Reference Ranges for Respiratory Ciliary Function for Chinese Children May Not Be Extrapolated From European Data

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Research

Keywords: beat frequency, beat pattern, Chinese children, nasal cilia, reference data, ultrastructure

Posted Date: June 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-34578/v1

Version of Record: A version of this preprint was published on October 9th, 2020. See the published version at https://doi.org/10.1186/s12931-020-01506-w.

Abstract

Background:To aid in the diagnosis of Primary Ciliary Dyskinesia (PCD) and to evaluate the respiratory epithelium in respiratory disease, normal age related reference ranges are needed for ciliary beat frequency (CBF), beat pattern and ultrastructure. These reference data are not available for Asian or Chinese children. Our aim was to establish reference ranges for healthy Chinese children.

Methods:Ciliated epithelial samples were obtained from 135 health Chinese children aged below 18 years by brushing the inferior nasal turbinate. CBF and beat pattern were analysedfrom high speed video recordings. Epithelial integrity and ciliary ultrastructure were assessed using transmission electronic microscopy.

Results:The mean CBF from 135 children studied was 10.1 Hz (95% CI 9.8 to 10.4). Approximately 20% (ranged 18.0-24.2 %) of ciliated epithelial edges were found to have areas of dyskinetically beating cilia. Normal beat pattern was observed in ciliated epithelium from all subjects. We did not find any effect of exposure to second hand smoke on CBF in our subjects. Microtubular defects were found in 9.3% of all of the cilia counted in these children while other ciliary ultrastructural defects were found in less than 3%.

Conclusions: We established the reference range for CBF, beat pattern and ultrastructure in healthy Chinese children. Using similar methodology, we found a lower overall mean CBF than previously obtained European values. This study highlights the need to establish normative data for ciliary function in different populations.

Introduction

Primary ciliary dyskinesia (PCD) is a rare genetic condition with ciliary ultrastructural defects leading to ineffective ciliary movement and impaired mucociliary clearance. This results in sinusitis, recurrent chest infections leading to bronchiectasis and respiratory failure in affected individuals [1,2]. Majority of patients have persistent symptoms from birth or early infancy. However, a diagnosis is often made at a late stage, when significant bronchiectasis and permanent loss of lung function already occurred [3].Early diagnosis facilitates implementation of management strategies for new cases with the hope that morbidity can be reduced and lung structure and function preserved.

The European Respiratory Society (ERS) task force suggests a combination of nasal nitric oxide (NO) measurement and digital high speed video microscopy (HSVM) assessment of ciliary beat frequency (CBF) and pattern as the first step in diagnostic testing for PCD [4]. Although CBF can be measured by photomultiplier and photodiode techniques, the values were significantly lower than by digital HSVM [5] and they do not allow assessment of ciliary beat pattern. HSVM has the additional advantage of allowing analysis of beat pattern that has been shown to be abnormal in PCD [6]. HSVM has been shown to have a high sensitivity and specificity for the diagnosis of PCD [7-9]. However, expertise is required to interpret ciliary function using HSVM as secondary epithelial damage following viral infection [10] and in severe asthma may result in significant secondary ciliary dyskinesia [11,12].

Transmission electron microscopy (TEM) is advocated as the next step if HSVM or nasal NO are abnormal and is regarded as a highly specific test to confirm PCD. Yet, some patients with PCD have an apparently normal ultrastructure [13].As such, diagnostic armentarium should include both HSVM and TEM.

Normal ranges are essential to help interpret findings and to date normal data on ciliary beat pattern has only been established for a European population [1]. The aim of this study was to establish normal age related reference ranges for CBF and pattern using digital HSVM and ultrastructure of the ciliated epithelium and ciliary axonemes using TEM in Chinese children. It also allowed comparison with normative data from a European study using the same methodology.

Methods

Study design and subjects

This cross-sectional study recruited healthy Chinese school children aged less than 18 years and adult volunteers in Hong Kong (HK). Subjects with a history of chronic respiratory or nasal disease, symptomatic upper respiratory tract infection during the previous 6 weeks, those requiring long-term medication or known cigarette smokers were excluded [1]. Exposure to second hand smoking (SHS) at home were explored. Subjects were examined to exclude obvious nasal defects with an auroscope. The inferior turbinate of subjects was brushed with a 3mm cytology brush to obtain nasal epithelial cells. Nasal brushings were placed in medium 199 (pH7.3) supplemented with streptomycin 50μg/ml and penicillin 50IU/ml (Gibco, UK).

Analysis of CBF and beat pattern by HSVM [1,6] and ultrastructure by TEM [7]

Ciliated strips of epithelium were suspended in a chamber created by glass slide and cover slips. The slide was then placed on a heated stage (37°C) of a Leica light microscope mounted on an anti-vibration table. Specimens were examined using a x100 interference contrast lens. Undisrupted ciliated strips of about 50μm in length and devoid of mucus were studied. Beating ciliated edges from a side-view profile, were recorded using a digital high speed video camera (IDT, model NX4) at a rate of 500 frames per second. The recorded video sequences, projected onto a high resolution monitor were played back at reduced frame rates or frame by frame to determine CBF. The ciliated edge was divided into 5 adjacent areas measuring 10μm. Two measurements of CBF were recorded in each area, resulting in a total of 10 measurements for each edge. A maximum of 10 edges were analysed per subject. The number of frames for groups of beating cilia required to complete 10 cycles were recorded. CBF was converted using a calculation (CBF= 500/(number frames for 10 beats) x10). The reproducibility for inter-observer (measured independently by 2 observers) and intra-observer (measured by the same observer after 2 days) CBF measurements were evaluated [1]. Normal ciliary beat pattern denoted a coordinated cilium beating in a forward backward motion along the whole epithelial edge. Edges with dyskinetically beating cilia were counted and percentage of these edges was calculated.

