CHEST

Bardet Biedl Syndrome
Motile Ciliary Phenotype

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VIDEO

BACKGROUND: Cilia line the surface of the respiratory tract and beat in a coordinated wave to protect the lungs against infection. Bardet Biedl Syndrome (BBS) is a rare condition attributed to cilia dysfunction. Murine models of BBS suggest a respiratory phenotype; however, no reports have studied the translation of these findings in patients.

METHODS: We assessed the clinical symptoms of motile cilia dysfunction and the histology of ciliated respiratory epithelium in patients with BBS.

RESULTS: We report an increased prevalence of neonatal respiratory distress at birth (12%), general practitioner-diagnosed asthma (21%), otitis media (33%), and rhinitis (36%) in patients with BBS. These symptoms, however, occurred at a significantly reduced prevalence compared with patients with known motile cilia dysfunction (primary ciliary dyskinesia). Respiratory epithelial assessment revealed cellular damage, significant ciliary depletion (on 60% of ciliated cells), and goblet cell hyperplasia in patients with BBS (50% goblet cells). These findings were quantifiably similar to those of patients with asthma ($P > .05$). Surprisingly, motile cilia function and ultrastructure were grossly normal with the exception of occasional unique inclusions within the ciliary membrane.

CONCLUSIONS: In conclusion, motile ciliary structure and function are essentially normal in patients with BBS.

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ABBREVIATIONS: BBS = Bardet Biedl Syndrome; GP = general practitioner; NO = nitric oxide; PCD = primary ciliary dyskinesia; ppb = parts per billion

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Bardet Biedl Syndrome (BBS) is clinically characterized by rod-cone dystrophy, truncal obesity, postaxial polydactyly, cognitive impairment, genital anomalies, and renal abnormalities.[1] The phenotype of this genetic condition is thought to occur because of a loss of function in nonmotile primary cilia resulting from an absence of BBS proteins. These proteins are located at the base of the cilium, some in a complex called the BBSome, which is involved in the movement of particles in and out of the cilium by a process known as intraflagellar transport.[1] Dysfunction of nonmotile primary cilia in this condition is well described; however, evidence from murine models suggest that BBS proteins are also colocalized with motile cilia on the epithelial surface of the respiratory tract, suggesting that this ciliopathy may also affect motile cilia.[2] Murine models with defects in BBS proteins 1, 2, 4, and 6 demonstrate dysfunction of the ependymal cilia of the brain, sperm flagella, and motile cilia in the respiratory tract.[2,3] Reports show slow ciliary beat frequency within the respiratory tract increased variation in ciliary length and swollen, paddle-shaped cilia tips containing vesicles on electron microscopy.[2] The translation and clinical relevance of these murine findings have never been reported in human patients with BBS.

Dysfunction of motile cilia in humans usually results in a well-defined phenotype known as primary ciliary dyskinesia (PCD). Symptoms include neonatal respiratory distress, otitis media, rhinosinusitis, and chronic wet cough. Recurrent chest infections eventually lead to permanent scarring of the lung in the form of bronchiectasis. Motile cilia structure is similar to the flagella of sperm tails, and consequently, men with PCD are often subfertile.[4] Occasionally, PCD has been reported in patients with nonmotile cilia dysfunction.[5,6] The aim of this study was to assess the structure and function of respiratory ciliated epithelium and the clinical implications of respiratory ciliary dysfunction in a cohort of patients with BBS.

Materials and Methods

Subjects

A retrospective review of clinical data was analyzed for all patients attending BBS clinics at Great Ormond Street and Guys Hospital who were referred for respiratory cilia function tests. Results from 46 patients were analyzed, of which 24 were men (57%). Ages ranged from <1 year to 48 years, with an average age of 22 years.

Study Design

Respiratory history was recorded from patients with BBS and their families, concentrating on factors implicated in motile respiratory ciliary dysfunction such as respiratory distress in the neonatal period; frequency of chest infections; and ear, nose, and throat symptoms. Patients were screened for ciliary dysfunction using a nasal nitric oxide (NO) screening test. Individuals in which PCD was indicated because of symptoms leading to high clinical suspicion or low nasal NO (<250 parts per billion [ppb]) were followed up with a nasal brush biopsy ($n=16$). Microscopy assessments of respiratory cilia were made by a blinded observer and were compared with three groups: patients with PCD and static cilia caused by an outer dynein arm defect, patients with asthma, and healthy control subjects.

Investigations

Clinical tests for ciliary dysfunction were performed according to national and international standards for the diagnosis of PCD following the standard operating protocols of the Royal Brompton Hospital, a National Health Service nationally designated specialist diagnostic center.[7] These are described in the following sections.

Nasal NO: Nasal NO was measured by chemiluminescence on a portable Logan analyzer at a flow rate of 250 mL/s (Logan Research Ltd). Patients were asked to hold their breath, and NO was measured when there was a 10-s plateau in value. Correct technique was assessed by a simultaneous CO_2 trace. When subjects were unable to perform breath hold with satisfactory technique ($n=7$), a tidal breathing method was used, in which subjects breathed normally with their mouths open. NO testing was not performed in children under 2 years of age.

Nasal Brush Biopsy: Nasal brush biopsies were collected from the nasal inferior turbinate using a modified 3-mm bronchial cytology brush (Diagmed Healthcare) and were suspended in Media199.

Light Microscopy: Nasal brushings were assessed for ciliary beat pattern and frequency using high-speed video microscopy.[8] Cilia were recorded at 37°C under a 100× oil immersion lens using a high-speed video camera at 500 fps (Fastcam Troubleshooter XS; Lake Imaging Systems). Movies were then played back at 60 fps, and motion analysis was performed on the top and side profiles.

Electron Microscopy: Nasal brush biopsies were fixed in 2.5% glutaraldehyde in cacodylate buffer and were processed as previously described.[9] Briefly, cells were washed in sodium cacodylate buffer, postfixed with 1% osmium tetroxide, and centrifuged in 2% agar to generate a pellet. Using a series of increasing concentrations of methanol followed by propylene oxide, cells were dehydrated before being embedded in Araldite resin. Sections of 70 to 90 nm in size were cut using a Reichert Ultracut-E ultramicrotome, mounted onto copper grids, and stained with methanolic uranyl acetate and lead citrate. Assessment of the respiratory epithelium and ciliary ultrastructure was made on a Hitachi 7000 transmission electron microscope. A clinical electron microscopist blinded to the case information quantified cells, determined their microtubular arrangement in the axoneme, and investigated the presence of dynein arms.

Ethics Statement

This study was conducted in accordance with the amended Declaration of Helsinki. The National Research Ethics Service Committee London, Bloomsbury, approved the protocol (study number 08/H0713/82). Signed patient consent was not required.