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Changes of Motile Ciliary Phenotype in Patients with Primary Ciliopathies

Miroslava Brndiarova, Julia Kvassayova, Jarmila Vojtkova, Matus Igaz, Tomas Buday, and Jana Plevkova

Abstract

Primary ciliopathies are a group of disorders associated with abnormal formation and function of primary cilia. Many cilia-associated proteins found in primary cilia are also present in motile cilia. Such proteins are important for the ciliary base, such as the transition zone or basal bodies, and the intraflagellar transport. Their exact role in the respiratory motile cilia is unsettled. In this prospective clinical single-center study, we investigated the hypothesis that these proteins regulate the function of motile cilia. We addressed the issue by defining the motile cilia beat frequency in the respiratory tract of patients with primary ciliopathies accompanied by chronic kidney disease and comparing it in those without kidney involvement. Ciliary beat frequency in the nasal mucosa samples was evaluated by the ciliary analysis software LabVIEW. Both children and their parents with primary ciliopathies and kidney involvement had significantly lower median airway ciliary beat frequencies than those without kidney involvement who have normal ciliary motility. Further, the ciliary beat frequency is inversely associated with the serum creatinine level. These findings strongly suggest that kidney involvement in patients with primary ciliopathy may underlie the development of motile cilia dysfunction in the respiratory tract, potentially increasing respiratory morbidity.

Keywords

Cilia · Ciliary beat frequency · Ciliopathy · Kidney disease · Respiratory morbidity

1 Introduction

Cilia play a vital part in human physiology. They are divided into two main types. Motile cilia are found in the respiratory tract, ventricles of the central nervous system, and the middle ear. These cilia have a rhythmic beating motion and, for instance, in the respiratory tract, they are an important component of the airway defense by sweeping mucous out of the lungs (Kempeneers and Chilvers 2018). Primary cilia are nonmotile and typically appear on the apical surface of most eukaryotic cells as microtubule-based organelles covered with the plasma membrane. These cilia lack the two central microtubule singlets

M. Brndiarova (✉), J. Kvassayova, J. Vojtkova, and M. Igaz
Department of Pediatrics, Jessenius Faculty of Medicine and University Hospital, Comenius University in Bratislava, Martin, Slovakia
e-mail: brndiarova1@uniba.sk

T. Buday and J. Plevkova
Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University in Bratislava, Martin, Slovakia
e-mail: tomas.buday@uniba.sk; jana.plevkova@uniba.sk

characteristic of motile cilia. Primary cilia play an essential role for chemosensory (Bloodgood 2010), mechanosensory, for instance, detecting luminal flow in renal tubules, biliary ducts, and vessels (Nauli et al. 2003), and photosensory retinal functions (Senior et al. 1961). Primary ciliopathies are a group of disorders associated with abnormal formation and function of primary cilia. The clinical phenotypes of ciliopathy are diverse. A common feature of some primary ciliopathies is a variable extent of kidney damage or cyst formation (Braun and Hildebrandt 2017). Typical extra-renal manifestations include hepatic cysts and fibrosis (Schueler et al. 2015), retinal degeneration (Senior et al. 1961), skeletal deformities, dysmorphic facial features (Sensenbrenner et al. 1975), laterality defects, and congenital heart defects (Otto et al. 2003). Respiratory manifestations of primary ciliopathies like bronchiectasis, respiratory infection, or lung cysts are rare and their exact cause is unclear (Ibrahim and Rasoul 2015; Driscoll et al. 2008). Nonetheless, the respiratory tract is quite often affected by the disturbance of motile cilia manifest by impaired function of mucociliary clearance. The ciliary beating is coordinated in metachronal waves. It is a complex process that requires intertwined molecular interactions influenced by intracellular Ca^{2+} content and pH change, and protein kinase activation (Joskova et al. 2020; Marusiakova et al. 2020; Bayless et al. 2019; Duricek et al. 2019). Many cilia-associated proteins found in primary cilia are also present in motile cilia. Yet it is unclear to what extent proteins forming the ciliary basal body and the transition zone, and those engaged in the intraflagellar transport, could be concurrently affected in both types of cilia. Therefore, this study aims to evaluate the ciliary beat frequency (CBF) of the airway motile cilia in patients with a primary ciliopathy in an organ distal to the respiratory tract. We investigated the hypothesis that the airway CBF could be affected in primary ciliopathies involving kidneys, which would manifest in reduced airway mucus clearance. We addressed the issue by investigating the pediatric and adult (children's parents) patients with primary ciliopathies affecting kidneys.

