Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 9 (58) No. 2 - 2016

PRIMARY CILIARY DYSKINESIA: AN OVERLOOKED ORPHAN DISEASE?

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Abstract: *Primary Ciliary Dyskinesia (PCD) is a genetically heterogeneous disorder of motile cilia which affects the mucociliary clearance. This leads to chronic problems of the upper and lower respiratory tract and in around 50% of cases to mirror image arrangement. Ciliary dysfunction results in an array of clinical manifestations including chronic bronchitis leading to bronchiectasis, chronic rhino-sinusitis and otitis media, male infertility. The low estimated incidence situates PCD among orphan diseases. Establishing diagnosis is a difficult task and is usually based on clinical suspicion followed by a series of investigations (nasal nitric oxide measurements, in vivo tests of ciliary motility examination of cilia by electron microscopy, epithelial cultures and genetic testing) that will be described in this article. Despite new genetic findings, PCD is still underdiagnosed disorder. An overview of the literature is made, followed by illustration of suggestive case reports.*

Key words: *cilia, chronic respiratory disease, dynein, situs inversus, primary ciliary dyskinesia*

1. Introduction

Primary ciliary dyskinesia (PCD), also known as immotile cilia syndrome (ICS; OMIM 242650) and Kartagener syndrome (KS; OMMIM .244400) is a genetically heterogeneous disorder of the motile cilia which affects mucociliary clearance.[2], [11] Usually it is an autosomal recessive disorder characterised by abnormal ciliary beat pattern, often with low frequency and detectable ciliary ultra-structural abnormalities.[8]

The main consequence of dysfunctional cilia is the impairment of mucociliary clearance in the airways, leading to chronic airway infection and bronchiectasis, chronic rhino-sinusitis and otitis media.

Approximately 50% of affected patients have situs inversus, and those that associate chronic sinusitis and bronchiectasis have been referred to as Kartagener syndrome.

The altered function of cilia/flagellae in different cell types may be clinically expressed as male infertility (sperm tail), hydrocephalus (ependymal cilia), complex heart disease (nodal cilia), biliary atresia, retinal degeneration. [4]

Motile nodal cilia are responsible for normal right-left orientation, but may have also other developmental roles in the embryo. [1], [13]

The estimated incidence of PCD is

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about 1/16 000 births and is based on the prevalence of situs inversus and bronchiectasis, but may be higher, according to new genetic findings.

Establishing diagnosis may be delayed in a significant proportion of patients, even characteristic symptoms are present from early childhood (chronic rhinitis and wet cough, situs inversus) and this usually leads to poorer outcomes.[12]

The clinical phenotype in PCD is broad and overlaps with other chronic airway diseases, as cystic fibrosis (CF), other supurative respiratory disorders.

Establishing a final diagnosis may be a difficult task for the clinician and requires examination of cilia by electron microscopy, nasal nitric oxide measurements, genetic testing in specialized centres.

2. Ciliary structure and function

Cilia and flagella are ancient organelles with conserved structure and function across the phylogenesis (fig.1). They are recognised for the role in cell motility and fluid transport over mucosal surfaces. It has been recently recognized also a sensory function of cilia that modulates parts of development and cell function.[11]

Motile and sensory cilia are composed of arrays of nine peripheral microtubule doublets arranged around two central microtubules (9+2 axoneme) (fig. 2). [6]

