CHEST

Original Research

BRONCHIECTASIS

Autosomal Dominant Polycystic Kidney Disease Is Associated With an Increased Prevalence of Radiographic Bronchiectasis*

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Background: **Autosomal dominant polycystic kidney disease (ADPKD) is a common disease with several known extrarenal manifestations, although no known pulmonary features. The formation of renal cysts in ADPKD has been attributed to dysfunction of primary cilia and the primary cilia-related proteins polycystin-1 (in 85% of cases) and polycystin-2 in renal epithelial cells. The goals of this study were to characterize the normal expression of polycystin-1 in the motile cilia of airway epithelial cells and to evaluate lung structure in ADPKD patients.**
Methods: **Airway epithelium from non-ADPKD patients was immunostained to localize polycystin-1 expression, and lung tissue from ADPKD patients was examined for pathologic changes. CT scans from ADPKD patients (n = 95) and a control group of non-ADPKD chronic kidney disease patients (n = 95) were retrospectively reviewed for the presence of bronchiectasis using defined criteria.**
Results: **Immunostaining revealed polycystin-1 expression in the motile cilia of non-ADPKD airway epithelial cells. Lung tissue from one of five available ADPKD patient autopsies revealed histologic changes of bronchiectasis. Review of CT scans revealed a threefold-increased prevalence of bronchiectasis in the ADPKD group compared to the control group (37% vs 13%, p = 0.002).**
Conclusions: **ADPKD patients demonstrate an increased prevalence of radiographic bronchiectasis, a previously unrecognized manifestation of the disease. This association suggests that patients with primary cilia-associated diseases may be at risk for airway disease.** ***(CHEST 2008; 133:1181–1188)***

Key words: airway; autosomal dominant polycystic kidney disease; bronchiectasis; cilia; CT; epithelial cells; polycystin-1

Abbreviations: ADPKD = autosomal dominant polycystic kidney disease; CBF = ciliary beat frequency; ESRD = end-stage renal disease; hTEC = human tracheal epithelial cells

Autosomal dominant polycystic kidney disease (ADPKD) is a common disease associated with defective primary cilia function in renal epithelial cells. ADPKD affects between 1/400 and 1/1,000 people and accounts for 5 to 10% of all cases of end-stage renal disease (ESRD) in the United States.[1,2] In addition to the renal manifestations of the disease, there are several known extrarenal features. Hepatic cysts are the most common extrarenal feature, noted in up to 94% of ADPKD patients aged 35 to 46 years.[3] Seminal vesicle, pancreatic, and arachnoid membrane cysts also occur, although much less commonly than hepatic cysts.[1,2] Noncystic manifestations occurring at increased frequency in ADPKD patients include cardiac valve abnormalities (*eg*, mitral valve prolapse, approximately 26%), intracranial vascular aneurysms (approximately 4%), diverticulosis (approximately 83%), and abdominal wall hernias (approximately 45%).[4–7] Pulmonary manifestations have not been described.

Two genes, *PKD1* and *PKD2*, have been implicated in the pathogenesis of ADPKD. Of these, mutations in *PKD1* account for approximately 85% of cases.[1] The proteins encoded by *PKD1* and *PKD2*, respectively termed *polycystin-1* and *polycystin-2*, are expressed in the primary cilia of renal epithelial cells, where they exist as a complex, such that loss of function either protein results in a polycystic kidney phenotype. Primary cilia are immotile structures capable of sensing fluid flow, a process mediated by

polycystin-1 and polycystin-2.[8,9] In ADPKD, impaired primary cilia sensing results in abnormal intracellular signaling, cell hyperproliferation, and cyst formation.[1,10,11] Thus, ADPKD is one of a growing number of diseases related to defects in genes expressed in cilia (so-called *ciliopathies*).[12]

Primary cilia are solitary structures that are distinct from motile cilia, which are abundant on airway epithelial cells (approximately 250 per cell). Because polycystin-1 is known to be expressed in renal primary cilia, we examined its expression in airway motile cilia. We found that polycystin-1 specifically localizes to motile cilia and noted changes consistent with bronchiectasis in ADPKD autopsy samples. Thus, we hypothesized that an increased prevalence of bronchiectasis occurs in ADPKD patients, and that this could be detected by CT scanning. Accordingly, we performed a retrospective review of CT scans from patients with ADPKD, evaluating for the presence of bronchiectasis.