Transmission electron microscopy analysis

For TEM, nasal brushing samples were fixed in 2% glutaraldehyde and processed through resin by standard techniques [1]. Ultrathin sections were cut at 100 nm, collected on 200 mesh thin bar copper grids, stained in 1% uranyl acetate, counterstained in Reynold's lead phosphate.

Evaluation of ciliary structure and function

The epithelial and ciliary ultrastructural changes of epithelium were assessed in a blinded fashion [7]. The number of ciliated cells, mucous cells, and dead cells of the epithelium were expressed as a percentage of total number of cells examined. Disruption and damage to the tissue were quantified using a scoring system that assess the degree of loss of cilia from ciliated cells, projection of cells from epithelial edge, cytoplasmic blebbing and mitochondrial damage with a summation epithelial integrity which incorporated all these measurements to assess the overall epithelial damage: 0=no damage, 1=minor, 2=mild, 3=moderate, 4=major, 5=severe damage [1]. Cilia in less than perfect cross-section but in which the microtubular arrangement could be recognised were recorded as being normal (9+2 microtubules) or defective. The results were recorded in batches of 100 counts, assembling as many such batches from a single section, and thus any pattern would be revealed. Dynein arms were assessed in any high-quality cross-sections encountered during the recording of microtubular arrangements and documented as showing the presence of both arms, only an outer or inner arm, or neither arm. If the presence of an arm was equivocal, it was counted as being present [14].

Data analysis

To calculate the sample size to establish age-related reference in children aged <18 years with a local population size of around 1,000,000 [15], allowing 9% margin of error from the mean at 5% confidence level, 120 subjects was required. This sample size of 120 would have a power of 80% and 5% level of significance (two-sided) to detect a 10% difference of mean CBF between the Chinese and European population [1]. Assuming 10% of sample insufficiency and loss during preparation, a total of 135 subjects were recruited. The mean and standard deviation (SD), 5th and 95th percentiles of CBF, the mean percentage, 5th and 95th percentiles of edges exhibiting dyskinetically beating cilia, cells with loss of cilia, cellular projections, cytoplasmic blebbing, mitochondrial damage and ultrastructural defects including microtubular, dynein arm or other defects of the 3 individual age groups and the whole group were calculated. A one way analysis of variance (ANOVA) was performed to detect a significant difference among individual groups. The mean CBF between children <18 years with adults between subjects with and without SHS exposure in each individual age group and between subjects in current study and the European study were compared using t-test.Inter-observer and intra-observer difference of CBF measurement were calculated by comparing the 95% confidence interval (CI).

Results

Analysis of ciliary beat frequency and beat pattern measurements

We recruited 164 children (88 males, age range 2-17 years) and 50 adult volunteers from December 2015 to November 2016. We excluded 34 subjects because of known underlying diseases or inadequate samples and 1 adult subjects who was an active smoker. Samples from the remaining 135 children subjects (67 males, age range 3-17 years) and 44 adults (25 males, age range 18-60 years) were analysed. Forty-six children were exposed to SHS at home. Ten adults were exposed to SHS at home while 34 did not. The mean CBF and the percentage of dyskinetically beating edges for all subjects were summarized in Table 1. No significant difference was found in mean CBF between individual age groups (ANOVA, p=0.542) and dyskinetically beating edges between individual age groups (ANOVA, p=0.212).The normal ciliary beat pattern from a subject showed a coordinated ciliary beating in a forward backward motion along the whole epithelial edge (see Video 1) and the ciliated edges analysed that exhibited areas of dyskinetically beating cilia ranged from 18.0 to 24.2% (representative images of ciliated edge exhibited static cilia, seeVideo 2). There was also no significant difference in mean CBF between children with and without exposure to SHS for the 3 different age groups (ANOVA, p=0.89 for children aged 2-6, p=0.29 for children aged 7-12, p=0.58 for children aged 13-17) but there was slightly higher mean CBF in adults with SHS exposure compared to adults without (ANOVA, p=0.04) (Table 2). The mean CBF for children aged < 18 years was slightly higher than the adult group but it did not reach statistical significance [10.1Hz (95% CI 9.8 to 10.4) versus 9.5Hz (95% CI 8.9 to 10.0), ANOVA, p=0.05]. Yet, it was lower than that of the European study [12.8Hz (95% CI 12.3 to 13.3, ANOVA, p<0.05) [1].

Table 1
Analysis of ciliary beat frequency and beat pattern measurements

Age (years)	N	Mean*	SD	5th, 95th percentiles	Dyskinetically beating edges (%)¶
<18	135	10.1	2.0	6.3, 13.5	20.9 (0.0, 56.4)
2-6	51	10.3	2.0	6.9, 13.8	24.2 (0.0, 60.0)
7-12	43	10.1	2.1	5.8, 13.5	18.0 (0.0, 50.0)
13-17	41	9.9	1.9	6.1, 13.1	19.8 (0.0, 50.0)
≥18	44	9.5	1.9	5.4, 11.9	14.9 (0.4, 44.7)

*Mean ciliary beat frequency(Hz), standard deviation (SD), and 5th, 95th percentiles
¶mean (5th, 95th percentiles) percentage of edges exhibiting areas of ciliary dyskinesia

UK reference CBF mean for <18 years 12.8 (95% CI 12.3 to 13.3)[1] vs ours <18 mean CBF 10.1 (95% CI 9.8 to 10.4) (p<0.05, t-test)