2 Methods

2.1 Study Design and Population

This is a prospective clinical single-center study. We had four groups of patients: two each of children and adults with and without chronic kidney diseases, CKD(+) and CKD(−), respectively. Additionally, we had two groups of healthy age-matched children and adults with no kidney disease for the reference measurement of CBF. The group stratification with basic demographic data is shown in Table 1.

Inclusion criteria were the lack of acute respiratory infections in the preceding 4 weeks, no smoking, no allergy, and no use of antihistamines. The degree of chronic kidney disease (CKD) in adults was evaluated according to the National Kidney Foundation's CKD Epidemiology Collaboration (CKD-EPI) creatinine equation (Levey and Stevens 2010) and in children according to the bedside Schwartz equation (Schwartz and Work 2009). Basic demographic data, detailed respiratory history, laboratory indices data (plasma creatinine, urinalysis), abdominal ultrasonography, echocardiography, spirometry, and chest computer tomography were obtained from medical records. Exclusion criteria were adenoid hypertrophy, acute or chronic infections, and anatomical deformities identified during the ear-nose-throat examination.

Table 1 Stratification of study groups

	Primary ciliopathy—children		Primary ciliopathy—adults		Healthy children	Healthy adults
	CKD(+)	CKD(−)	CKD(+)	CKD(−)	CKD(−)	CKD(−)
n	3	16	3	5	43	21
Age (years)	14 (12–16)	9 (6–11)	54 (37–59)	37 (35–43)	10 (7–13)	42 (34–49)

Data are medians (IQR). *CDK(+)* chronic kidney disease, *CKD(−)* lack of chronic kidney disease, *n* number of subjects

Other exclusion criteria were gastroesophageal reflux disease, endocrine, metabolic, or oncological diseases.

2.2 Investigations

Samples of ciliated epithelium in nasal mucosa were obtained by a cytology brush (Cytobrush plus, Medscand Medical; CooperSurgical, Inc., Trumbull, CT) and suspended in the Roswell Park Memorial Institute (RPMI) 1640 medium. The temperature was maintained at 20 °C. Motile cilia were observed using a digital high-speed video camera (Basler A504kc; Basler AG; Ahrensburg, Germany) at a frame rate of 256–512 per sec. The camera was connected to an inverted phase-contrast microscope (Zeiss Axio Vert. A1; Carl Zeiss AG: Göttingen, Germany) and a computer. Specimens were examined using a 40× objective lens providing a magnification of 400×. Video analysis was performed using cilia analysis software (LabVIEW National Instruments Corp, Austin, TX) (Hargaš et al. 2011). Each preparation was evaluated for a maximum of 10 min, with 10 short video sequences recorded. The median and range of CBF were provided. Only undisrupted ciliated epithelia were analysed.

Genetic analysis was performed to confirm the presence of primary ciliopathy using the next-generation sequencing method using NextSeq System (Illumina Inc., San Diego, CA). The entire coding and splice-relevant regions of genes were evaluated.

2.3 Statistical Analysis

Data were expressed as medians and interquartile ranges (IQR). Differences were assessed using Student's *t*-test for unpaired samples. A *p*-value <0.05 defined statistically significant changes. The analysis was performed using a commercial SYSTAT v11.0 package (Systat Software Inc., San Jose, CA).

3 Results

3.1 Airway Ciliary Beat Frequency (CBF)—Motile Phenotype

The airway CBF in healthy children and their adult parents without primary ciliopathy and chronic kidney diseases was, on average, between 6 and 8 Hz and was alike in both groups as detailed in Table 2. This value of CBF was taken as a reference level for comparison to that in patients with primary ciliopathies and chronic kidney involvement. Children and their parents with CKD had the median airway CBF, on average, between 4 and 5 Hz, which was a highly significant decline compared to the patients without CKD ($p < 0.001$). There were no appreciable differences in CBF between the two age groups of patients in the presence or absence of CKD (Table 3). The CBF in patients without CKD was at the level of that present in the control healthy subjects.

