cases.

Mutational analysis on candidate genes encoding other axonemal structural components has excluded the dynein genes for the heavy chain DNAH9, the intermediate chain DNAI2, the light chain TCPX2, and the gene encoding the central complex protein, hPF20 as major causes of PCD. In one report, an absence of DNAH7 protein in cilia from a PCD patient was shown but no coding mutations were found, suggesting the likely involvement of another gene as the primary defect. Other candidate genes involved in ciliary function have also been investigated, including the genes for the transcription factor FOXJ1 and DNA polymerase lambda (DPCD).

Genetic work is aided by the selection of candidate disease genes with a conserved homolog in Chlamydomonas reinhardtii, a biflagellated eukaryotic unicellular alga and an established ciliary model organism. Dysmotile Chlamydomonas strains with axonemal defects similar to those of PCD patients have been and will be valuable in identifying genes associated with PCD. Furthermore, advances in proteomics and comparative bioinformatics provide comprehensive sets of ciliary genes and proteins, which comprise excellent new PCD candidates.

Therapeutic Considerations

No specific therapeutic modalities are available to correct ciliary dysfunction in PCD. Management should include aggressive measures to enhance clearance of mucus, prevent respiratory infections, and treat bacterial infections in the airways, sinuses, and middle ear. Few clinical trials have been conducted because PCD is rare and most centers follow only a few PCD patients.

Approaches to enhance mucus clearance from the lung in PCD include chest percussion with postural drainage, mechanical oscillatory Vest percussion, vigorous aerobic exercise, and other maneuvers to encourage cough and deep breathing. Bronchodilators such as albuterol may aid mucus clearance in patients who are bronchodilator responsive. Inhaled corticosteroids have been used, but the role of anti-inflammatory agents has not been defined.

Measures to prevent respiratory tract infection and irritation should be considered, including routine immunizations (for pertussis, measles, H. influenzae type b, S. pneumoniae, and influenza) and preventive counseling to avoid exposure to respiratory pathogens, tobacco smoke, and other irritants.

Prompt institution of antibiotic therapy for bacterial infections (bronchitis, sinusitis, and otitis media) can prevent or delay irreversible damage. Sputum culture results can direct appropriate antimicrobial therapy. In some patients, symptoms recur within days to weeks after completing a course of antibiotics. This subgroup may benefit from extended use of a broad-spectrum antibiotic. If detected and treated early, P. aeruginosa colonization of the airways can be eradicated; however, long-standing pseudomonal infection of bronchiectatic airway is unlikely to clear, even with long-term intravenous antibiotic therapy.

Use of tympanostomy with ventilation tube placement may benefit children with chronic ear infections and conductive hearing impairment. Nasal polypectomy and/or sinus drainage may provide short-term relief of symptoms in severe sinusitis without response to antibiotic therapy. However, long-term benefits are unclear. Lobectomy should be considered only in special cases. In patients with end-stage lung disease, lung transplantation has been performed successfully.

Prognosis

Chronic lung disease with bronchiectasis may progress to severe disability and eventually respiratory failure. The rate of disease progression is variable. A number of individuals have experienced a normal or near-normal life span. Better diagnostic tools are needed to facilitate earlier diagnosis and institution of therapy, thereby improving the overall health and prognosis for these patients.

See also: Bronchiectasis. Symptoms of Respiratory Disease: Cough and Other Symptoms.
PRIMARY MYELOFIBROSIS

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Abstract

Chronic idiopathic myelofibrosis refers to an acquired clonal hematopoietic stem cell disorder that is characterized by hyperplasia of progenitor cells in the bone marrow, reactive bone marrow fibrosis, characteristic changes in the blood and extra-medullary hematopoeisis (EMH). Complications include thrombotic disease, bleeding and leukemic transformation. Respiratory complications are less common and include thromboembolic disease, pulmonary hypertension and EMH in the lung. The long-term prognosis is poor. Current treatments are largely palliative; however, experimental treatments targeted at the underlying pathogenesis are likely to become available shortly.

Introduction

Chronic idiopathic myelofibrosis (CIMF) is an acquired clonal hematopoietic stem cell disorder that is characterized by hyperplasia of progenitor cells in the marrow, including dysplastic megakaryocytes and clonal monocytes, which secrete growth factors that lead to varying degrees of fibrosis, osteosclerosis, and new vessel formation in the bone marrow. Extra-medullary hematopoeisis (EMH), also known as agnogenic myeloid metaplasia, is usually present and refers to ectopic hematopoeisis that occurs most often in the liver and spleen but may be seen in any organ, including the lungs and pleura. This is most likely a consequence of the replacement of normal hematopoietic tissue by collagen fibrosis, the abnormal mobilization of hematopoietic progenitors into the peripheral blood and their localization in other organs.

Pathology

The diagnosis of CIMF is based on morphologic findings and the exclusion of other pathologies known to cause fibrosis of the marrow (see Table 1). It is important to be rigorous in the application of diagnostic criteria as marrow fibrosis and EMH are not specific for CIMF. Bone marrow fibrosis may also be seen in other myeloproliferative disorders, such as polycythemia vera and essential thrombocythemia, as well as in association with metastatic bone marrow tumors.

Examination of the blood film frequently suggests the diagnosis of CIMF. Patients in the fibrotic phase of the illness are usually anemic and have characteristic changes on the blood film, such as leukoerythroblastosis (a left-shift in the granulocyte count and nucleated red cells, which are normally absent in the blood) and marked red cell anisopoikilocytosis that includes tear drop cells (see Figure 1). These findings are suggestive but not diagnostic of CIMF. They may also be seen in conditions that replace normal bone marrow that may or may not have associated marrow fibrosis.