Table 2
Effect of exposure to second-hand smoking on ciliary beat frequency

Age (years)	Family member(s) smoked				
	Yes		No		
	N	*Mean ± SD	N	*Mean ± SD	P value
<18	46	9.9 ± 1.68	89	10.2 ± 2.16	0.284
2-6	11	10.4 + 1.53	40	10.3 ± 2.09	0.886
7-12	21	9.7 ± 2.13	22	10.4 ± 2.15	0.289
13-17	14	9.7 ± 0.81	27	10.0 ± 2.33	0.575
≥18	10	10.5 ± 1.65	34	9.2 ± 1.82	0.044
*Mean ciliary beat frequency(Hz), standard deviation(SD)					

For ciliary beat pattern, 1 subject in the 13-17 year group had a mixed ciliary beat pattern with dyskinetic cilia, cilia with a normal pattern and cilia with a circular beat pattern when viewed from above on occasional ciliated cells(see Video 3).The mean CBF and TEM was normal. Normal beat pattern was observed in the ciliated epithelium from all other subjects.

To establish a reference range, mean CBF was plotted against age of each subject (Figure 1A). A weak negative correlation was found between mean CBF and increasing age (r^2 = 0.021). As cilia were found to beat at different frequencies within each sample, we also plotted sample variation in CBF, the ciliated edges with the highest and lowest CBF against age. The highest mean CBF of edges ranged from 7.3 to 24.3Hz (Figure 1B) with 93% of subjects having a maximal CBF of >10 Hz. The lowest mean CBF of edges ranged from 2.3 to10.4Hz(Figure 1C) with 45% of subjects having a minimum CBF of >6 Hz.

No significant difference was found for inter-observer and intra-observer measurements of CBF. The mean (SD) for inter-observer measurement was 0.57 (1.87) (95% CI -0.77 to 1.9; range -2 to 4) and intra-observer was 0.01 (1.4) (95% CI -1.02 to 1.03; range -3 to 3).

Transmission electron microscopy examination of cell types

Some subjects had an inadequate sample for ultrastructural analysis as tissue might have been lost during initial HSVM assessment performed before sample processed for TEM [1]. Among 179 subjects analysed with CBF and beat pattern, 121 subjects had sufficient tissue for epithelial integrity measurements and 159 subjects had tissue processed for ultrastructure analysis. The percentages of different cell types observed in the ciliated epithelial strips were summarized in Table 3. There was no significant difference between the percentages ofdifferent cells types across age groups (ANOVA, p>0.1). Ciliated cells formed about 50% of the cell population.

Table 3
Transmission electron microscopy examination of cell types

Age (years)	n	Ciliated cells (%)	Unciliated cells (%)	Mucous cells (%)	Dead cells (%)
<18	95	52.0 (0.0, 100.0)	35.8 (0.0, 100.0)	12.2 (0.0, 52.0)	0.0 (0.0, 0.0)
2-6	37	53.6 (0.0, 100.0)	37.1 (0.0, 100.0)	9.3 (0.0, 34.3)	0.0 (0.0, 0.0)
7-12	30	46.0 (0.0, 100.0)	38.3 (0.0, 100.0)	15.7 (0.0, 78.7)	0.0 (0.0, 0.0)
13-17	28	56.4 (0.0, 100.0)	31.3 (0.0, 77.8)	12.3 (0.0, 55.5)	0.0 (0.0, 0.0)
≥18	26	51.0 (17.8, 93.0)	36.5 (3.6, 77.5)	12.3 (0.0, 43.0)	0.2 (0.0, 2.7)
Results are expressed as the mean percentage (5th, 95th percentiles) for each age group.					

Assessment of ciliary epithelial integrity and ultrastructure by transmission electron microscopy

The integrity of ciliated epithelium was assessed by examining factors including loss of cilia, cellular extrusion, cytoplasmic blebbing, and mitochondrial damage in Figure 2. Normal with a normal healthy mitochondrion (arrow, bar=1μm) in Figure 2A. Loss of cilia, grade 3, and a cell with a damaged mitochondrion (arrow, bar=1μm) in Figure 2B. Cellular extrusion, grade 2, (bar=2μm) in Figure 2C. Cytoplasmic blebbing, grade 2 (arrow, bar=2μm) in Figure 2D. Evidence of minor epithelial damage was observed and the analysis was summarised in Table 4.There was no significant difference of epithelial integrity score across age groups (ANOVA, p=0.07). Normal nasal epithelium with an intact ciliated surface and minimal disruption (epithelial integrity score=0, bar=2μm) was shown in Figure 3Aand abnormal epithelium with severely disrupted cell surface and marked loss of cilia (epithelial integrity score=4, bar=2μm) in Figure 3B.

Table 4
Assessment of epithelial integrity by transmission electron microscopy

Age (years)	n	Cells with loss of cilia (%)	Cells extruding from epithelial edge (%)	Cells with cytoplasmic blebbing (%)	Cells with mitochondrial damage (%)	Epithelial integrity
<18	95	41.4 (0.0, 56.8)	31.5 (0.0, 67.0)	21.1 (0.0, 100.0)	16.3 (0.0, 100.0)	1.83 (0.8, 3.0)
2-6	37	55.1 (0.0, 100.0)	26.1 (0.0, 67.0)	10.8 (0.0, 50.0)	14.9 (0.0, 100.0)	1.8 (0.0, 3.1)
7-12	30	44.4 (0.0, 100.0)	41.0 (0.0, 90.9)	29.2 (0.0, 100.0)	14.4 (0.0, 100.0)	2.1 (0.0, 3.5)
13-17	28	20.3 (0.0, 56.8)	28.6 (0.0, 67.0)	25.9 (0.0, 100.0)	20.2 (0.0, 100.0)	1.6 (0.0, 3.0)
≥18	26	49.3 (0.0, 100.0)	40.2 (6.0, 67.0)	24.7 (0.0, 61.1)	13.4 (0.0, 100.0)	2.0 (1.0, 3.0)

Results are expressed as the mean percentage (5th, 95th percentiles) for each age group.

