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Primary ciliary dyskinesia: Ultrastructural defects and clinical features*

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SUMMARY

Primary ciliary dyskinesia is a genetically determined disorder characterized by immotility or poor motility of the cilia in the airways and elsewhere. Certain specific defects in the ciliary axoneme can be found, which are pathognomonic of the syndrome. The defects include missing dynein arms, abnormally short dynein arms, spokes with no central sheath, missing central microtubules, and displacement of one of the nine peripheral doublets. We have reviewed 19 cases of primary ciliary dyskinesia diagnosed by transmission and scanning electron microscopy. The age distribution ranged from five to 15 years, and there were six males and 13 females. All 19 cases had abnormal cilia which consisted of Ia (three cases), Ib (three cases), isolated Id (three cases), isolated II (one case), isolated III (two cases), and Id + other types (seven cases), according to Sturgess' classification. The most pronounced clinical manifestations are chronic paranasal sinusitis (52%) and chronic bronchiectasis (52%), followed by bronchopneumonia (26%), chronic bronchitis (21%), and nasal polyps (15%).

Key words: primary ciliary dyskinesia, electron microscopy, clinical features

INTRODUCTION

Primary ciliary dyskinesia is an autosomal genetical disorder characterized by impaired ciliary movement throughout the entire body of the affected individual. Since Afzelius (1976) first described ultrastructural dynein-arm defects as the underlying cause of this rare entity, many variations of the original description as well as its association with Kartagener's syndrome have been recognized (Hartline and Zelkouitz, 1971; Turner et al., 1981; Chao et al., 1982; Richard et al., 1989).

This syndrome is usually manifested as childhood-onset paranasal sinusitis, bronchiectasis, and chronic bronchitis (Turner et al., 1981; De Boode et al., 1989), and is diagnosed by ultrastructural study of the ciliated epithelium (Van der Baan et al., 1987). We have aimed to characterize primary ciliary dyskinesia in Koreans, both ultrastructurally and clinically.

MATERIAL AND METHODS

Nineteen patients who were diagnosed as having primary ciliary dyskinesia by electron microscopy at the Seoul National University Hospital from January 1984 through December 1993 were reviewed. Eighty-six patients had been referred from the Department of Paediatrics with suspicion of primary ciliary dyskinesia.

Nasal mucosal biopsy was done from the middle portion of the inferior turbinate. Clinical features as well as ultrastructural defects were analyzed. The final diagnosis of primary ciliary dyskinesia was made by the pathologists at the Department of Pathology. Sixty-seven cases were excluded from this ultrastructural study, because the number of cilia was too low or because of the presence of metaplastic epithelia.

For ultrastructural study, the specimens were fixed in 2.5% glutaraldehyde. Four 1-mm^3 pieces were taken for transmission electron microscopy (TEM). The remaining specimens were prepared for scanning electron microscopy (SEM). Adequacy of the specimens were confirmed by SEM. Specimens with deficient cilia or low ciliary counts were discarded. We adopted the classification system suggested by Sturgess et al. (1979).

For SEM, the specimens were washed in 0.85% saline solution prior to fixation. After fixation in 2.5% glutaraldehyde solution and post-fixation in 1% OsO_4 solution, the specimens were dehydrated in a graded series of ethanol, substituted with isoamylacetate, and dried with liquid CO_2 in a Hitachi HCP-2 critical-point drier. The specimen were coated with gold-platinum alloy (Eiko, 3B) and observed in a Hitachi S-520 scanning electron microscope.

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For TEM, the specimens were post-fixed in 1% OsO_4 solution and dehydrated in a graded series of ethanol. The specimens were embedded in epoxy resin, and sections (0.5–2 μm) were stained with toluidine blue. After selecting an area with the highest ciliary population, ultrathin sections (70–90 nm) were cut with a Sorvall MT6000 ultratome, and examined in a Hitachi H-600 transmission electron microscope.