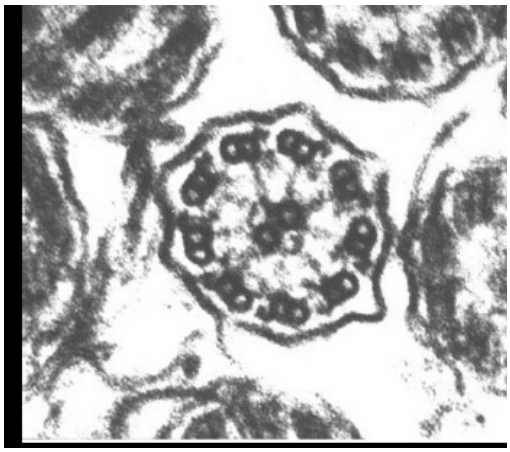

Fig. 1. *Structure of cilia*

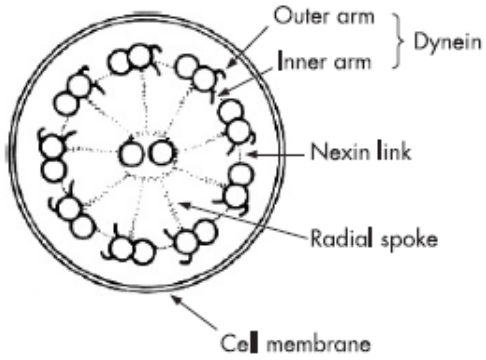

Fig. 2 *Schematic diagram of structure of cilia*

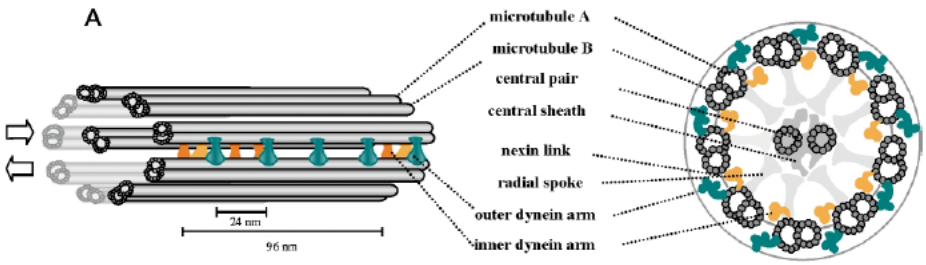

Fig. 3. *Axoneme structure and components.*

In figure 3, the schematic diagram of the cilium axoneme in length and in cross section is indicated, with the different axonemal components. Nine microtubule doublets (microtubule A and B) surround two central microtubules (central pair), which are enclosed by the central sheath. The microtubules are interconnected by nexin links, radial spokes and dynein arms. Cilia beating originates from the sliding of microtubules doublets (double arrow on the left), which is generated by the ATPase activity of the dynein arms. The dynein arms are periodically distributed along the axoneme; outer dynein arms (green) with a 24 nm periodicity and inner dynein arms (light and dark orange) with a 96 nm periodicity. The dynein arms are multiprotein complexes that project from the A microtubule of every doublet; the outer arms (green) face towards the boundary of the axoneme; and the inner arms (orange) face the central sheath.

The central apparatus and radial spokes provide the structural interface for transmitting regulatory signals to the arms.

3. Normal ciliary beat frequency

There are there basic groups of cilia; motile 9+2 cilia with attendant dynein arm structures (respiratory epithelial cells) nonmotile 9+0 primary cilia lacking dynein arms (kidney tubules) and 9+0 motile primary cilia possessing dynein arms (embryonic node)'.

There are two distinct types of motility for the 9+2 and 9+0 motile cilia. Ciliated epithelial cells bear approximately 200 motile (9+2) cilia that move with both intracellular and intercellular synchrony. The pattern of beat in 9+2 motile cilia is in a waveform with a forward effective stroke followed by a return stroke.[11] The direction of stroke is related to the directional orientation of the central microtubules. In normal cells, individual cilia are very plastic and move fluidly, sometimes deforming briefly when encountering particles transported over the mucosal surface. Cilia are embedded in a watery periciliary fluid with low viscosity. The range of ciliary beat frequency varies between 8-20 Hz and may be accelerated by irritants (tobacco smoke). The mechanisms whereby beat frequency is accelerated has been suggested to be regulated through the activity of No synthases localized in the apical cytoplasm.

4. Genetic heterogeneity

Dysfunction of the axonemal structure has been linked to a class of disorders known as "ciliopathies" with include the following: PCD Kartagener syndrome, Bardet-Biedl syndrome, Hydrocephalus, polycystic kidney disease, polycystic liver disease, nephrolithiasis, Meckel-Gruber syndrome, Joubert syndrome, Alstrom syndrome, Jeune syndrome and laterality defects.

PCD was the first human disorder linked to dysfunction of motile cilia.

5. Diagnosis/testing

The diagnosis of PCD requires the presence of the characteristic clinical phenotype and either one specific ciliary ultrastructural defects identified by transmission electron microscopy in biopsy samples of the respiratory epithelium or two mutation in one of seventeen genes known to be associated with PCD: *DNAI1, DNAAF3, DNAH5, HYDIN, NME8, DNAH11, DNAI2, DNAAF2* (*C14orf104*), *RSPH4A*, *RSPH9, DNAAF1* (*LRRC50*), *CCDC39, CCDC40, DNAL1, CCDC103, HEATR2,* and *LRRC6*.

Some other gene candidates undergo research.