Ultrastructural analysis was summarised in Table 5. Abnormal cilia were observed in some subjects. Microtubule defects including disarranged tubules (0.7%), extra-tubule including extra-single tubule and extra-microtubular pair (2.1%), 8+gap (1.0%) and single tubule (5.5%); central microtubules defects including extra-inter tubule (0.3%) and central pair damage (1.2%); compound cilia (0.5%) and combination of defects (0.9%) were identified in all of the cilia counted in children aged <18 years (Figure 4 B-I). The commonest defect identified was single micro-tubule (Figure 4E).

The mean microtubule defects in 13-17 year group (14.4%) were significantly higher than those in the other age groups (8.3% in 2-6 year group, 5.3% in 7-12 year group; ANOVA, $p<0.01$). Central microtubules defects, compound cilia and other microtubule defects referring to a combination of defects were found in less than 3% of all of the cilia counted in children aged <18 years (Table 5). There was no significant difference between groups for the central microtubules defects, compound cilia and other microtubule defects (ANOVA, $p>0.05$).

Table 5
Analysis of ciliary ultrastructure by transmission electron microscopy

Age (years)	n	Outers	Inners	Dynein arm defects (%)	Microtubules defects (%)	Central microtubules defects (%)	Compound cilia (%)	Other defects (%)
<18	121	8.5 (7.5, 9.0)	7.8 (6.5, 8.6)	0.0 (0.0, 0.0)	9.3 (0.0, 28.4)	1.5 (0.0, 9.1)	0.5 (0.0, 5.8)	0.9 (0.0, 6.6)
2-6	46	8.5 (7.8, 9.0)	7.8 (6.9, 8.5)	0.0 (0.0, 0.0)	8.3 (0.0, 27.9)	1.5 (0.0, 10.4)	0.2 (0.0, 0.0)	0.5 (0.0, 7.0)
7-12	36	8.3 (6.9, 9.0)	7.6 (4.2, 8.5)	0.0 (0.0, 0.0)	5.3 (0.0, 22.6)	2.7 (0.0, 19.7)	1.0 (0.0, 9.2)	0.3 (0.0, 3.1)
13-17	39	8.7 (8.3, 9.0)	8.1 (6.7, 8.8)	0.0 (0.0, 0.0)	14.4 (0.0, 37.8)	0.4 (0.0, 3.7)	0.4 (0.0, 6.3)	1.8 (0.0, 11.1)
≥18	38	8.5 (7.0, 9.0)	7.3 (5.8, 8.5)	0.0 (0.0, 0.0)	10.1 (0.0, 31.5)	1.9 (0.0, 16.1)	0.7 (0.0, 6.3)	0.9 (0.0, 13.1)

Results are expressed as the mean percentage (5th, 95th percentiles) for each age group.

Discussions

We established the reference ranges for ciliary function and structure in healthy Chinese children using HSVM and TEM. We found an overall mean CBF of 10.1 Hz in children aged <18 years. CBF did not vary with age in these children.

We did not find a significant effect of SHS exposure on CBF of nasal cilia in our children but adults with SHS exposure had a significantly higher mean CBF compared to adults without. Previous studies had been carried out in adults only and the results were conflicting. Nasal CBF in active or passive smokers was significantly lower than that in non-smoke exposed in one study [16]. However, this study included subjects with middle ear diseases and healthy control. Our finding concurred with more recent studies that showed higher mean CBF in nasal epithelial biopsies or air liquid interface cultures among active smokers and non-smokers exposed to SHS than non-smokers [17,18].

We found a lower overall mean CBF in healthy Chinese children compared to the European study using the same methodology [1]. The HK team was trained by the European study team in England. A member of that team, who developed normal ranges for the UK, visited the HK laboratory to ensure the methods used were similar, with samples observed using similar slide chambers and with a heated stage that would negate differences due to laboratory temperature and humidity. It was possible that the difference was due to the different status of the subjects as the samples were obtained from awake Chinese children and

from European children immediately after anaesthetic induction for elective surgery. However, Propofol used in the anaesthetic induction was not shown to affect CBF [19].Subclinical laboratory-confirmed respiratory infections due to respiratory syncytial virus (RSV) and influenza are common in children during influenza seasons in HK [20,21]and they might have affected ciliary function in some cases. Yet, similar exclusion criteria were used to the European study to minimise this effect. The other possibility was an observer bias but the difference of CBF of our intra-observer and inter-observer mean was smaller than the European study. Thus, our finding may represent a genuine genetically determined difference between European and Chinese children.

One patient had a mixed ciliary beat pattern with dyskinetic cilia, cilia with normal pattern and cilia with circular beat pattern on occasional ciliated cells. Circular beat pattern is observed in ciliary trans-position defect and central microtubular agenesis [6,22], but the cilia of all the ciliated cell show dyskinetic beat pattern when viewed from above in these cases. We invited the subject to repeat the test, which showed normal CBF and normal beat pattern in 3 different beating planes including sideway profile, towards the observer and view from above [23]. The percentage of dyskinetic edge decreased from 33.3% to 10%. Thus, we believed that the initial abnormal finding was secondary in nature, possibly due to a preceding subclinical respiratory infection.

