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► To cite this version:

Jeanne-Marie Perotin, Christelle Coraux, Eymeric Lagonotte, Philippe L. Birembaut, Gonzague Delepine, et al.. Alteration of primary cilia in COPD. European Respiratory Journal, 2018, 52 (1), pp.1800122. 10.1183/13993003.00122-2018 . hal-02431953

HAL Id: hal-02431953

<https://hal.univ-reims.fr/hal-02431953>

Submitted on 20 May 2020

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Alteration of primary cilia in chronic obstructive pulmonary disease

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Key words: Airway epithelial cells; Chronic Obstructive Respiratory Disease; Cilia; Histopathology

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1 To the Editor,

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3 Chronic obstructive pulmonary disease (COPD) is a major economic and social concern

4 worldwide because of its impact on mortality and morbidity [1]. COPD is characterized by

5 airway epithelium remodelling, a hallmark of dysregulated airway epithelium plasticity [2].

6 There are currently no available therapeutics to restore the integrity and functionality of the

7 epithelium. Therefore, novel sources of investigation are becoming crucial to understand the

8 alterations at the root of COPD initiation. Non-motile primary cilium (PC) is a solitary sensor

9 organelle playing a critical role in cell cycle control, proliferation, polarity and differentiation,

10 particularly of ciliated cells possessing motile cilia [3-4]. PC are assembled on different types

11 of human cells depending on their state and activities in response to cellular quiescence where

12 they relay extracellular signals and retract upon cell cycle re-entry [5]. Alterations of PC

13 structure and function are responsible for ciliopathies [6-7]. PC may be crucial in determining

14 outcomes during airway epithelial cell differentiation thus we hypothesized that PC are

15 present in adult epithelial cells and may play a key role in airway plasticity. First, we

16 investigated the presence and localization of PC in the bronchial epithelium. Secondly, we

17 analysed the relationships between PC and clinical, functional and histological characteristics

18 of non-COPD and COPD patients.

19 Patients scheduled for lung resection for cancer (University Hospital of Reims, France) were

20 prospectively recruited following standards approved by the institutional review board (IRB

21 Reims-CHU-20110612). Informed consent was obtained from all the patients. Clinical

22 assessment and pulmonary function tests were performed. Emphysema quantification on the

23 resected lobe was performed visually on thoracic CT-scan by two independent investigators

24 as previously described [8-9]. Formalin-fixed paraffin-embedded (FFPE) lung tissues distant

25 from the tumour were stained with hematoxylin and eosin for bronchial epithelium analysis.

26 Immunofluorescence was performed on FFPE lung tissues with the following primary

27 antibodies: anti-Arl13b (ProteinTech, 1:200); anti- γ -tubulin (Sigma, 1:200); anti-acetylated- α -

28 tubulin (Sigma, 1:1000), anti-GT335 (AdipoGen 1:500), and anti-p63 (R&D systems, 1:100).

29 Images were taken by Confocal Zeiss LSM710 microscope (63xDIC/1.40oil). Primary cilia

30 were analysed on 10 random fields per stained slide. Differences between groups were

31 determined using the Student *t* test or Fisher exact test and associations between variables

32 were analysed using the Spearman rank correlation test. A p-value <0.05 was considered

33 significant.

Thirty-six patients were included, 19 COPD patients (GOLD 1 n=5, GOLD 2 n=12, GOLD 3-4 n=2; aged 66 years [59-73]; FEV₁: 71%[62-81]), and 17 non-COPD patients (aged 69 years [66-76]; FEV₁: 89%[83-102]). CT emphysema score for the resected lobe was significantly higher in COPD group compared to non-COPD group (0[0-0] vs 1[0-2], p<0.0001).

Cilia were identified and localized on epithelia with a specific staining for the cilia axoneme and membrane (GTPase Arl13b) and the basal body/centrosome (γ -tubulin) (Figure 1a). Motile cilia were readily identified at the surface of epithelial cells pointing towards the lumen, while solitary cilia (PC) were found on non-differentiated cells of the pseudostratified epithelium as seen during mouse lung development [10]. We confirmed the identification of PC with two additional and well characterized markers of the axoneme (acetylated α -tubulin and GT335) [11] (Figure 1a and data not shown).

