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IMMOTILE CILIA SYNDROME-ULTRASTRUCTURAL AND IMMUNOGENETIC ANALYSIS

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SUMMARY: **Immotile cilia syndrome-Ultrastructural and Immunogenetic analysis.**

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Immotile Cilia Syndrome (ICS) is a congenital disease due to a disorder of the ciliar movements. Usually the affected patients show recurrend upper and lower airway infections. The Authors describe 2 new cases with ICS. Diagnosis was done through clinical symptoms , radiologic findings and both ultrastructural and immunogenetic examination. Analysis by Electron Microscopy of nasal mucosa biopsi es revealed structural anomalies of the cilia, characterized by isolated single microtubuli and couples of tubuli arranged abnormally in the assonema. HLA study of all members of the cases showed significant association between ICS and aplotype HLA-DR7, DQW2, comm on in all siblings with the disease (p=0.0099, RR=25.94).

RIASSUNTO: **La sindrome della ciglia immobili-analisi ultrastrutturale ed immunogenetica.**

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La sindrome delle ciglia immobili (ICS) è un disordine congenito dei movimenti ciliari. I pazienti colpiti mostrano ricorrenti infezioni delle basse ed alte vie aeree. Gli autori descrivono due nuovi casi con ICS. La diagnosi è stata posta con l'ausilio dei dati radiologici, ultrastrutturali ed immunogenetici oltre alla valutazione dei sintomi clinici. L'analisi con il microscopio elettronico di biopsie della mucosa nasale ha rivelato anomalie strutturali delle ciglia, caratterizzate da singoli microtubuli isolati e coppie di tubuli disposti in modo anomalo nell'assonema. Lo studio dell'HLA in tutti i membri della famiglia ha dimostrato un'associazione significativa tra la ICS e l'aplotipo HLA-DR7, DQW2, presente in tutti i fratelli colpiti della famiglia (p=0.0099, RR=25.94).

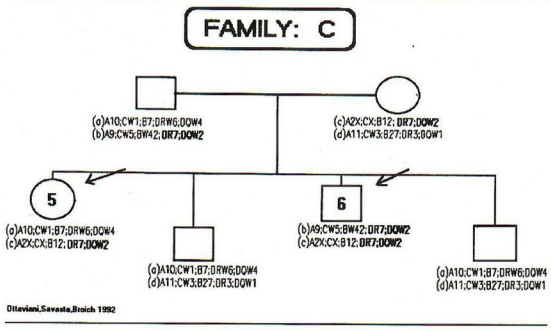
KEY WORDS: Immotile cilia syndrome, ultrastructural ciliar anomalies, HLA.
Sindrome delle ciglia immobili, anomalie ultrastrutturali ciliari, HLA.

Introduction

The Immotile Cilia Syndrome (ICS) is a hereditary disorder of the motion of the cilia of the mucous membranes, part of a larger grou p of Primary Ciliary Diseases (PCS) (Rossman et al. 1984). In the worst cases complete absence of ciliar movement can be seen due to : 1) partial or complete loss of the dinein (Afzelius 1976); 2) absence of the radial spokes (Sturgess et al. 1979); 3) loss of the central microtubuli (Howel et al. 1980); 4) transposition of the external tubular pairs (Sturgess et al. 1980). Besides this less co mplete forms can be found, in which the ciliary activity is not absent but only impaired, due to absent microtubuli or supranumerary pairs, irregularly oriented in the assonema (Antonelli et al. 1973). Recently (Niggerman et al. 1992, Afzelius et al. 1991) cases have been described in which the dysfunction was not linked to ultrastructural changes in the microtubuli but to their greater length. The most frequent consequences of ciliary disfunction are recurrend upper airway infections and sinusitis from early age on, as we ll as male sterility and in about 50% of cases situs viscerum inversus. It is probable that this clinical polimorphicity reflects a genetic variability and that the involved genes are located on different chromosomes. Studies specifically oriented to establish a relationship between the different kinds of ciliar malfunction and specific biochimical and genetic abnormalities are still scarse today (Cagnon et al. 1982). ICS comprises a clinically not homogeneous entity, deriving probably from a range of different underlying genetic diseases.