Materials and Methods

Study Protocol

Described human and animal studies were approved by the institutional committees of the Washington University School of Medicine.

Cell Culture and Immunostaining

Primary human tracheal epithelial cells (hTEC) from lung transplant donor trachea and mouse tracheal epithelial cells from C57BL/6J mice were cultured using air-liquid interface conditions to generate ciliated and nonciliated cells as described.[13,14] Cultured cells and lung tissue sections were immunostained as previously described.[15,16] Primary antibodies included rabbit anti-human polycystin-1 LRR (1:50, gift from Dr. O. Ibraghimov-Beskrovnaya, Genzyme; Framingham, MA),[17] goat anti-human polycystin-1 (A-20; 1:50; Santa Cruz Biotechnology; Santa Cruz, CA), and mouse anti-acetylated tubulin (1:1000; Sigma-Aldrich; St. Louis, MO). Antibody binding was detected using Alexa 488-conjugated (Invitrogen; Carlsbad, CA) or Cy3-conjugated (Jackson ImmunoResearch Labs; West Grove, PA) species-specific antibodies. Nuclei were identified using 4′,6-diamidino-2-phenylindole. After immunostaining, cultured cells were mechanically dislodged from membranes for further imaging. Microscopy images were captured and composed using a charge-coupled digital camera and software (QCapture Pro; Qimaging; Surry, BC, Canada; and Photoshop; Adobe; San Jose, CA).

Protein Blot Analysis and Real-time Polymerase Chain Reaction

Cell lysates from differentiated hTEC were separated on Tris-Acetate 3–8% gradient gels and subjected to standard protein blot analysis using the polycystin-1 LRR antibody (1:150, 18 h, 4°C) as previously described.[16] RNA was isolated from differentiated mouse tracheal epithelial cells, reverse transcribed, then amplified using mouse *Pkd1*-specific primers (5′-AAGCACAGGAGCAATGTCG-3′ and 5′-AAGTTCTCAGTATCCCACACAGG-3′) and SYBR Green PCR Master Mix (Invitrogen) in a Gene AMP 5700 Sequence Detection System (Applied Biosystems; Foster City, CA).

Lung Tissue Samples

Mouse lung was embedded in freezing media (Triangle Biomedical Science; Durham, NC). Non-ADPKD lung tissue from explanted lungs of patients who had undergone transplantation for COPD was freshly fixed in buffered formalin. Lung tissue from ADPKD patients was obtained from noninflation fixed autopsy samples collected at Barnes-Jewish Hospital from 1996 to 2006.

Study Populations and CT Scans

Patients with ADPKD who had undergone CT scanning from January 2000 to July 2006 at Barnes-Jewish Hospital were identified by searching the Department of Radiology database using the term *polycystic kidney*. As a control, a second group was identified using the search terms "end-stage renal disease," "ESRD," "chronic kidney disease," and "chronic renal insufficiency." All scans had been obtained for clinical indications using multidetector CT scanners (≥ 16 rows). All images were reconstructed as 5-mm-thick sections and by 5-mm intervals. When multiple examinations were identified for an individual patient, only the most recent study (abdominal or chest CT scan) was evaluated. All CT scans were reviewed by a chest radiologist (S.B.) and a pulmonologist (J.A.D.), with final interpretations made by consensus. Renal images were first reviewed to identify cases of ADPKD. For the purpose of this study, a definition of ADPKD required that all of the following CT scan criteria were met: (1) the presence of five or more cysts in each kidney, (2) cysts involving both the medullary and cortical regions of each kidney, and (3) bilateral kidney enlargement.[1,18,19] Studies lacking kidney images (due to previous nephrectomy or inadequate abdominal cuts on chest CT scans) or containing fewer than four lung cuts (on abdominal CT scans) were not included. The lung images were then reviewed for the presence of bronchiectasis or bronchiolectasis (hereafter termed *bronchiectasis*). A diagnosis of bronchiectasis required that at least two different airways in areas of nonconsolidated lung met one or more of the following criteria: (1) inner diameter of airway lumen larger than diameter of accompanying pulmonary artery, (2) airway visible within 1 cm of pleural edge/chest wall (excludes mediastinal pleural reflections), or (3) nontapering of airway for at least 2 cm beyond last branch point.[20,21] Abdominal and chest images were commonly

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