Since it has been suggested that PC were absent from adult lung tissues [10] while they are present on other human cells in resting phase [12], we considered to distinguish between histologically “normal” epithelia and “remodelled” epithelia [13]. A normal epithelium was defined as a pseudostratified epithelium (i) presenting the three main cell types (basal, ciliated and goblet cells), (ii) lacking hyperplasia or metaplasia (iii) and showing at least 50% of ciliated cells at the surface.

We analysed bronchial epithelium in non-COPD and COPD groups (Figure 1b). Taking the epithelium as a whole, COPD patients presented a tremendous increase in PC numbers compared to non-COPD patients (68.1[52.2-88.2] vs 9.5[6.1-18.3], p<0.0001) (Figure 1c). Interestingly, the normal epithelium of COPD patients showed an increase in PC numbers compared to non-COPD patients (56.5[48.2-67.1] vs 6.2[2.9-19.5], p<0.0001) and this increase was more pronounced in remodelled areas (80.7[51.1-109.4] vs 10.7[7.9-14.8], p<0.0001). It was particularly striking in basal cell hyperplasia (116.9[91.2-188.2] PC/mm of epithelium in COPD patients vs 15.5[8.9-32.5] in non-COPD patients, p<0.0001). Except for one subject, a number of 40 PC/mm in normal epithelia and 50 PC/mm in remodelling epithelia were identified as cut-off between COPD and non-COPD status (r=0.9215, p<0.0001) (Figure 1d). Interestingly, a significant increase of PC was associated with smoking status (p=0.01) but not with smoking history (p=0.08), respiratory symptoms including dyspnea score (p=0.0003) and chronic bronchitis (p=0.04), the severity of airway obstruction (FEV₁: p=0.002 and FEV₁/FVC: p=8.7 10⁻⁷) and the presence (p=0.008) and the severity of emphysema (p=0.0009). Each of these clinical or functional characteristics of patients coupled with the analysis of PC sat apart patients with a COPD as for example the

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1 FEV₁ (%) (Figure 1e, the differences between elevations of linear regressions were extremely
2 significant for each parameter in non-COPD vs COPD groups, p<0.0001).
3 Localization of PC in the lung is consistent with their known functions including acquisition
4 of polarity, migration, differentiation and cell cycle control [14]. PC were rare in epithelia
5 from non-COPD patients suggesting that either PC did not stabilize long enough to be
6 observed or only a few cells were quiescent. The increase of PC among COPD patients may
7 pave the way to a novel understanding of cell plasticity in the context of this disease: (i) if PC
8 are cell cycle progression indicators, are the basal cells no longer able to divide to renew the
9 epithelium? (ii) Is the onset of COPD responsible for the apparition of PC or vice versa? (iii)
10 Can an increase in PC be considered as a marker of abnormal and dysfunctional bronchial
11 epithelium that would be involved in the development of airway obstruction and/or
12 emphysema, especially regarding to airway regeneration anomalies?
13 Interestingly, PC numbers were altered between a normal and a remodelled area indicating
14 that PC may appear during the renewal or the repair of the epithelium [10]. This is also
15 consistent with the known role of PC in cell migration and tissue homeostasis [15].
16 Abnormalities of bronchial epithelial wound closure have been shown in severe COPD, and
17 were associated with the severity of airway obstruction and emphysema [8]. Likewise,
18 anomalies of PC could be involved in this phenomenon.
19 Some limitations must be pointed out in our study. Despite the novelty of the findings, these
20 analyses are exploratory and conducted in a monocentric study including a relatively low
21 number of patients. Thus sample selection and cohort's size represent biases, as well as the
22 lack of "normal controls" which cannot be circumvent in studies conducted from lung
23 resection tissues. Moreover, further investigations are clearly needed to precise the underlying
24 mechanisms of PC alteration.
25 In conclusion, we have shown an increase of PC on bronchial epithelia associated with
26 clinical, morphological, functional and histological parameters defining COPD. As the
27 presence of PC correlates with clinical features representative of COPD, understanding the
28 dysregulation of PC expression and function would provide additional clues in this complex
29 pathology where the role of the epithelium appears increasingly important. To our knowledge,
30 it is the first observation of non-motile PC in human adult airway epithelia. Whether an
31 accumulation of primary ciliated cells is a disease-driving process or a consequence of
32 epithelium alterations will require further investigations.

