SUPPLEMENTARY MATERIAL

NASAL NITRIC OXIDE AND NITRIC OXIDE SYNTHASE EXPRESSION IN PRIMARY CILIARY DYSKINESIA

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MATERIALS AND METHODS

Nasal nitric oxide

Nasal nitric oxide evaluation was performed using standard methodology [1] which is also applicable to very young non-cooperative children [2]. Nasal air was sampled continuously with a constant transnasal flow of 1.8 mL/s for ≥ 30 s (CLD 88 Exhalyzer; EcoPhysics, Duernten, Switzerland).

Nasal brush biopsy

Samples, obtained from the inferior turbinate using a cytology brush (Microvasive, Milford, MA, USA), were suspended in 2 ml of Medium 199 fluid cell culture or in 2 ml of normal saline (2 samples), for immediate light-microscopic studies, transmission electron microscopy (TEM) evaluation, and NOS 2 and NOS 3 gene expression measurements. Ciliary motion analysis, ultrastructural assessment, and NOS 2 and NOS 3 mRNA measurements were performed by independent, blinded operators**.**

Ciliary motion analysis and ultrastructural studies

Samples for ciliary motion analysis were kept at 37°C and immediately transferred to a variable-thickness culture chamber. Ciliary morphology, motion pattern and beat frequency were quantitatively evaluated according to standardised methodology [3, 4]. Samples for ultrastructural studies were also prepared according to standardised methodology [5]. Cilia were studied by TEM at a final magnification of median 157,000 IQR 17,000. A mean of 14 ciliated cells and 120

transversely sectioned cilia were examined for each specimen. Quantitative analysis of ultrastructural alterations (expressed as per cent of observed abnormalities) was performed for the absence or shortening of dynein arms and for gross abnormalities in the central apparatus (central pairs/nexin links/radial spokes) [4]. A high proportion of these abnormalities, in association with specific motion patterns, a significant reduction in ciliary beat frequency, and a progressive worsening of respiratory disease were considered diagnostic of PCD. Other ciliary abnormalities were believed to be secondary to chronic inflammation and compatible with the diagnosis of SCD. In atypical cases, the diagnosis of PCD was confirmed by ciliary activity evaluation after ciliogenesis in culture [6].

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