RESULTS

Morphology of cilia

Ultrastructurally (TEM), various types of abnormal cilia were observed. Patients under the age of 11 had a tendency to a solitary type of defect, while patients over the age of 12 had a tendency to a combined type (Table 1). Classification of ciliary defects in 19 patients with primary ciliary dyskinesia is shown in Table 2. Total or partial defects of the dynein arms were most common, and observed in 16 of 19 patients (Figures 1–3). Three patients had outer arm defects only (Ia), and three patients inner arm defects only (Ib). Complete defects of outer and inner arms (Ic) were not observed. Incomplete defects of both outer and inner arms (Id) was observed in 10 out of 19 patients (Figure 1), as a solitary form in three patients, and as a combined form in seven patients. Seven patients had radial spoke defects, as a solitary form in one patient, and a combined form in six patients. Microtubular transpositions were observed in eight patients (Figure 4), and six of them were combined with other types of defects.

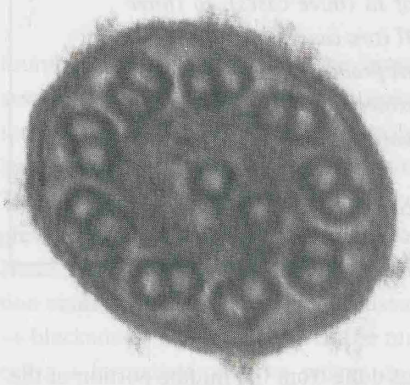

Figure 1. Transmission electron micrograph demonstrating Sturgess' type Id defect, with a cilium containing incomplete defects of both inner and outer dynein arms (×240,000).

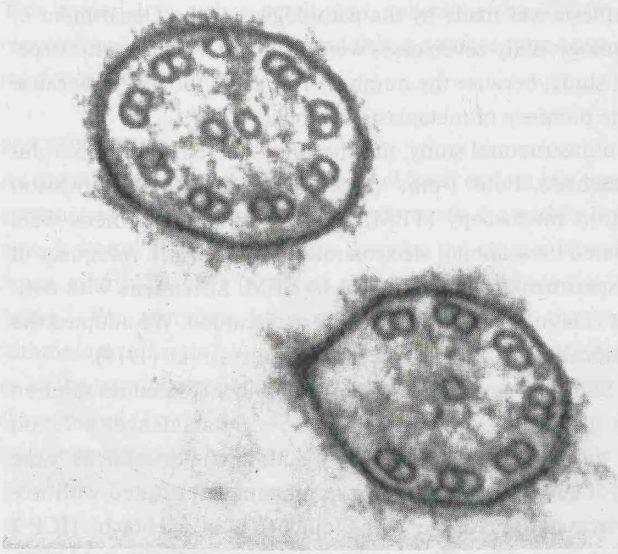

Figure 2. Transmission electron micrograph showing complete absence of both inner and outer dynein arms. Note the absence of two pairs of outer microtubules, resulting in a "7+2" configuration (×160,000).

Table 1. Clinical and ultrastructural characteristics of patients (PCD: primary ciliary dyskinesia).

patient	sex	age (years)	type of PCD
1	F	5	Ia
2	M	6	Id
3	F	6	III
4	F	6	III
5	F	8	Ib
6	F	9	II
7	F	9	Id + III
8	F	10	Id
9	M	11	Ib
10	F	11	Ib
11	M	12	Ia
12*	M	12	Id + II + III
13	M	12	Id + II + III
14	F	13	Id
15	F	13	Id + II
16*	F	13	Id + II + III
17*	F	13	Id + II + III
18	F	14	Ia
19	M	15	Id + II + III

*: siblings

Table 2. Classification of defects in primary ciliary dyskinesia and distribution of patients according to ciliary defect.

classification		number of patients (%)
solitary form:		
type I (dynein arm defect):		
Ia	outer dynein arms	3 (15)
Ib	inner dynein arms	3 (15)
Ic	outer and inner dynein arms (complete)	0 (0)
Id	outer and inner dynein arms (incomplete)	3 (15)
type II	radial spoke defect	1 (5)
type III	microtubular transposition	2 (10)
type IV	normal ultrastructural organization with functional impairment	0 (0)
combined forms:		
type I d + II		1 (5)
type I d + III		1 (5)
type I d + II + III		5 (26)
total		19 (100)