The involved genes may be located on several different chromosomes. Mutations in only one gene may perhaps be the cause of an abnormal ultrastructure and its deriving ciliar malfunction. The gene for the beta-tubulin has been located on Chromosom 6 in the segment

TABELLA 1



6p21-6pter (Floyd-Smith et al. 1985). This is also the area were the HLA genes are located.

For the examined cases our results support the hypothesis of a primary disease of the beta-tubulina due to mutation or its abnormal expression due to influences from the nearby HLA genes. The described cases of supranumerary tubules may be due to excess production of tubulin or excess transport. It can be suggested that the disease may be given by a rare mutation of a gene of the tubulin or of the genes of a carrie r protein, in linkage disequilibrium with the haplotype HLA-DR7; DQW2 in the persons of our study. Familial incidence and the having localized the gene responsible for the beta chains of tubulin on the short arm of chromosome 6 (Floyd-_Smith et al. 1985) brought us to look for a possible presence of a gene for ICS near to the HLA region. Being the tubulin the principal proteic component of the microtubuli, a mutation of the structural gene may result in an excessive or reduced formation of that protein.

Materials and methods

Two patients came to our attention due to recurrent upper and lower airway infections, resistant to proper medical treatment. The patients were examined for clinical history of recurrent upper airway infections in otherwise normal persons, sweat test, white blood cell count, sedimentation rate, rheumatic factor, immunoglobulins and its subclasses, alfa-1-antitripsin, positivity for tubercolin intradermoreaction, chemiotaxis, phagocytosis and chest x-rays. The hypothesis has already been studied by one of us (SS) in other cases (Bianchi et al. 1992).

Electron microscopy

Biopsies have been taken from the mucosa of the middle turbinate and have been examined by electron microscopy. The specimen was immediately fixed in 2,5% glutaraldehyde and 2% paraformaldehyde in 0.1M sodium cocodilate buffer at pH 7.4 for four hours. Postfixation was done in 1.33% osmium colloidin-tetraoxyd for 1.5 hours. The ultrathin sections have been stained with uranylacetate and lead citrate. The examination was done with a Zeiss EM 109 transmission electronmicroscope at 80kV and lens opening of 60 micrometers. A stroboscopic technique has been used to better study the dinein arms, the radial spokes and the nexin bridges (Markham 1963). The plane passing through the central couple of microtubuli, which is perpendicular to the stroke direction of the cilia, has been marked and its angle with a conventional plane has been measured.

Immunogenetic study and linkage analysis

The search for changes in class I and II of the HLA complex has been done with the technique of microlinphotoxicity (NIH) on suspens ions enriched with lymphocytes B and T. HLA typization has been done. Linkage analysis has been done with the sub-pair test (Penrose 1935), resulting from the observation that if pairs of siblings with a specific disease are taken from a series of families with multiple cases of that disease, some pairs will be identical for the studied genetical character with a larger frequency if there is a genetic linkage between the examined gene and the gene responsible for the disease than as there would have been in case of genetic independency. If the gene responsible for ICS maps in the HLA region, it will segregate with the HLA in the affected children in a different way than it would have done based on the medelian laws alone. If there is a statistically significant difference between the expected association of couples and the real presence seen in the examined cases, a linkage between the two genes can be assumed. The test of Fisher has been used to validate the presence of certain HLA phenotypes in cases of ICS.

Results

In both cases the white blood cell count (WBC) and sedimentation rate were high, all were negative at tests for immunoglobulins and subclasses, rheumatic tests, alfa-1-antitripsin, chemotaxis and phagocytosis, tubercolin intradermoreaction and sweat test. We had four siblings in our family, two normal and two with recurrent disease, the affected male age 7 showed changes due to chronic bronchitis at the chest x-ray, especially in the hill. The fema-

le age 9 suffered from recurrent otitis and sinus problems.

