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CASE REPORT

Guillermina Bautista Harris
Juan Carlos Rodriguez Bermejo
Miguel Castillo Suarez

Different frequency of cilia with transposition in human nasal and bronchial mucosa. A case of acquired ciliary dyskinesia

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Abstract Nasal and bronchial cilia and spermatozoa of a patient with a high clinical suspicion of a ciliary dyskinesia syndrome were ultrastructurally studied and quantified. Defective cilia showed two types of axonemal patterns: 9d+0s and 8d+1d. Of these, 9d+0s cilia prevailed in the proximal region, whereas 8d+1d prevailed in the distal region. Translocation of a peripheral doublet to the central position occurred at the middle region of cilia lacking the central pair, probably to compensate for its absence. Quantitative analysis showed that the percentages of anomalous cilia were 5.32±0.93 in nasal samples and 43.17±2.34 in bronchial samples. Spermatozoa without the central pair or with a translocated microtubular doublet were rarely observed, but a variety of nonspecific defects were seen. Even though transposition is generally considered to be an inherited ciliary defect and one of the causes of primary ciliary dyskinesia, in this case quantitative ultrastructural analysis and clinical data indicate that this is an acquired ciliary defect.

Keywords Acquired ciliary dyskinesia · Bronchial cilia · Nasal cilia · Spermatozoan flagella · Transposition

Introduction

The mucociliary system of the respiratory tract constitutes a significant defense mechanism, which protects the airways against potentially injurious inhaled particles. Cilia of the respiratory epithelium play an important part in mucociliary clearance, transporting the mucus towards the pharynx, where it is swallowed [22]. Morphological and/or functional alterations of respiratory cilia produce decreasing efficiency of the mucociliary clearance, causing respiratory troubles such as sinusitis and bronchitis, which can degenerate in bronchiectasis.

Ciliary abnormalities may be primary, that is to say inherited, or secondary (acquired), when it is caused by environmental factors. Absent or reduced inner and/or outer dynein arms [1, 17], absent radial spokes [24], translocation of microtubular doublets [25], and recently added ciliary disorientation [6, 18, 20] are considered primary or inherited ciliary defects and the cause of the primary ciliary dyskinesia syndrome (PCDS). Other abnormalities, such as compound cilia [13], addition and deletion of microtubules [10, 21], disorganized axonemes, absence of the central pair [14], internalized or shed axonemes [13], very long cilia [2, 16] or ciliary aplasia [5], have been stated to be nonspecific or acquired structural abnormalities.

Ultrastructural studies for the diagnosis of primary ciliary dyskinesia (PCD) are based on respiratory ciliated cells, because clinical symptoms affect principally the respiratory tract. It has been reported that the percentage of abnormal cilia with the main ultrastructural defect is similar in the upper and the lower airways [3, 11, 26]. Most ultrastructural studies are performed on nasal samples only, on the assumption that the results can be generalized to the lower airways.

In this paper we present the case of a patient who had suffered with respiratory troubles since early childhood and been diagnosed with infertility at least 10 years before. As his clinical condition led to the suspicion of ciliary dyskinetic syndrome (CDS) the ultrastructure of the nasal cilia was studied. The percentage of anomalous cilia was low enough (<5%) to be considered normal. In a second study of nasal and bronchial cilia and of spermatozoa, a high percentage of bronchial cilia were found to be characterized by the specific defect of transposition,

G. Bautista Harris (✉)
Departamento de Morfología, Centro de Ciencias de la Salud,
Universidad de Las Palmas de Gran Canaria,
P.O.B. 550 35080 Las Palmas, Spain
e-mail: gbautist@cicei.ulpgc.es
Tel.: +34-928-451465, Fax: +34-928-453420

J.C. Rodriguez Bermejo
Servicio de Neumología,
Hospital Universitario Insular de Gran Canaria

M. Castillo Suarez
Servicio de Analisis Clinicos, Laboratorio de Andrologia,
Hospital Materno Infantil de Las Palmas, Las Palmas, Spain

while the percentage of nasal cilia with this defect was low and it was only an occasional finding in spermatozoa.