Our results of ultrastructural analysis were in agreement with other reports on the number of outer and inner dynein arms visible, number of ciliated cells and epithelial integrity [1,24].Nevertheless, we found a higher percentage of microtubular defects, especially in 13-17 year group compared to the European study. The reasons for this are unclear. As discussed above, some children may have had unrecognized subclinical or be in the recovery phase of a mild respiratory infection. Microtubular defects have been reported in the recovery phase of mild respiratory infection in some patients up to 10 weeks after acute phase [25,26]. The damage to the nasal ciliated epithelium might also occur during sampling. Culture of ciliated epithelial cells at air-liquid interface, with re-differentiation and re-analysis of ciliary function and ultrastructure may eliminate secondary damage but was not feasible during this study [27].

We had a large sample size of healthy subjects and none were on medication. Our methodology was robust as we followed the European study [1] and were closely supervised. Our study was conducted to avoid peak seasons for viral infections. However, laboratory surveillance data showed influenza was still prevalent in March 2016 with adenovirus, RSV and rhinovirus circulating in our community for that whole year [28]. We might have included some subjects with subclinical infections or recovering from very mild infection. We also identified subjects with undiagnosed allergic rhinitis and asthma. We excluded them in the analysis but might still include some with very mild allergic rhinitis with local inflammation leading to secondary ciliary dyskinesia.

In conclusion, we established normal reference range for respiratory ciliary function and ultrastructure in healthy Chinese children. Unexpectedly, we found a lower mean CBF in our children aged <18 years compared to a Europeanstudy. The reasons remain uncertain but could include potential genetic basis. Our results suggest the necessity to establish normal reference ranges for different populations.

Abbreviations

PCD	Primary ciliary dyskinesia
ERS	European Respiratory Society
NO	Nasal nitric
HSVM	High speed video microscopy
CBF	Ciliary beat frequency
TEM	Transmission electron microscopy
HK	Hong Kong
SHS	second hand smoking
HKU/HA HKW	The University of Hong Kong/Hospital Authority Hong Kong West Cluster
SD	Standard deviation
ANOVA	Analysis of variance
CI	Confidence interval
UK	United Kingdom
RSV	respiratory syncytial virus

Declarations

Ethics approval and consent to participate

Ethics approval for this study was granted from the institutional review board (HKU/HA HKW).

Consent for publication

Parents or subjectsgave informed written consent.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the Health and Medical Research Fund (HMRF No.02133316) under the Government of the Hong Kong Special Administrative Region and supported by the NIHR GOSH BRC.

Authors' contributions

SL designed, supervised the study and wrote the manuscript. CO supervised and critically reviewed the manuscript. YLcritically reviewed the manuscript.CW did the data acquisition and analysis. All of the authors read and approved the manuscript to be published.

Acknowledgements

We thankMr. Wilfred Hing-Sang WONG, Ms Susanna FOK, Ms. Sau-Man CHAN and Ms. Eva Man-Yin FONG for the assistance throughout the project and Dr Kenneth TSANG for his advice on preparing samples for TEM. We also thank the Boys' & Girls' Clubs Association of Hong Kong (BGCA), Jockey Club Southern District Children & Youth Integrated Services Centre (Wah Fu Centre, WahKwai Centre), The Cheerland Nursery School cum Kindergarten (Kowloon Bay) and Henrietta Secondary School for their support. We are grateful to all the subjects and their parents.

References

1. Chilvers MA, Rutman A, O'Callaghan C. Functional analysis of cilia and ciliated epithelial ultrastructure in healthy children and young adults. Thorax 2003 Apr;58(4):333-8. [publised online first: 2003/04/02]
2. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. Am J RespirCrit Care Med 2004 Feb 15;169(4):459-67. doi: 10.1164/rccm.200303-365OC [published online first: 2003/12/06]
3. Ellerman A, Bisgaard H. Longitudinal study of lung function in a cohort of primary ciliary dyskinesia. EurRespir J 1997 Oct;10(10):2376-9. [published online first: 1997/12/05]
4. Kuehni CE, Lucas JS, Diagnosis of primary ciliary dyskinesia: summary of the ERS Task Force report. Breathe 2017 Sep;13(3):166-178. doi: 10.1183/20734735.008517.
5. Chilvers M, O'Callaghan C. Analysis of ciliary beat pattern and beat frequency using digital high-speed imaging: comparison with the photomultiplier and photodiode methods. Thorax 2000 Apr;55(4):314-7
6. Chilvers MA, Rutman A, O'Callaghan C. Ciliary beat pattern is associated with specific ultrastructural defects in primary ciliary dyskinesia. J Allergy ClinImmunol 2003 Sep;112(3):518-24
7. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. Am J RespirCrit Care Med 2010 Feb 15;181(4):307-314
8. Papon J-F, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B.Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. Orphanet J. Rare Dis 2012 Oct 11; 7:78.