6. Clinical manifestations/diagnosis

The first link between cilia and left-right determination was described by Kartagener who observed that patients with situs inversus (heart and viscera positioned in reverse mirror-image), had also respiratory problems. This is why the condition in these patients was called Kartagener's syndrome and encounters about 50% of the patients with primary ciliary dyskinesia (PCD).

Recent advances in the diagnosis of patients with primary ciliary dyskinesia (PCD) have included networks of specialists developing protocol-driven testing , international consensus guidelines and rapid expansion of known PCD-related genes. [6].

There is no "gold-standard" test for PCD, hence European consensus guidelines (2009) recommend a combination of tests including nasal nitric oxide (nNO) screening, high-speed video microscopy analysis (HSVMA) of ciliary beat frequency (CBF) and pattern (CBP), and transmission electronmicroscopy (TEM) analysis of ciliary ultrastructure Reanalysis following submerged [9] or air-liquid interface (ALI) [5] culture may be useful to exclude secondary ciliary dyskinesia or confirm PCD when analysis of the primary sample is abnormal, and may provide additional cilia if the primary sample is inadequate.

PCD may be suspected because of respiratory manifestations that are present already in the neonatal period, but even so, diagnosis is usually made late. This is partially, because many of the symptoms (rhinitis, cough, secretory otitis media) are common in childhood and overlap other respiratory diseases.

General clinicians must be aware of the condition and must take a detailed, careful history, more so, when heterotaxy is present.

In the newborn period, almost 70% of babies present with respiratory distress, nasal obstruction. Continuous rhinorrhea from the first day of life is highly likely to be due to PCD.[2]

In childhood, chronic productive cough, (after excluding other conditions as cystic fibrosis), associated with mirror image arrangement may be a clue to suspect PCD.

"Atypical asthma", non-responsive to treatment, mainly associated with wet cough or idiopathic" bronchiectasis, constant rhinitis and rhinosinusitis episodes, are features of PCD.

Recurrent otitis media with effusion, even after insertion of ventilation tubes, is usually not responding to treatment and may progress to hearing loss.

In adolescence and young adults, features presented in childhood may be associated with ectopic pregnancies or decreased fertility in women; immotile sperm is even seen in 50% of male PCD patients.

PCD may be associated with other diseases and should be considered when the following diagnosis are made: complex congenital heart disease, polycystic kidney disease and liver disease, hydrocephalus, biliary atresia, severe oesophageal disease (reflux and atresia), retinal degeneration (including retinitis pigmentosa).

7. Screening for PCD

Most popular tests for screening in PCD are the saccharin test and nasal nitric oxide measurement.

The sacharin test involves the introduction of a microtablet of sacharin in the inferior turbinate and recording of time taken for the patient to feel the taste of it. It may be unreliable in children and also difficult to perform. More so, an abnormal test must be confirmed with further tests.

In PCD, nasal and exhale NO is low for

still unclear reasons, but measurement of them is a good screening test (overlap with other respiratory conditions should always prompt to further testing).

In order to confirm PCD it will be mandatory to do further testing, which includes: ciliary beat frequency measurement, ciliary beat pattern analysis, electron microscopy of ciliary ultrasructure, measurement of ciliary disorientation, cell culture with re-growth of ciliated epithelium.

All these mentioned investigations have to be done in specialized centers and interpretation of results has to be made by experienced specialist.

Viral infections may temporarily affect ciliary function and test should be repeated if this may lead to a false positive result and should be carefully interpreted.

8. Management of PCD

PCD being mainly represented by respiratory involvement, appropriate medical therapy has been shown to prevent deterioration in lung function. [3], [12]

This should be done by experienced specialists in appropriate care centers and consists of: regular respiratory monitoring, airway clearance (combination of physiotherapy and physical exercise), aggressive antibiotic treatment of upper and lower respiratory infections. In order to correctly do this, regular monitoring of respiratory microbial colonization has to be performed (as in cystic fibrosis), taking into account that the common infecting organisms in children are *Haemophylus influenzae* and *Staphylococcus aureus*. *Sterptococcus pneumoniae, Pseudomonas aeruginosa* and non-tuberculous mycobacteria are also reported.

Investigation of bronchiectasis should be performed using high resolution CT scans of the lung to define disease progression.

Prophylactic antibiotics have not been shown to be beneficial in the majority of cases, but should be considered when frequent courses of antibiotics are needed. Choice of antibiotics should be made based on sputum culture and high doses of oral antibiotics should be initiated from first signs of respiratory worsening and lung function deterioration.