Clinical features

The patients' ages ranged from 5 to 15 years with an average of 10.4 years. There were six males and 13 females, and male to female ratio was 1:2.2. Presenting symptoms were cough, sputum, nasal obstruction, rhinorrhoea, fever, and postnasal drip, in order of decreasing frequency (Table 3). The most common presenting symptom was cough which was present in all

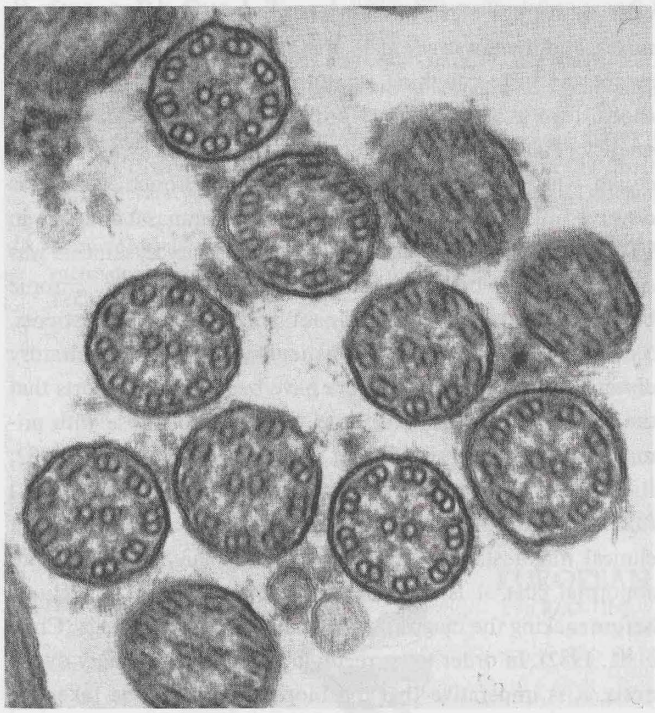

Figure 3. Transmission electron micrograph demonstrating cilia with variable defects of their dynein arms, with some showing complete defect and others showing partial defect of one of the dynein arms. One of the cilia has a "double central doublet" (×107,000).

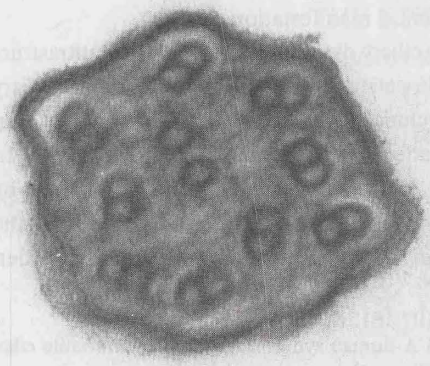

Figure 4. Transmission electron micrograph showing Sturgess' type IV defect, in which the microtubules are transposed within the cilium (×260,000).

patients. On plain chest radiographs and paranasal sinus computerized tomograms, bronchiectasis was observed in 10 out of 19 patients (52%), and paranasal sinusitis in 10 out of 19 patients (52%; Table 4). Two out of 19 patients (10%) fulfilled Kartagener's triads. Otitis media with effusion was observed in two out of 19 patients (10%), and tuberculosis was associated in two out of 19 patients (10%). One 6-year-old female patient underwent surgery for tracheo-oesophageal fistula and the plain radiograph showed spina bifida. There was no correlation between ultrastructural types of cilia abnormalities and clinical manifestations. However, three siblings with the same type of ciliary defect had similar symptoms and signs. Two patients with a solitary microtubular transposition (Sturgess' type III) had no nasal symptoms at all. The symptoms and signs were more severe as the type of ciliary abnormality was more complex (Table 3).