Materials and methods

Respiratory cilia and flagella of the patient were studied with electron microscopy because of the clinical suspicion of a ciliary dyskinesia.

Nasal samples were obtained by biopsy after local anesthesia with 10% tetracaine and 1% adrenaline. Bronchial samples were obtained by biopsy fiberoptic endoscopic examination performed under local anesthesia with 2% lidocaine. Sperm samples were collected by masturbation after a 72-h abstinence. The ejaculates were allowed to liquefy at room temperature and 0.5 ml was used for routine seminal analysis following the WHO criteria [27]. The number of spermatozoa per milliliter of ejaculate and their motility were recorded. Motility study was performed in vivo and the study of spermatozoa morphology was made after Diff-Quick staining (triple stain DADE). The remainder of the ejaculate was centrifuged at 1500 *g* for 10 min and pelleted spermatozoa were processed for the electron microscopic study.

Electron microscopy

All the samples were fixed in 2.5% glutaraldehyde in cacodylate buffer 0.1 M pH 7.4 for 20 h at 4°C. Then they were postfixed with 1% osmium tetraoxide (OsO4), dehydrated in ethanol and embedded in Epon 812 (Embed 812).

Initial evaluation of the specimens was done by light microscopy of semithin sections stained with toluidine blue. Ultrathin sections were cut on an ultramicrotome Reichert Ultracut S, stained with uranyl acetate and lead citrate, and examined in an electron microscope (Philips EM301, 60 kV). Zones with cilia transversely sectioned were microphotographed.

Quantitative analysis

For quantitative analysis, only cilia in which axonemal microtubules were sharply visible were considered [29]. Cilia sectioned near either the basal end or the distal tip were excluded from the study because the microtubular organization of the axoneme 9d+2s changes at these zones. Selection of cilia for counting was random except for the necessity for sharp cross sections that permit counting of microtubules.

The number of ciliary cross sections evaluated was 582 in a total of nine electron micrographs (between 32 and 114, mean 64.66) for nasal cilia and 447 in a total of seven electron micrographs (between 44 and 100, mean 63.85) for bronchial cilia.

The results for abnormal cilia are expressed as a percentage of the two main abnormal axonemal patterns: 9d+0s and 8d+1d, among the total number of cilia analyzed. Percentages and standard deviation of the mean were calculated in each electron micrograph, and for all nasal and all bronchial samples separately.

Clinical history

A 34-year-old man who had suffered meconium aspiration during delivery had a history of nasal obstruction, rhinorrhea, anosmia, recurrent maxillary sinusitis and episodic bronchitis from childhood. He was also affected by progressive onset of daily cough productive of sputum. At the age of 21 he had a medical diagnosis of medial lobe bronchiectasis, and a right lobectomy was carried out. Since then he has continued to suffer from persistent infectious bronchitis with impaired tracheobronchial clearance and occasional hemoptysis.

He came to our hospital with an acute illness involving hemoptysis, albeit not massive, purulent bronchitis, and fever without signs of bloody mucosal injury on bronchoscopic exploration.

The physical examination was normal except that scattered wheezes, ronchi and rales were heard on lung auscultation. Lung function tests were performed using a Sensormedics Vmax 22 spirometer and body plethysmography. The first lung function test presented a mild airways obstruction, with forced expiratory volume in 1 s (FEV1) of 79%, forced vital capacity (FVC) of 97%, and Tiffeneau index (FEV1/FVC) of 68% compared with the predicted values. Predicted values were calculated according to age, sex height and ethnic origin from standard sources.

Routine laboratory tests, serum immunoglobulins and the concentration of $alpha_1$ antitrypsin were normal except for mild leukocytosis. Repeated chloride sweat tests showed normal values. No signs of cardiac pathology neither hypoxemia were found.

Chest X-ray demonstrated diffuse bronchiectasis. A film of paranasal sinuses revealed frontal sinus hypoplasia and opacifications of the right maxillary sinus.