If *P aeruginosa* is isolated, treatment regimens are similar to those recommended in CF.

High calories nutrition is recommended. Respiratory physiotherapy should be individually tailored.

Frequent ear problems with recurrent infections should be treated non aggressively, avoiding tympanic membrane perforation and using local antibiotic ear-drops with proper cleaning of the ear. Saline douches are used for chronic mucoid rhinorrhea.

All childhood immunizations, including influenza and anti-pneumococcal should be done.

Lung transplant is rarely needed for severe lung disease or lobectomies for extensive bronchiectasis.

Fertility problems in men should be managed in specialized centers and is totally attributed to poor motility of sperm.

9. PCD European Respiratory Society Task Force 2009

The European survey has analyzed data sent from 223 centers, from 26 countries, including Romania and reported 1009 patients aged under 20 years. Adjusted age at diagnosis was 5 years in Western Europe and was strongly correlated with general government expenditure for health.[10]

TABLE 3 Characteristics of diagnosed primary ciliary dyskinesia patients aged 0–19 yrs at time of the survey

Country	Total# n	Female sex % (95% CI)	*Situs inversus* % (95% CI)	Age at diagnosis# yrs	
				Total n	Median (IQR)
Austria	36	42 (25-59)	53 (36–70)	28	4.8 (0.3–8.2)
Cyprus	20	40 (16-64)	45 (21–69)	19	10.1 (7.0–13.9)
Denmark	51	45 (31-59)	35 (22–49)	51	4.1 (0.8–7.9)
Hungary	35	31 (15-48)	34 (18–51)	35	5.5 (4.2–8.3)
Slovakia	7	43 (0-92)	100	7	2.6 (1.8–10.3)
Switzerland	61	39 (27-52)	56 (43–69)	49	3.8 (1.0–6.7)
Greece	20	55 (31–79)	50 (26–74)	20	4.6 (2.0–7.5)
Finland	4	75 (0–100)	50 (0–100)	4	3.7 (1.7–5.0)
Israel	61	41 (28-54)	57 (44–70)	56	1.9 (0.2–6.5)
The Netherlands	6	50 (0–100)	50 (0–100)	6	1.9 (0.5–4.7)
Portugal	6	33 (0–88)	83 (40–100)	6	6.1 (0.5–12.3)
Spain	104	40 (31-50)	10 (4–16)	96	6.1 (3.0–7.7)
Belgium	17	53 (26–79)	29 (5–54)	16	5.8 (2.6–8.5)
France	97	55 (45-65)	39 (29–49)	96	3.3 (0.8–6.5)
Subtotal¶	**525**	**44 (40–48)**	**40 (35–44)**	**489**	**5.0 (1.2–7.5)**
UK	80	43 (31-54)	45 (34–56)	53	3.2 (0.7–5.2)
Italy	128	41 (33-50)	46 (38–55)	127	4.7 (0.7–8.2)
Sweden	46	35 (20-49)	29 (16–43)	43	6.4 (1.5–10.2)
Serbia	16	25 (1–49)	31 (6–57)	16	8.3 (2.3–12.4)
Czech Republic	13	54 (22-85)	45 (10–81)	13	5.1 (3.9–11.2)
Romania	8	75 (36–100)	88 (58–100)	8	1.1 (0.3–8.0)
Turkey	102	46 (36-56)	71 (62–80)	102	7.3 (3.7–10.5)
Germany	57	33 (21–46)	46 (33–60)	38	5.0 (1.4–8.6)
Norway	28	43 (23-62)	32 (14–51)	9	5.2 (3.2–6.9)
Total	1,003	43 (40–46)	44 (41–47)	897	5.3 (1.2–8.2)

n=1,009. #: date of diagnosis, sex, or information on *situs inversus* were missing for some patients; ¶: the subtotal summarises results for all countries with a response rate of >60%. IQR: interquartile range.

Fig. 4. (table 3): *adapted from ERS Task Force survey 2008* [10]

Low diagnostic rates were reported from low health expenditure countries (fig.4), and overall data suggested, at the time of the survey, that PCD is still underdiagnosed.

More so, treatment is not standardized across Europe and specific investigations are available only in specialized centers.

The aim of the consensus statements of the ERS Task Force and of the following research groups (Bestcilia) was to formulate recommendations regarding diagnostic and therapeutic interventions for a more accurate approach in PCD patients.