Table 2 Airway ciliary beat frequency in healthy control subjects without primary ciliopathy and chronic kidney diseases (CKD−)

	CKD(−)
Ciliary beat frequency (Hz)	
Children	6.7 (6.0–7.7)
Adults	6.2 (5.3–7.3)
p (children/adult)	> 0.05
Creatinine (μmol/l)	
Children	55 (47–66)
Adults	73 (67–79)
p (children/adult)	0.03

Data are medians (IQR)

Table 3 Airway ciliary beat frequency and serum creatinine level in patients with and without chronic kidney diseases, CKD(+) and CKD(−), respectively

	CKD (+)	CKD (−)	*p*
Ciliary beat frequency (Hz)			
Children	4.0 (3.9–4.8)	6.8 (6.5–7.1)	< 0.0001
Adults	4.9 (4.3–5.0)	6.9 (6.7–7.1)	< 0.001
p (children/adult)	0.45	0.39	
Creatinine (μmol/l)			
Children	296 (225–299)	57 (47–78)	0.00003
Adults	216 (186–315)	62 (47–81)	0.003
p (children/adult)	0.43	0.39	

Data are medians (IQR)

3.2 Respiratory Manifestations

Respiratory manifestations were seen in 100% of children with CKD(+) and 6.3% of children with CKD(−). Concerning the adult patients, respiratory manifestations were seen in 33.3% with CKD(+) and in none with CKD(−). Patients with nephronophthisis type I (NPHP type 1) did not suffer from respiratory infections but had a restrictive lung disorder and nonspecific nodules up to 5 mm in diameter in the upper lobes of lung parenchyma (segments: S10 bilateral and S6 in the right lung). Overall, a restrictive lung disorder was seen in 50% of patients with CKD. A 10-year-old boy with Sensenbrenner syndrome (SBS) and low CBF had mild recurrent rhinosinusitis. Two patients with autosomal recessive polycystic kidney disease (ARPKD) had recurrent rhinitis. One of them belonged to the CKD(+) group and had a strongly decreased lower CBF of 3.8 (3.2–6.0) Hz. Another CKD(+) patient with the autosomal dominant polycystic kidney disease (ADPKD) and a restrictive lung disorder also had a low CBF of 3.8 (3.2–5.9) Hz. Computer tomography of the lungs did not detect bronchiectasis in the patients.

3.3 Genetic Analysis

The presence of primary kidney ciliopathy was confirmed in the genetic analysis. We found that patients with ADPKD commonly had a mutation in the *PKD1* gene (88.0%). We identified 6 new pathogenic variants of this gene found in 11 patients (Table 4).

4 Discussion

This study demonstrates that patients with primary ciliopathies with developed CKD had reduced ciliary beating frequency in the airway mucosal cells. The most likely underlying reason could be an impaired ciliary structure caused by mutations of the genes encoding ciliary proteins that are identical for both primary and motile cilia. Several hypotheses of the origin of respiratory manifestations in patients with primary kidney ciliopathies are presented in the literature. One of them is a disorder of calcium homeostasis leading to reduced cellular calcium signaling in patients with polycystic kidney diseases. Calcium plays a critical role in regulating CBF (Mangolini et al. 2016; Nauli et al. 2003; Braiman and Priel 2001). Wu et al. (2009) have suggested that damage to primary cilia in airway smooth muscle cells could underlie motile cilia dysfunction. Bronchiectases are frequently associated with irregular bronchial wall thickness. Primary cilia of airway smooth muscle cells may be mechanical pressure sensors and play a role in cell migration, injury repair, and possibly in ciliogenesis. Motile ciliated cells originate from primary ciliated cells. When ciliogenesis is impaired, cilia exhibit slow and dyskinetic motion (Jain et al. 2010). An unresolved question is whether the sensory function of primary cilia is also present in motile cilia (Jain et al. 2012).

The present findings are comparable to those of Shoemark et al. (2015) who have found that patients with Bardet-Biedl syndrome have normal CBF. Likewise, Fliegauf et al. (2006) have found that in the absence of nephrocystin, a molecular

Table 4 Pathogenic mutations in the *PKD1* gene that encodes the protein polycystin-1 active in kidney primary cilia. Individual subjects, renal and extrarenal manifestations, as well as the patients' airway ciliary beat frequency (CBF) are shown. New pathogenic mutations unraveled are bold out

