Ultrastructural Changes in the Cilia of Experimental Mice

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Mice lacking dynein arms in the cilia were examined; the strain was obtained by inactivation of dynein heavy chain gene in chromosome 7. The cilia of these mice were examined by electron microscopy and compared to the cilia of random-bred mice. No statistically significant differences or typical disorders in the outer or inner dynein arms were detected. The number of inner dynein arms was lower, in some cilia secondary changes presenting as swelling of the outer part of the ciliary membrane or formation of complex cilia were seen.

Key Words: *primary ciliary dyskinesia; transmission electron microscopy; experiment*

Primary ciliary dyskinesia (PCD) is a group of autosomal recessive hereditary diseases characterized by complete or partial disturbances of the mucociliary clearance. The disease is diagnosed by ultrastructural methods and evaluation of functional disorders (frequency of epitheliocyte cilia pulsation). Many aspects of PCD remain unclear. Animal models can be used for studying some aspects of the pathogenesis of this disease.

Ciliary cells of the bronchial epithelium are responsible for mucus transport along airways, which ensures the mucociliary clearance, a defense mechanism of the respiratory system. PCD is a rare congenital disease associated with impairment of the mucociliary function. It is accompanied by characteristic ultrastructural changes in the axonema [1,3]. The most incident are disorders in the structure of the inner and outer dynein arms (DA) [8]. Clinically mucociliary dysfunction are associated with frequent repeated infections of the upper and lower airway [3,10].

Evaluation of ultrastructural and functional parameters of the cilia and study of ciliogenesis in epitheliocyte culture are important for the diagnosis of PCD [9]. Though molecular investigations of this disease can yield interesting data, genetic analysis is not yet acknowledged as a diagnostic method [2,13].

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Experiments on animal models can be useful for the description of genetically determined diseases. A model with inactivated dynein heavy chain gene was described. This condition is characterized by ciliary dysfunction and defective production of the semen [11, 12]. The inactivated gene (MDHC7) is responsible for the formation of inner DA. However, complex ultrastructural studies including description of the defect incidence were never carried out on this mouse model.

Here were examined cilia of mice hetero- and homozygotic by this mutation under an electron microscope.

MATERIALS AND METHODS

Dynein defect was created by replacing four exons of MDHC7 gene encoding ATP-binding site with a neomycin-resistant gene cassette [11,12]. Random-bred, hetero- and homozygotic mice (5 per group) were used in the study.

Samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 2 h. After postfixation in OsO_4 and uranyl acetate contrasting, the sections were dehydrated in ascending alcohols and embedded in epon. Semithin sections were stained with methylene blue for 2 min and examined under a light microscope. Ultrathin sections of selected regions were made on a Reichert OM U3 ultramicrotome, stained with lead citrate, and exa-

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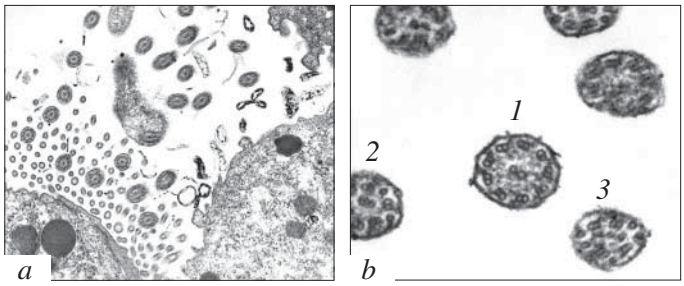

Fig. 1. Random-bred mice. Cross-sections of a cilia. Here and in Figs. 2, 3: transmission electron microscopy. *a*) some cilia without special features in their structure, ×12,000; *b*) *1*: cross-section of a cilia with 9+2 structure with several outer dynein arms, one inner dynein arm; *2* and *3*: cilia with several dislocated tubules, ×5000.