10. Overview of some pediatric PCD cases (Clinical Children's Hospital of Brasov- personal records of the author)

Case 1: 15 years old female known from birth with situs inversus and congenital nystagmus (fig. 5, 6, and 7). Medical history includes: delayed menarchae, frequent LRTIs since infancy, chronic cough with intermittent purulent sputum, permanent nasal obstruction, low BMI, no specialized regular follow up and treatment for respiratory exacerbations, horizontal nystagmus, poor quality of life (QoL).

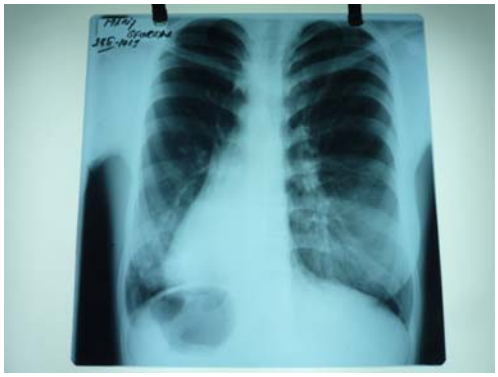

Fig. 5. *Chest radiography (case 1) dextrocardy, hilar bilateral interstitial markings, bilateral central and lower lobes bronchiectasis*

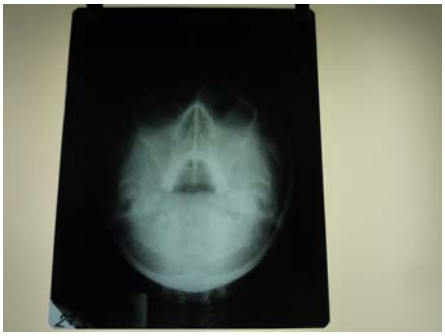

Fig. 6. *Total opacifiation of sinuses, chronic maxillary sinusistis (case 1)*

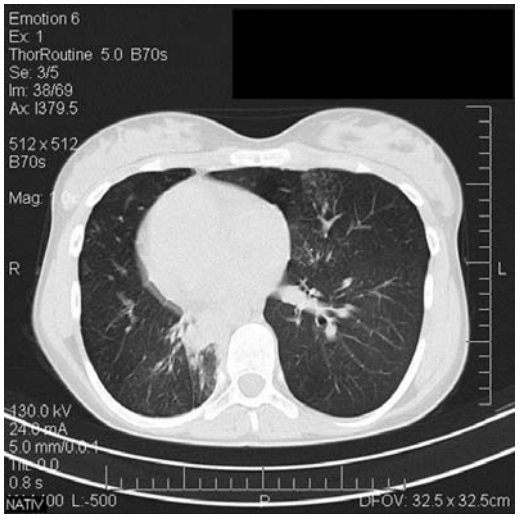

Fig.7. *Tubular left lower lobe bronchiectasis, left hilar mucus impactation (case1)*

Other investigations done in case 1 did not reveal any other malformations (dextrocardia with no structural abnormalities), lung function FEV_1 71% of predicted, FVC 79% of predicted.

Poor adherence to treatment regimens was noted, usually prescribed by the general practitioner, poor understanding of chronic disease. Associated treats in the family: brother with Leiner Moussous dermatitis (genetic autosomal recesive disease).

Case 2*:* 7 years old female, premature birth at 27 weeks of gestation, birth weight 1300 g, hospitalized for 2 months in NICU (long term oxygen need, bronchopulmonary dysplasia?), known from birth with situs inversus (fig. 8, 9 and 10).

Medical history included frequent LRTIs and otitis media in early childhood (actual hypoacusia), failure to thrive, chronic nasal obstruction and cough, chronic adenoids currently treated by the GP.

First degree cousin has cystic fibrosis (homozygous for F508 del).

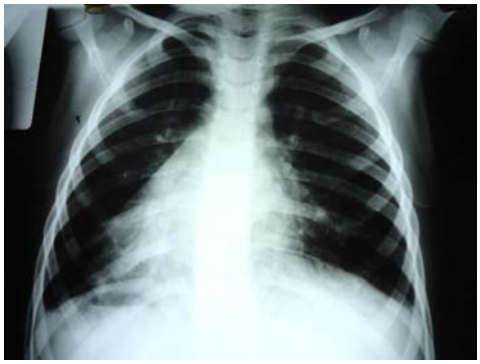

Fig. 8. *Chest X-ray (case 2): dextrocardia, interstitial markings, central bronchiectasis, RLL consolidation*