Table 3. Clinical symptoms according to type of primary ciliary dyskinesia.

symptoms	Ia	Ib	Id	II	III	Id+II	Id+III	Id+II+III	no. of patients (%)
coughing	3	3	3	1	2	1	1	5	19 (100)
sputum	2	2	1	1	1	1	1	4	13 (68)
nasal obstruction	2	1	2	1		1	1	3	11 (57)
rhinorrhoea		1	2			1	1	4	9 (47)
fever			1		2			4	7 (36)
postnasal drip	1	1	1				1	2	6 (31)
dyspnoea		1			1			3	5 (26)
headache	1		2					1	4 (21)
hearing loss			1				1		2 (10)

*: numbers are not mutually exclusive

Table 4. Associated diseases with primary ciliary dyskinesia.

findings	no. of patients (%)*
chronic paranasal sinusitis	10 (52)
chronic bronchiectasis	10 (52)
bronchopneumonia	5 (26)
chronic bronchitis	4 (21)
nasal polyp	3 (15)
otitis media with effusion	2 (10)
Kartagener's syndrome	2 (10)

*: numbers are not mutually exclusive

DISCUSSION

The normal kinocilium is composed of an axoneme core which is surrounded by the cell membrane (Figure 5). It contains one pair of central microtubules surrounded by nine doublets of microtubules, and retains a constant "9+2" pattern. The double microtubules have two subfibres, A and B. The dynein molecules are attached to subfibre A and form two arms, an inner and an outer arm, both of which point toward the next doublet's subfibre B. These arms are long enough to bridge the span between the microtubules.

The results of this study showed a similar pattern of distribution of ciliary abnormalities to that reported by Sturgess et al. (1979) and Chao et al. (1982). We observed that Sturgess' type I was most frequent and that isolated solitary abnormalities and combined abnormalities almost occupied equal proportions. In three siblings with the same type of defect had similar symptoms and signs as reported by others (Antoneli et al., 1981; Greenstone et al., 1983; De Boode et al., 1989). They had radial spoke defects resulting from defects in the autosomal chromosome. We observed megacilia, defects in the ciliary membrane, decreased numbers of cilia, and oedematous cilia in areas of intensive inflammation as reported by others (Greenstone et al., 1983). Rautiainen et al. (1990) have studied the orientation of the cilia in 43 patients with primary ciliary dyskinesia and reported that ciliary orientation is clearly more random in patients than in controls. Random ciliary orientation may be one of ultrastructural abnormalities in primary ciliary dyskinesia. Further pooling of cases may permit a better perspective on the pattern of distribution of ciliary abnormalities. Various abnormalities of the cilia in patients with primary ciliary dyski-