9. Jackson CL, Behan L, Collins SA, Goggin PM, Adam EC, Coles JL, Evans HJ, Harris A, Lackie P, Packham S, Page A, Thompson J, Walker WT, Kuehni C, Lucas JS. Accuracy of diagnostic testing in primary ciliarydyskinesia. Eur. Respir. J 2016; 47: 699-701. doi: 10.1183/13993003.00749-2015.
10. Chilvers MA, McKean M, Rutman A, Myint BS, Silverman M, O'Callaghan C.The effects of coronavirus on human nasal ciliated respiratory epithelium.EurRespir J 2001 Dec;18(6):965-70
11. Thomas B, Rutman A, Hirst RA, Haldar P, Wardlaw AJ, Bankart J, Brightling CE, O'Callaghan C. Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. J Allergy ClinImmunol 2010 Oct;126(4):722-729
12. Wan WY, Hollins F, Haste L, Woodman L, Hirst RA, Bolton S, Gomez E, Sutcliffe A, Desai D, Chachi L, Mistry V, Szyndralewiez C, Wardlaw A, Saunders R, O'Callaghan C, Andrew PW, Brightling CE. NADPH oxidase 4 overexpression is associated with epithelial ciliary dysfunction in neutrophilic asthma. Chest: 2016 Jun: 149(6): 1445-1459
13. Shoemark A, Hogg C. Electron tomography of respiratory cilia.Thorax2013 Feb; 68(2): 190-191
14. Shoemark A, Dixon M, Corrin B, Dewar A. Twenty-year review of quantitative transmission electron microscopy for the diagnosis of primary ciliary dyskinesia. J ClinPathol. 2012 Mar;65(3):267-71.
15. Census and Statistics Department, Hong Kong SAR. 2011 population census.https://www.census2011.gov.hk/en/interactive-visualisations.html
16. Agius AM, Smallman LA, Pahor AL. Age, smoking and nasal ciliary beat frequency. ClinOtolaryngol Allied Sci.1998 Jun;23(3):227-30
17. Zhou H, Wang X, Brighton L, Hazucha M, Jaspers I, Carson JL.Increased nasal epithelial ciliary beat frequency associated with lifestyle tobacco smoke exposure. Inhal Toxicol.2009 Aug;21(10):875-81. doi: 10.1080/08958370802555898
18. Carson JL, Lu TS, Brighton L, Hazucha M, Jaspers I, Zhou H.Phenotypic and physiologic variability in nasal epithelium cultured from smokers and non-smokers exposed to secondhand tobacco smoke. In Vitro Cell Dev Biol Anim. 2010 Jul;46(7):606-12. doi: 10.1007/s11626-010-9310-6.[published Online First: 2010/04/10]
19. Hann HC, Hall AP, Raphael JH, Langton JA. An investigation into the effects of midazolam and propofol on human respiratory cilia beat frequency in vitro. Intensive Care Med.1998 Aug;24(8):791-4. [published Online First: 1998/10/03]
20. Kutsaya A, Teros-Jaakkola T, Kakkola L, Toivonen L, Peltola V, Waris M, Julkunen I. Prospective clinical and serological follow-up in early childhood reveals a high rate of subclinical RSV infection and a relatively high reinfection rate within the first 3 years of life. Epidemiol Infect 2016 Jun;144(8):1622-33. doi: 10.1017/S0950268815003143 [published online first: 2016/01/07]
21. Centre for Health Protection DoH, HK. Detection of pathogens from respiratory specimens.https://www.chp.gov.hk/en/index.html

[cited 2017 28 August]

22. Stannard W,Rutman A, Wallis C,O'Callaghan C.Central microtubular agenesis causing primary ciliary dyskinesia. Am J RespirCrit Care Med 2004 Mar 1;169(5):634-7; doi: 10.1164/rccm.200306-782OC
23. Kempeneers C, Seaton C, ChilversMA.Variation of ciliary beat pattern in three different beating planes in healthy subjects. Chest 2017 May; 151(5):993-1001. doi: 10.106/j.chest.2016.09.015 [published online first: 2016/09/29]
24. Jorissen M, Willems T, Van der Schueren B, Verbeken E. Dynein arms and spokes after ciliogenesis in cultured respiratory epithelial cells from non-PCD individuals. ActaOtorhinolaryngol Belg. 2000;54(3):325-332
25. Carson JL, Collier AM, Hu SS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. N Engl J Med 1985 Feb 21;312(8):463-8. doi: 10.1056/NEJM198502213120802 [published online first: 1985/02/21]
26. Giorgi PL, Oggiano N, Braga PC, Catassi C, Gabrielli O, Coppa GV, Kantar A. Cilia in children with recurrent upper respiratory tract infections: ultrastructural observations. PediatrPulmonol 1992 Dec;14(4):201-5. [published online first: 1992/12/01]
27. Hirst RA, Rutman A, Williams G, O'Callaghan C. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia. Chest 2010 Dec;138(6):1441-7. doi: 10.1378/chest.10-0175 [published online first: 2010/07/10]
28. Yaghi A, Dolovich MB. Airway Epithelial Cell Cilia and Obstructive Lung Disease.Cells 2016 Nov 11;5(4). doi: 10.3390/cells5040040 [published online first: 2016/11/16]

Figures

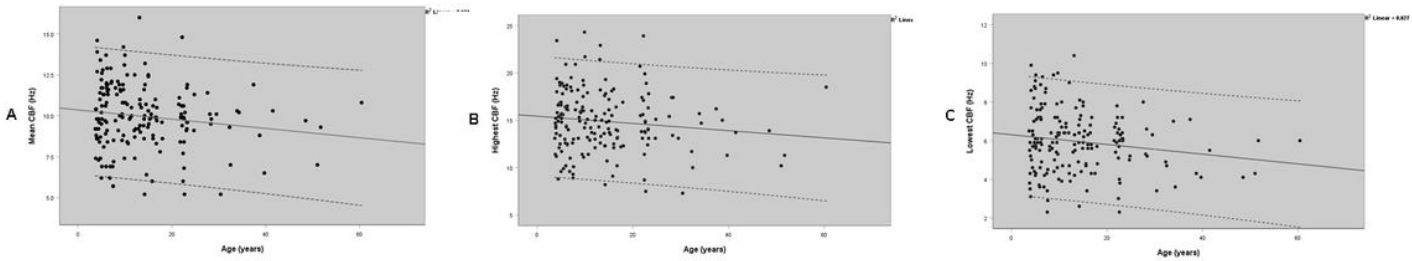

Figure 1

Relationship of the ciliary beat frequency(CBF) with age(years). (A) Mean CBF plotted against age for all subjects showing a negative correlation between increasing age and a reduction in CBF. Mean (solid line) and ±1.96 standard deviation (dashed line) regression lines were indicated. Relationship of the ciliary beat frequency(CBF) with age(years). (B)Ciliated edges with the highest and CBF within a nasal sample plotted against age for all subjects. Mean (solid line) and ±1.96 standard deviation (dashed line) regression lines were indicated Relationship of the ciliary beat frequency(CBF) with age(years). (C)Ciliated edges with the

lowest CBF within a nasal sample plotted against age for all subjects. Mean (solid line) and ±1.96 standard deviation (dashed line) regression lines were indicated.