In the last 3 years he has suffered a clinical evolution of recurrent chest infections, multiple resistance to colonizing bacteria and chronic hemoptysis even with intensive physical therapy, use of anticholinergic bronchodilators and repeated series of antibiotic therapies guided by cultures. The last lung function tests showed a mixed obstructive-restrictive pattern with FEV1 of 62%, FVC of 74%, FEV1/FVC of 70%, total lung capacity (TLC) of 71%, functional residual capacity (FRC) of 88%, residual volume (RV) of 73%, RV/TLC ratio of 28% and airways resistance (RAW) of 3.85 cmH_2O/l per seg. Bronchodilator tests were always negative. The patient has recently suffered from pneumonia complicated with pleural empyema.

He has no direct relatives with antecedents of symptoms of chronic respiratory diseases. One of his three brothers is married and has a daughter. The patient, also married, had been attending the infertility service since the age of 24, presenting with oligozoospermia and astenozoospermia. Ten years after his first appointment there, the last semen analysis of the patient showed severe oligozoospermia with a sperm concentration distinctly below the normal range. Sperm viability was only 51%, with a low percentage of motile spermatozoa and the quality of forward progression much reduced. Morphological abnormalities of head and tail were observed in a high percentage. No fixed antibodies to spermatozoa were found (MAR test negative), and the biochemical study of seminal plasma of citric acid and fructose was normal. In spite of that a daughter has recently been born to him and his wife.

Results

Ultrastructructural study of respiratory cilia revealed that there were two main anomalous axonemal patterns: the 9d+0s (lack of the central pair of microtubules) and the 8d+1d (translocation of a peripheral microtubular doublet to the center; Fig. 1). Other ciliary abnormalities, such as compound cilia or supernumerary microtubules (Fig. 2), were occasionally observed and not considered for statistical analysis.

Favorable longitudinal sections of anomalous cilia show one of the peripheral microtubular doublet deflecting to the central position in the ciliary axoneme, apparently to compensate for the absence of the central pair (Fig. 3), so that the microtubular pattern of the axoneme varies along the length of the ciliary shaft. Cross sections of the proximal region, under the deflection zone, thus present the 9d+0s pattern (Fig. 1), while cross sections of the distal region above this level are 8d+1d (Fig. 4). The