Fig. 9. *Chronic maxillary sinusistis – (case 2)*

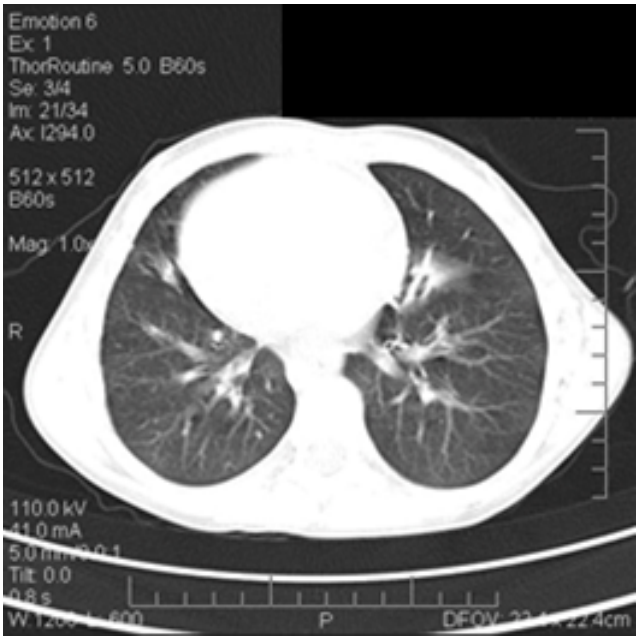

Fig. 10. *Lung CT (case 2): bilateral hilar mucus impactation, linear lower lobes bronchiectasis*

Other investigations revealed a perimembraneous ventricular defect.

Case 2 had no specialized treatment until age 7, invasive treatment for repeated otitis media with ongoing hearing loss, poor nutrition and QoL due to socioeconomic factors.

Case 3: 4 years old female known with situs inversus from birth, with history of frequent LRTIs mostly treated by the GP, good nutritional status (fig. 11).

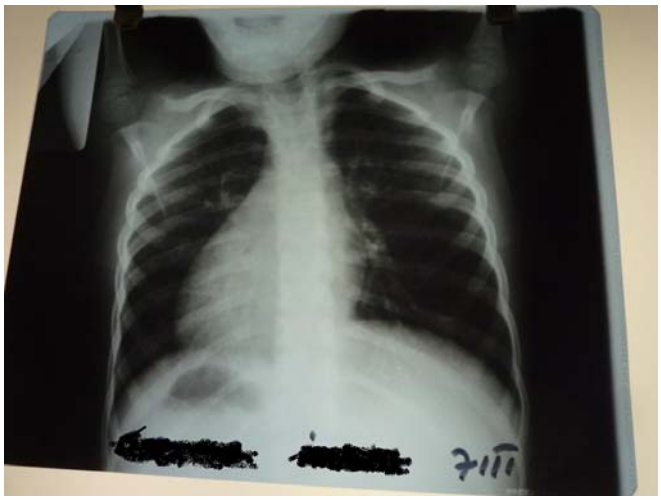

Fig. 11. *Chest X-ray (case 3): dextrocardia, bilateral interstitial markings, incipient lower lobes bronchiectasis.*

Investigations did not reveal any other malformations, lung CT was not done.

11. Conclusions

Diagnosis of PCD can be made by clinical suspicion, more so when associated with situs inversus and specific respiratory symptoms.

Specific diagnosis in Romania is still a problem, there are just a few centres with proper equipment for electron-microscopy and expertise in PCD.

Training of general practitioners and paediatricians is mandatory to increase awareness of such a diagnosis in order to offer proper care and follow up for patients.

Follow up of patients have to include regular visits in tertiary centres, improved nutrition, regular sputum cultures, ENT controls and chest physiotherapy, HR-CT scans and pulmonary function tests to evaluate lung damage.

Specialised care, in specialized centres with proper caregivers and teams for an overlooked orphan disease, may ensure good quality of life, preservation of lung function and normal social insertion for PCD patients.

PCD is now more often diagnosed in patients due to advance in research and improvement of techniques and genetics and represents an important subgroup of ciliopathies that are now become recognized as a multisystem disease. The current challenge for pediatricians is to increase diagnostic awareness and perform investigations before sustained lung damage is present [2].

The future is paved by basic science that may open the way for curative treatments, opposed to management of symptoms.

Nevertheless, a focused, proper history of respiratory symptoms has to be taken, by any specialist, in order to initiate an evaluation when suspecting PCD.

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