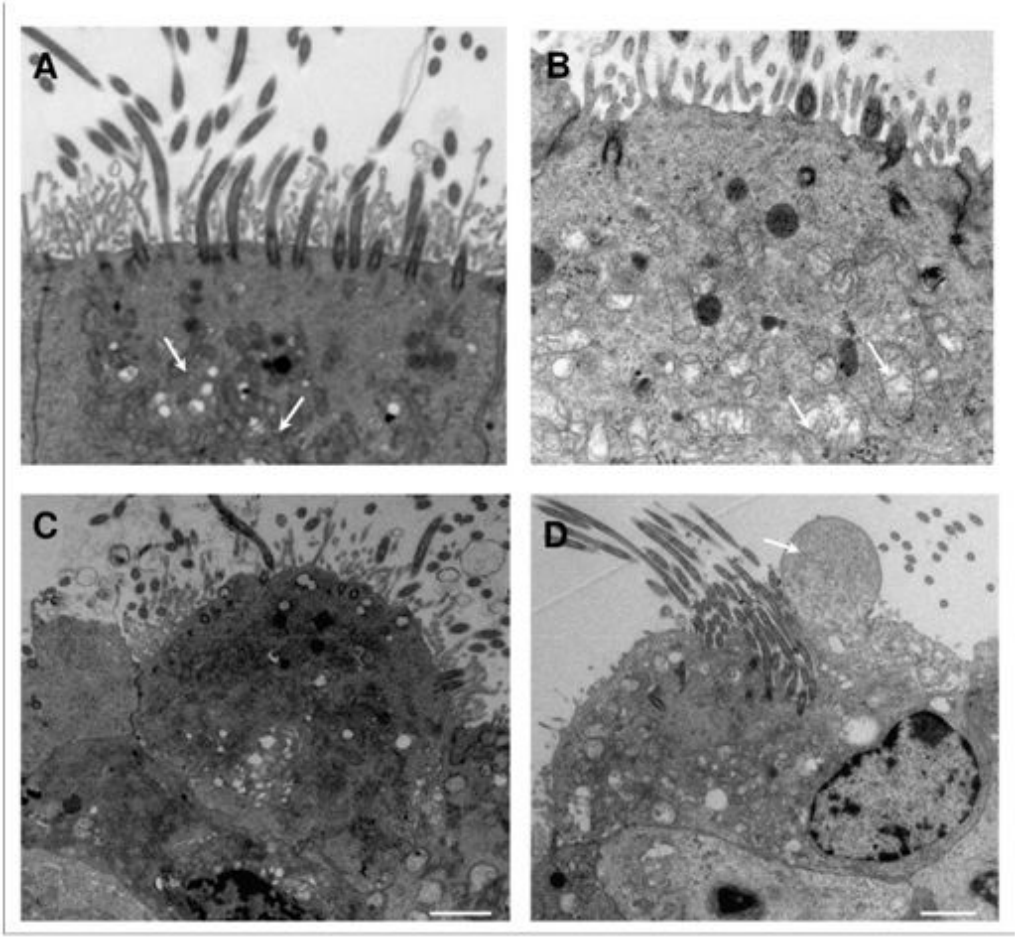

Figure 2

Transmission electron micrographs showing the normal epithelium and the parametersassessed to examine the epithelial damage. (A) Normal with a normal healthy mitochondrion (arrow, bar=1μm) (B)Loss of cilia, grade 3, and a cell with a damaged mitochondrion (arrow, bar=1μm) (C) Cellular extrusion, grade 2, (bar=2μm) (D)Cytoplasmic blebbing, grade 2 (arrow, bar=2μm)