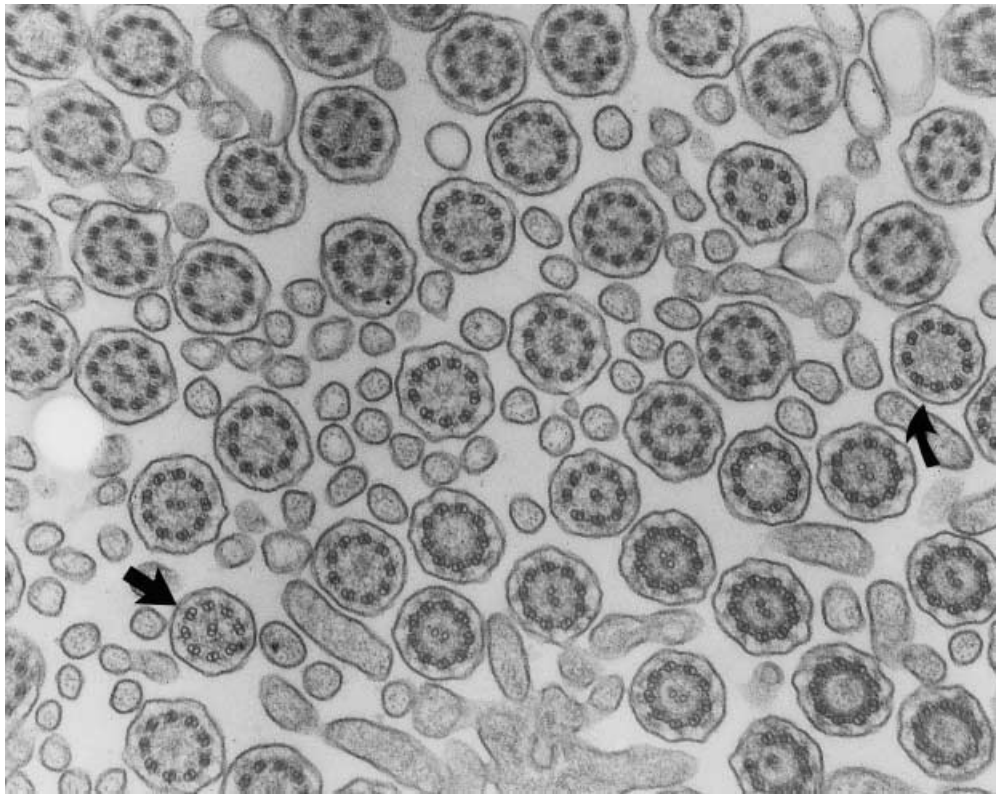

Fig. 1 Cross-sectioned cilia from bronchial mucosa. Among some normal cilia 9d+2s there are cilia with absent central pair of microtubules: 9d+0s (*curved arrow*) and cilia with transposition of a peripheral doublet: 8d+1d (*thick arrow*). Microvilli sections denote that it is a proximal region. ×50,700

Table 1 Normal and defective cilia in nasal and bronchial samples (*n* number of cilia, *SD* standard deviation)

Ciliary pattern	Nasal sample		Bronchial sample	
	n	(%±SD)	*n*	(%±SD)
9d+2s	550	(94.50±0.94)	270	(60.40±2.31)
9d+0s	27	(4.63±0.87)	141	(31.54±2.19)
8d+1d	4	(0.68+0.34)	52	(11.63±1.51)
(9d+0s)+(8d+1d)	31	(5.32±0.93)	193	(43.17±2.34)

deflection zone appears not to be at the same level for all the transposed cilia.

The results of quantitative analysis of abnormal cilia are summarized in Table 1. For nasal cilia the percentage of ciliary cross sections showing lack of the central pair (9d+0s) was 4.63±0.87 and the percentage of those with a doublet transposed (8d+1d) was 0.68±0.34. For bronchial cilia these percentages were 31.54±2.19 and 11.63±1.51, respectively.

As transposition of one peripheral doublet generally occurs in those cilia that lack the central pair, the percentage of anomalous cilia was the sum of both abnormal patterns (9d+0s and 8d+1d); this is 5.32±0.93 for nasal cilia and 43.17±2.34 for bronchial cilia.

Ultrastructural study of spermatozoa revealed that the lack of the two central microtubules and the transposition of a doublet were occasional findings among a variety of other defects. The major morphological abnormality was the tail coiling with the mitochondrial sheath and

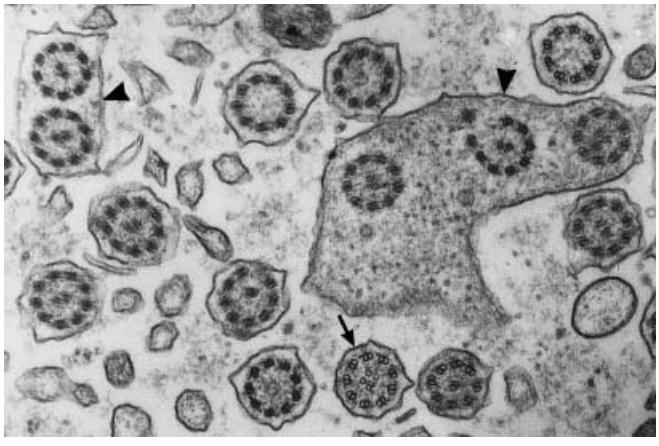

Fig. 2 Compound cilia (*arrowhead*) and cilia with supernumerary central singlets (*arrow*) are occasionally seen. ×42,000

the axoneme with the fibrous elements embedded in a droplet of cytoplasm (Fig. 5). Nuclear anomalies were also observed, such as chromatin condensed in coarse granules and detachment of the nuclear envelope.