Two different types of ciliar abnormality have been seen: 1) cilia with two axonemas within the same membrane; (2) microtubular disorganization in single abnormal cilia with an abnormal distribution of the microtubular pairs and unregular presence of single microtubuli. The orientation angle distribution resulted normal. The HLA haplotype segregated DR7 - DQW2. The sib-pair-analysis indicates a significant linkage of this HLA genes with the ICS gene.

Conclusions

The basic importance of the mucociliar transport system in the normal homeostasis of the upper and lower airways is well documented. The ciliary ultrastructure is of basic importance for a proper function of the respiratory system and its correlates. The ciliary movement depends on two major proteic components, the tubulin and the dinein, which form the microtubuli and its lateral arms. The better known ultrastructural changes involve the dinein arms and the radial spokes. Studies done by Rossman et al. (1980) suggest that not only the dinein arms but also other assonemic components may be of fondamental importance in the ciliar movement and whose changes may play an important role in the dysfunction of the cilia. A structural anomal or a functional insufficiency of these proteins may then result in ICS. This syndrome is clinically characterized by recurrent otitis media, rhinosinusitis, tracheitis, bronchopulmonary infections, bronchiectasis and situs viscerum inversus in about 50% of the cases. In a recent paper Niggeman et al. (1992) stress that a reduction of ciliary movement may also be not due to assonemic changes, but to an abnormal length of the cilia. Shimizu et al. (1992) report a decrease in ciliary length in hydrocephalic rats, with a lack of dinein arms and displacement of microtubuli.

In the cases presented here the diagnosis was given through clinical examination, x-rays and ultrastructural analysis as previously described by one of us (SS) (Bianchi et al. 1922). It is interesting to stress the polimorphism of the clinical symptoms in these patients. In patients with clinically evident ICS ultrastructural abnormalities of the cilia are not regulary seen, which are more of an exception. The finding of abnormal tubuli with excess microtubuli unregularly distributed in the axonema and delimitated by a single membrane has not been described up to now as responsible for changes in the ciliary movement in human ICS. The evidence of abnormal tubuli with excess microtubuli in ICS patients and their irregular distribution in the cilia, demonstrates that this ultrastructural abnormality can interfere with a regular ciliary movement and in consequence with the normal mucociliary clearance.

ICS is a multiform clinical entity with probably an underlying genetic heterogeneity. The gene for the beta subunits of tubulin maps on chromosom 6 near the region of the HLA complex. The forms of ICS with supranumerary microtubules may be the phenotypic expression of an altered protein synthesis or transport, as well as cilia of abnormal length are function but normal microtubular ultrastructure (Fig. 1). The results of the present work suggest that the disease is given by a mutation of the tubulin gene or of a gene that codifies for protein carriers, in linkage disequilibrium with the haplotypes DR7-DQW2 in the studied population.

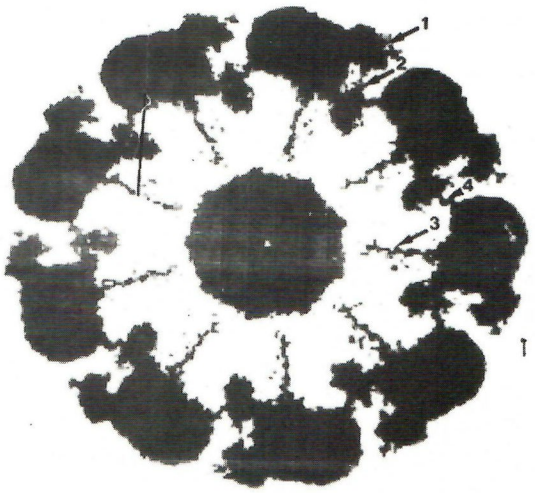


Fig. 1 - A structurally normal cilium examined with stroboscopic technique. 1) outer dinein arm; 2) inner dinein arm; 3) radial spoke; 4) nexin bridge.

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