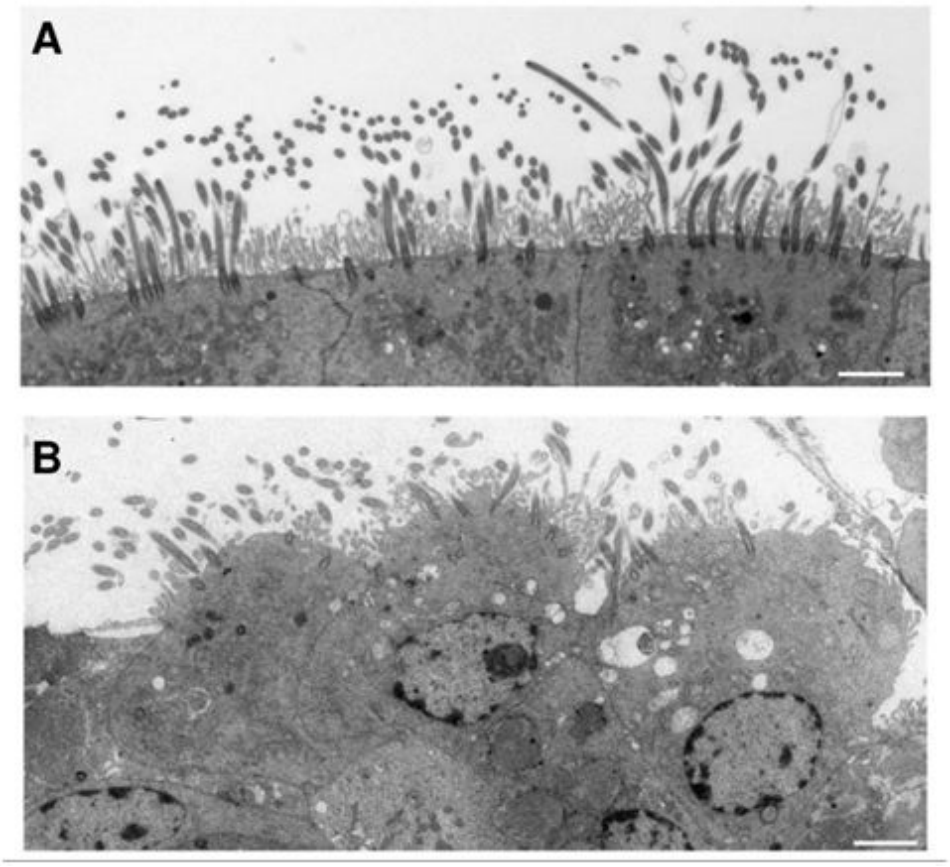

Figure 3

Transmission electron micrographs illustrating the assessment of epithelial integrity. (A) Normal nasal epithelium with an intact ciliated surface and minimal disruption(epithelial integrity score=0, bar=2μm) (B) Abnormal epithelium with severely disrupted cell surface and marked loss of cilia(epithelial integrity score=4, bar=2μm)

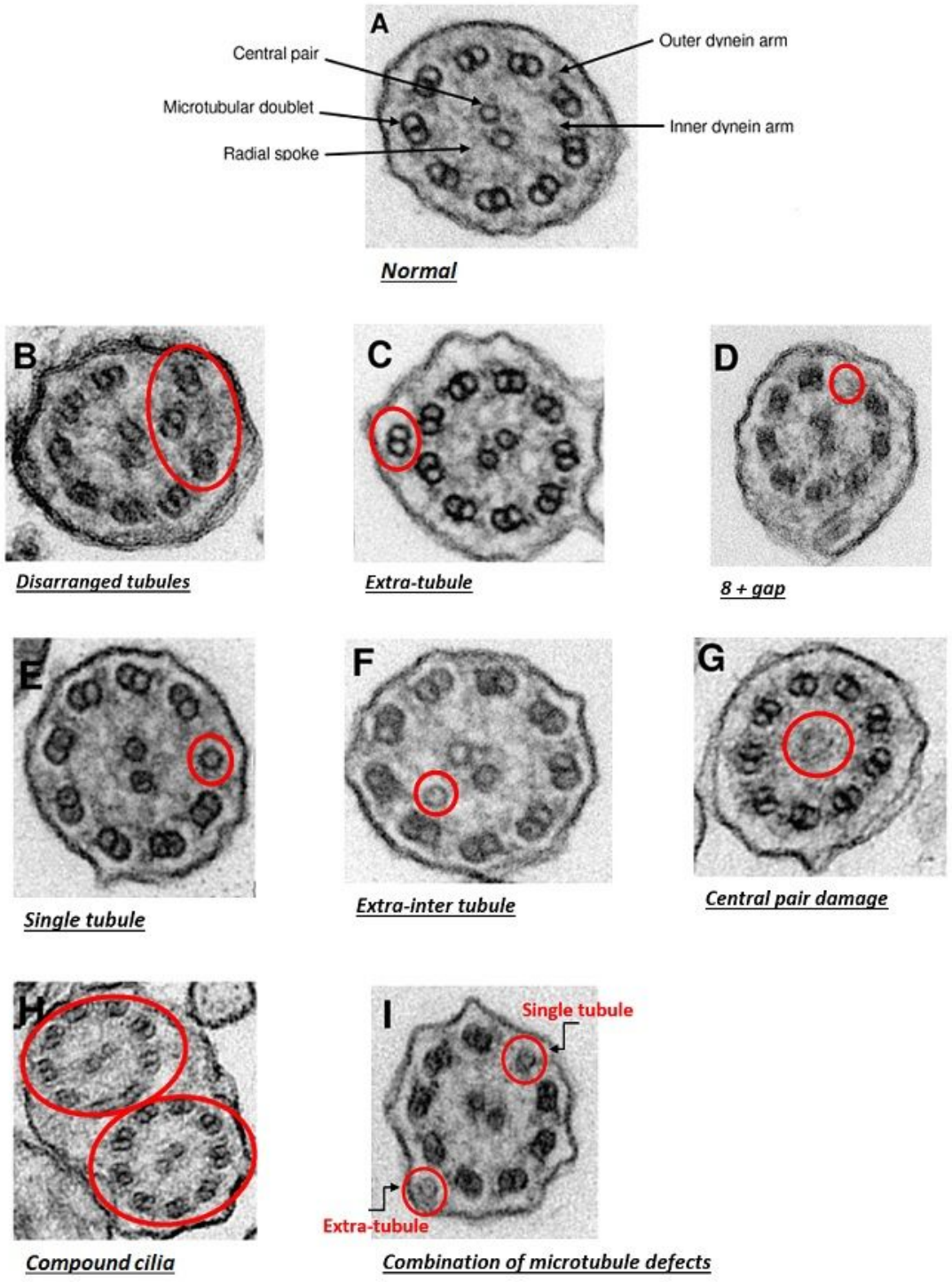

Figure 4

Transmission electron micrographs demonstrating the representative image of ciliary ultrastructural defects. (A) Normal (B-E) Microtubule defects (F-G) Central microtubules defects (H) Compound cilia (I) Combination of microtubule defects

Supplementary Files

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