Discussion

In the study of ciliary dyskinesia it has been generally assumed that nasal ciliary abnormalities reflect bronchial ciliary abnormalities, and most studies have been performed on nasal samples because such specimens are eas-

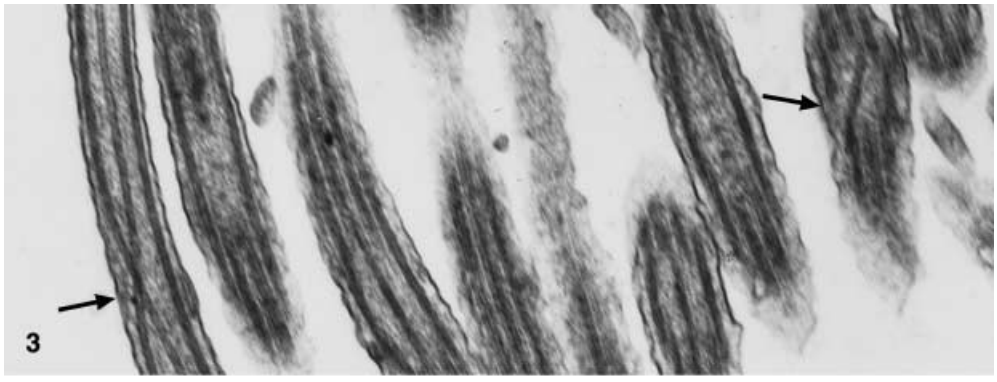

Fig. 3 In favorable longitudinal sections of cilia the deflection of the microtubular peripheral doublet can be observed (*arrows*). ×36,250

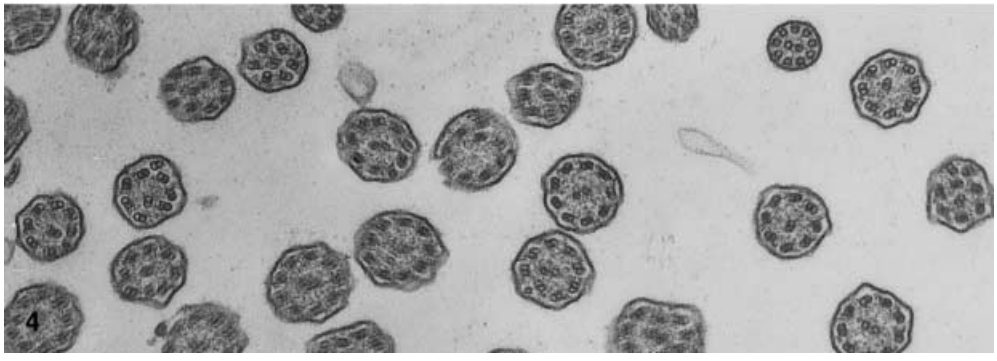

Fig. 4 Among cilia sectioned at the distal region transposed axonemes (8d+1d) prevail over axonemes lacking the central pair (9d+0s). ×36,250

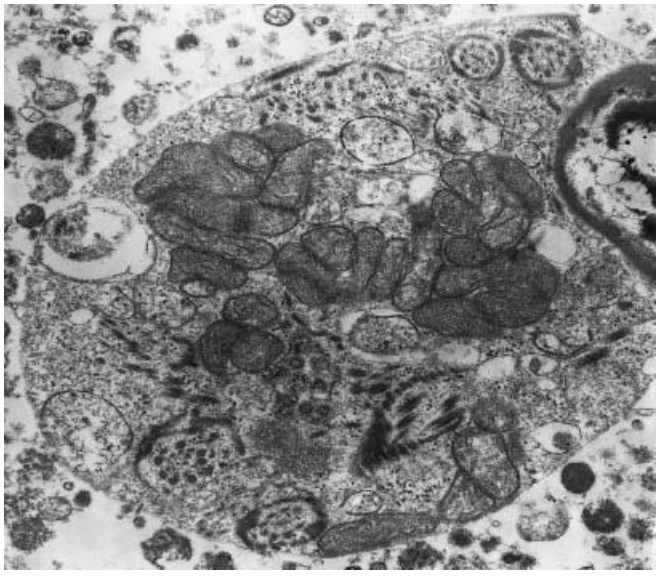

Fig. 5 A variety of nonspecific ultrastructural anomalies of spermatozoa were observed being the major morphological abnormality the tail coiling with the mitochondrial sheath and the axoneme with the fibrous elements embedded in residual cytoplasm. ×20,000

ier to obtain with little discomfort to the patient. Studies on the same patient to verify whether nasal and bronchial ciliary defects are similar have seldom been carried out. In this connection, Verra and coworkers [26] reported that in terms of the percentage of abnormal cilia and the main ultrastructural defect, ultrastructural ciliary abnormalities of nasal and bronchial samples are similar. When most of the cilia are abnormal, and especially when the dynein arms defects are predominant, the study of nasal ciliated cells is sufficient, but when the predominant defect affects the central complex bronchial cilia must be studied.