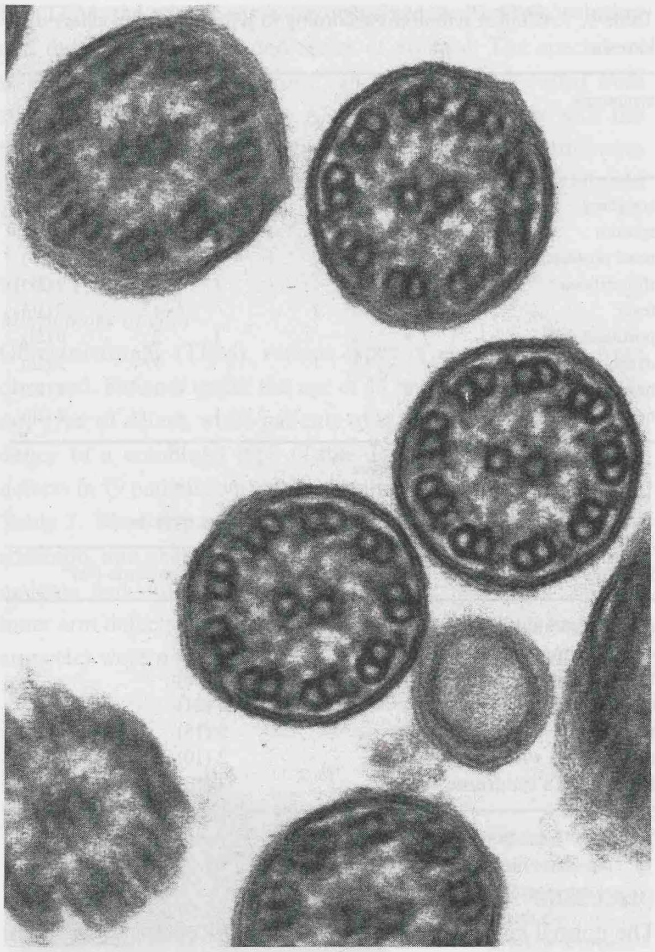

Figure 5. Transmission electron micrograph showing normal cilia with intact dynein arms and radial spokes (×160,000).

nesia may not be confined to the airway mucosa, and may take a generalized form.

Ciliary abnormalities of the Eustachian tube may result in impaired tubal function, leading to serous otitis media. In our series, otitis media with effusion was observed in two out of 19 patients. Infertility may ensue because of decreased motility of spermatozoa (Zamboni et al., 1991) and oviductal cilia (McComb et al., 1986).

Ciliary action in the respiratory mucosa removes foreign bodies for the maintenance of a physiological environment. However, as the proportion of non-functioning cilia increases, the clearing function of the respiratory mucosa is impaired with subsequent respiratory symptoms, such as chronic productive coughing, rhinorrhoea, and nasal obstruction. The defect in primary ciliary dyskinesia is a generalized abnormality of ciliary function throughout the respiratory tract. Greenstone et al. (1988) have reported that patients with very low ciliary motility scores during *in vitro* motility tests tend to have either outer dynein arm defects or both outer and inner dynein-arm defects. Ciliary motility studies *in vitro* may be a good diagnostical tool for primary ciliary dyskinesia. Patients with abnormal cilia often have bronchiectasis, paranasal sinusitis, pneumonia, and/or bronchitis, which are characterized by onset at early age and chronicity. In our series, the patients' ages ranged from five to 15 years with an average of 10.4 years. There were 13 females and six males, and there was a female predominance (2.2:1). Presenting symptoms were coughing, sputum, nasal obstruction, rhinorrhoea, fevers, and postnasal drips in order of decreasing frequency (Table 3). The most common presenting symptom was cough, which was present in all the patients. Bronchiectasis was observed in 10 of 19 patients (52%), and paranasal sinusitis in 10 of 19 patients (52%; Table 4). Primary ciliary dyskinesia was associated with bronchopneumonia in five patients, chronic bronchitis in four patients, and nasal polyposis in three patients. Since Kartagener reported five patients with chronic respiratory disease and situs inversus, there have been several reports that the incidence of situs inversus is up to 50% of those with primary ciliary dyskinesia (Turner et al., 1981; Chao et al., 1982; Richard et al., 1989). However, only two out of 19 patients (10%) fulfilled Kartagener's triad in our series. Since the degree of clinical manifestation is related to the relative proportion of abnormal cilia, it is desirable to observe at least 50 sections before making the diagnosis of primary ciliary dyskinesia (Chao et al., 1982). In order to correctly identify primary ciliary dyskinesia, it is imperative that the biopsy specimens be taken by proper technique and handling and be examined by both electron microscopy and ciliary motility studies. Although racial differences may account for the discrepancies observed, further pooling of cases is required to reach a conclusion. We could not observe any correlation between types of ultrastructural abnormality of cilia and clinical manifestations.

In summary, primary ciliary dyskinesia was diagnosed ultrastructurally in 19 out of 86 patients with suspicion of primary ciliary dyskinesia. Proper technique in nasal mucosal biopsy may help to diagnose more patients with primary ciliary dyskinesia. In addition, recognition of clinical signs and symptoms caused by impaired ciliary movement throughout the entire body of the affected individual can help to detect this rare genetical disorder.

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