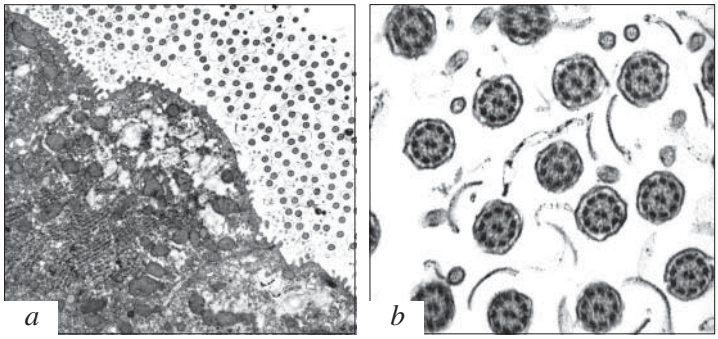

Fig. 2. Bronchial epithelium of MDCH7-deficient mice. *a*) even distribution of cilia on cell surface, ×7000; *b*) several transversely dissected cilia of 9+2 structure, outer and inner dynein arms are seen in all cilia, ×50,000.

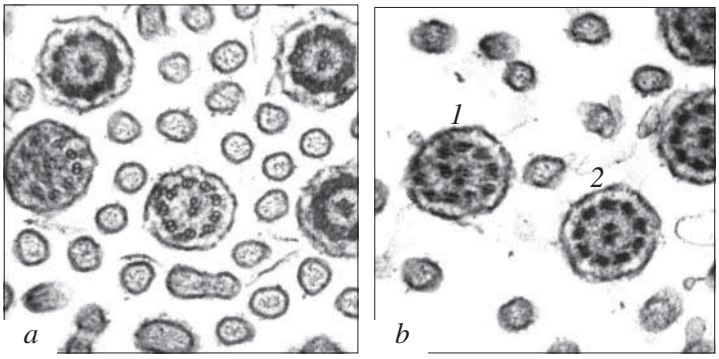

Fig. 3. Bronchial epithelium of MDCH7$^{-/-}$ mice. *a*) orthogonal section of cilia: 6 outer dynein arms, no inner dynein tubules, ×85,000; *b*) *1*: a cilium with dislocated tubule without ramification; *2*: transverse section of a cilium with 9+2 structure with inner and outer dynein arms, ×90,000.

mined under a transmission electron microscope (Zeiss Typ EM 900).

Fifty orthogonal sections of the cilia were made for each animal. The number of outer and inner DA was determined on each transverse section, the structure of the tubules was evaluated by the 9+2 scheme, radial spins and orientation of the basal system were studied.

RESULTS

Normal 9+2 tubules were generally detected in MDHC7$^{+/+}$, MDHC7$^{+/-}$, and wild random-bred mice. Outer and inner DA were detected virtually in all cilia (Fig. 1, *b*, *2*, *3*). Inner DA were absent in some MDHC7$^{+/+}$ and MDHC7$^{+/-}$ mice (Fig. 3, *a*). This defect was more incident than the absence of outer DA. The position of radial spins was typical. The distribution of the cilia on the cell surface was normal (Fig. 1, *a*; 2, *a*); no changes in the basal ciliary system were detected.

Secondary changes in cilia were detected in mice of all 3 genotypes in a different measure. Swelling of ciliary membranes was detected in some cilia. Only several complex cilia were detected. Changes (ramification and changed position of the tubules) were detected in some cilia.

TABLE 1. Number of DA per Cross-Section in Mice of Different Genotypes (*M*±*m*)

Mice	Outer DA		Inner DA	
	abs.	mean	abs.	mean
Wild type	2-6	3.66±1.77	1-3	1.62±0.70
MDHC7$^{+/-}$	2-5	3.36±1.17	0-2	1.56±0.58
MDCH7$^{-/-}$	2-6	4.56±1.21	0-3	1.95±0.68

Analysis of the number of the outer and inner DA in 50 cross-sections of the cilia showed negligible differences between wild type mice and MDHC7$^{+/-}$ and MDHC7$^{-/-}$ genotypes (Table 1).

Hence, despite disorders of the ciliary function in MDCH7 deficient mice, for example decreased rate of ciliary pulsation, we failed to detect characteristic primary ultrastructural changes [11]. It is well known that the number of outer DA on cross-sections is somewhat higher than the number of inner DA, which can be explained by their different orientation in the axoneme [8]. Our results confirm difficulties in understanding PCD. Sometimes it is impossible to detect ultrastructural changes in disorders of the mucociliary function. Presumably, there are some other genetic disorders playing an important role in the development of PCD, or the disease develops as a result of a combination of several disorders.

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