Sex	Mutation/gene	CBF (Hz)	Renal manifestations	Extrarenal manifestations
F	*PKHD1*	3.8	CKD G4, HT, P	Hepatic cysts and fibrosis, respiratory infections
M	*PKHD1*	6.5	HT	Hepatic cysts, respiratory infections
F	*PKHD1*	7.1		
F	*PKHD1*	6.0	HT	Hepatic fibrosis
F	***PKD1* c.2528C > G, p.(Ser843*) exon 11**	9.0		
M	***PKD1* c.7525_7539dup, p.(Val2509_Leu2513dup)**	8.3		
M	***PKD1* c.11425G > C, p.(Gly3809_Arg)**	8.7		
F	*PKD1*	7.0		
M	***PKD1* c.3114delA, p.(Leu1039*)**	7.0		
M	***PKD1* c.3400_3401delArg(p.Ser1134)**	6.4		
M	***PKD1* c.3400_3401delArg(p.Ser1134)**	6.9		
F	***PKD1* c9683dupG, p.(Leu3229Profs*24) exon 28**	6.7		
F	*PKD2*	6.6		
F	*PKD2*	7.2		
F	*PKD1*	3.0	CKD G4, HT, P	Hepatic cysts, restrictive lung disorder
F	*PKD1*	4.9	CKD G3b, HT, P	Hepatic cysts
M	*PKD1*	5.0	CKD, G3a, HT	
M	***PKD1* c.3400_3401delAG(p.Ser1134)**	6.7	HT	
M	*PKD1*	7.1		
F	*PKD2*	6.2	HT, P	Hepatic cysts
F	*PKD1*	6.9		
M	***PKD1* c.3114delA, p.(Leu1039*)**	7.9		
F	*NPHP1 (homozygote)*	4.0	CKD G5, P, HT, Tx	LVNC, nonspecific lung nodules, restrictive lung disorder
M	***WDR35* c.1922 T > G, p.(Leu641*), c.2522A > Tp.(Asp841Val)**	5.6	CKD G3b, HT, P	Foramen ovale, hypospadias, Cranioectodermal dysplasia, respiratory infections
M	***WDR35* c.1922 T > G, p.(Leu641*), c.2522A > Tp.(Asp841Val)**	6.5		Cranioectodermal dysplasia
M	*BBS9*	6.7		Syndactyly, Hirschsprung disease, hypogonadism
F	OFD type I	6.5		Syndactyly, brachydactyly

CKD chronic kidney disease, *Arg* arginine, *Asp* aspartate, *BBS* Bardet-Biedl syndrome, *Gly* glycine, *HT* hypertension, *Leu* leucine, *LVNC* left ventricular non-compaction cardiomyopathy, *OFD type I* orofaciodigital syndrome, *P* proteinuria, *Ser* serine, *Tx* kidney transplantation, *Val* valine

component of primary cilia, the structure of motile cilia remains unaltered, and the CBF is normal. The beating pattern may be slightly irregular, but the irregularity is not as severe as in primary ciliary dyskinesia. Therefore, the presumption that proteins that regulate ciliary motion would be damaged in patients with primary ciliopathies is hardly tenable.

Ciliopathies are a heterogeneous group of disorders. Phenotypic features are variable even in patients with the same mutations. Walczak-Sztulpa et al. (2017) have reported the intrafamilial variability in patients with Sensenbrenner syndrome with heterozygous variants in the *WDR35* gene. We observed two female siblings with ciliopathies having different phenotypic features (these two patients were not included in the present study group; unpublished observation). One girl had polycystic kidney disease. Her renal function was normal and the CBF remained in the normal range of 7.2 (6.5–8.4) Hz. The other girl had the CBF significantly reduced down to the level of 1.1 (0–2) Hz characteristic of primary ciliary dyskinesia. This girl had a normal renal function and no cystic kidney formations but suffered from recurrent respiratory infections. The plausibility is that the siblings had different clinical manifestations of the same cilia-elated disorder. However, the varied expression of relevant genes or genetic mutations ("genetic load") that can modulate clinical manifestation cannot be excluded (Arts and Knoers 2013; Bredrup et al. 2011).

It is generally known that patients with CKD are more likely to experience respiratory manifestations (Pierson 2006). A cross-sectional study of Kucur et al. (2016) who used saccharin tests suggests that chronic kidney failure is a risk for respiratory mucociliary dysfunction. According to that study, nasal mucociliary clearance time is significantly greater in patients with CKD than in healthy control individuals. No studies have yet directly evaluated the respiratory CBF in patients with kidney failure. There are several potential mechanisms of damage to respiratory cilia in this condition like free oxygen radicals or uremic toxins, endothelial dysfunction and vasoconstriction with reduced blood flow, decreased periciliary fluid flow, or increased mucus viscosity with electrolyte imbalance (Uluyol et al. 2016). The design of the present study did not make it possible to distinguish the exact molecular mechanism of respiratory cilia dysfunction. Yet we believe we have shown that motile cilia in the airways of patients with CKD are defunct as expressed by significantly reduced CBF. Further, we submit that genetic factors and disturbed body homeostasis in CKD may impair mucociliary transport function in the airways of patients with primary ciliopathies.

Conflicts of Interest The authors declare no conflicts of interest in relation to this study.

Ethical Approval All procedures performed in studies involving human participants were in accord with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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