In accordance with this, in the first study performed on nasal samples from our patient, ciliary anomalies were observed in such a low percentage that it was not considered indicative of a ciliary pathology. Since the patient's clinical condition was highly suggestive of CDS, bronchial, nasal, and sperm samples were then analyzed. Two types of anomalous axonemal patterns (9d+0s and 8d+1d) were observed, depending on the zone of the ciliary shaft that was sectioned. The 9d+0s pattern predominated at the proximal zone, while the 8d+1d pattern was more in evidence at the distal zone. Translocation of a peripheral doublet to the center occurred in cilia lacking the central pair.

The translocation of a peripheral doublet to the central position was first described as an hereditary specific ciliary defect and therefore one of the causes of PCDS. It was called transposition and was interpreted as an anomaly of the central sheath, affecting all the nasal and bronchial cilia and the sperm of the male of two siblings [25]. No further reports of all the cilia affected by this defect have been published since. Other cases of transposition have been described, and interpretations of the anomaly, such as the absence of radial spokes [24] or the absence of nexin links [15, 21], have been proposed. In our patient the transposition occurred in cilia lacking the two central singlets, with the peripheral doublet appearing to translocate to replace them. A similar case

was reported by Mierau et al. [14], and like them, we consider that the principal defect is the lack of the central pair, which has been frequently described as an acquired defect.

In an inherited disorder all the ciliated cells of a patient must be affected [2, 8], whereas in acquired ciliary defects focal lesions prevail in the upper or the lower respiratory tract, with more abnormal cilia in the more heavily infected area [19]. In addition, it has been reported that the ultrastructural abnormalities of the central complex are always expressed in less than half of the total population of cilia [9].

Our quantitative analysis shows that the ciliary anomaly is not expressed in all cilia and that the percentage of anomalous cilia is different in nasal and bronchial samples: 5.32±0.93 and 43.17±2.34, respectively. Anomalous axoneme were occasionally seen in spermatozoa.

Ciliary anomalies that in patients with PCD are inherited have also been reported as acquired defects [5, 6]. Transposition is generally considered an inherited defect; very few cases of transposition as acquired defects have been described [10, 14, 23].

Infertility resulting from sperm immotility may be a consequence of axonemal defects and when associated with respiratory symptoms should lead to the suspicion of PCDS. In this syndrome ultrastructural anomalies affecting both respiratory cilia and sperm flagella have been reported [2, 24, 25]. In this patient the specific defect was rarely observed in spermatozoa, but several findings, including tail coiling and disorganized axonemes, were felt to represent nonspecific defects [28].

In conclusion, it appears in this case that transposition defect is not specific for primary ciliary dyskinesia. Our findings suggest that in this patient the defect of transposition is an acquired defect, the principal defect being the absence of the central pair. The quantity of anomalous cilia is very different in each location studied. There were neither respiratory symptoms nor signs of infertility among the young man's relatives. Even though he had had symptoms of infertility for 10 years, he has recently become the father of a daughter. In addition, clinical data indicate that our patient had swallowed meconium at birth. There have been reports of ciliary defects such as transposition in children with acquired bronchiectasis after they have swallowing a foreign body [4].

We emphasize the importance of clinical suspicion in this pathology. This is the departure point for the diagnosis of the CDS, which must be corroborated by an ultrastructural study. When the nasal ultrastructural diagnosis is not in accordance with clinical data, cilia from other locations must be studied. Most cases reported as PCD with transposition have been diagnosed by ultrastructural study of only one location and without due consideration for whether the clinical symptoms have started in childhood or for the familial clinical background, all of which is very important for the medical diagnosis of PCD.

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