Competing interests: None declared

FIGURE LEGENDS

Figure 1: Primary cilia are present in adult bronchial epithelia and altered in COPD patients.

a) Examples of maximum intensity z-stack projection showing the presence of PC on confocal acquisition of the bronchial epithelia for two smoker COPD patients: DIC (grey), Arl13b (red) and γ -tubulin (up, green) or acetylated tubulin (down, green). Dashed lines indicate basal lamina. Boxed areas are shown as magnifications. Nuclei are stained with DAPI (blue).

b) Representative maximum intensity z-stack projections comparing PC on non-remodelled and remodelled areas for non-COPD and COPD patients on confocal acquisition for DIC (grey), Arl13b (red) and p63 (green). Arrowheads show PC localization. Dashed lines indicate basal lamina. Boxed areas are shown as magnifications. Nuclei are stained with DAPI (blue).

c) Box and whiskers plot showing means with IQR of the numbers of primary cilia per epithelium types in non-COPD patients and COPD patients on the epithelium as a whole (total), normal epithelium (normal), remodelled areas (remodelling), basal cell hyperplasia (BCH), mucous cell hyperplasia (MCH) and non-differentiated epithelium (NDE).

d) Graph depicting the repartition of non-COPD (white dots) and COPD (grey dots) patients according to the number of primary cilia per mm of normal (x axis) and remodelled (y axis) epithelium;

e) Graphs depicting the repartition of non-COPD (white dots) and COPD (grey dots) patients according to FEV₁ (%) (x axis) and the number of primary cilia per mm of epithelium (y axis) as a whole (left graph), normal (middle graph) or remodelled (right graph) areas. Lines indicate linear regressions.

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1. Rabe KF, Watz H. Chronic obstructive pulmonary disease. *Lancet*. 2017 May 13;389(10082):1931-1940.
2. Sohal SS. Epithelial and endothelial cell plasticity in chronic obstructive pulmonary disease (COPD). *Respir Investig*. 2017 Mar;55(2):104-113.
3. Fu J, Hagan IM, Glover DM. The centrosome and its duplication cycle. *Cold Spring Harb Perspect Biol*, 2015, 2;7(2):a015800.
4. Malicki JJ, Johnson CA. The Cilium: Cellular Antenna and Central Processing Unit. *Trends Cell Biol*. 2017 Feb;27(2):126-140.
5. Goto H, Inaba H, Inagaki M. Mechanisms of ciliogenesis suppression in dividing cells. *Cell Mol Life Sci*. 2017 Mar;74(5):881-890.
6. Mitchison HM, Valente EM. Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol*. 2017 Jan;241(2):294-309.
7. Braun DA, Hildebrandt F. Ciliopathies. *Cold Spring Harb Perspect Biol*. 2017 Mar 1;9(3).
8. Perotin JM, Adam D, Vella-Boucaud J, et al. Delay of airway epithelial wound repair in COPD is associated with airflow obstruction severity. *Respir Res*. 2014 Nov 27;15:151.
9. Washko GR, Criner GJ, Mohsenifar Z, et al. Computed tomographic-based quantification of emphysema and correlation to pulmonary function and mechanics. *COPD*. 2008 Jun;5(3):177-86.
10. Jain R, Pan J, Driscoll JA, et al. Temporal relationship between primary and motile ciliogenesis in airway epithelial cells. *Am J Respir Cell Mol Biol*, 2010 Dec, 43(6):731-9.
11. Hua K, Ferland RJ. Fixation methods can differentially affect ciliary protein immunolabeling. *Cilia*. 2017 Mar 24;6:5.
12. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol*. 2007 Nov;8(11):880-93.
13. Hirota N, Martin JG. Mechanisms of airway remodeling. *Chest*. 2013 Sep;144(3):1026-32.
14. Fry AM, Leaper MJ, Bayliss R. The primary cilium: guardian of organ development and homeostasis. *Organogenesis*. 2014 Jan 1;10(1):62-8.

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15. Veland IR, Lindbæk L, Christensen ST. Linking the Primary Cilium to Cell
Migration in Tissue Repair and Brain Development. *Bioscience*. 2014 Dec
1;64(12):1115-1125.

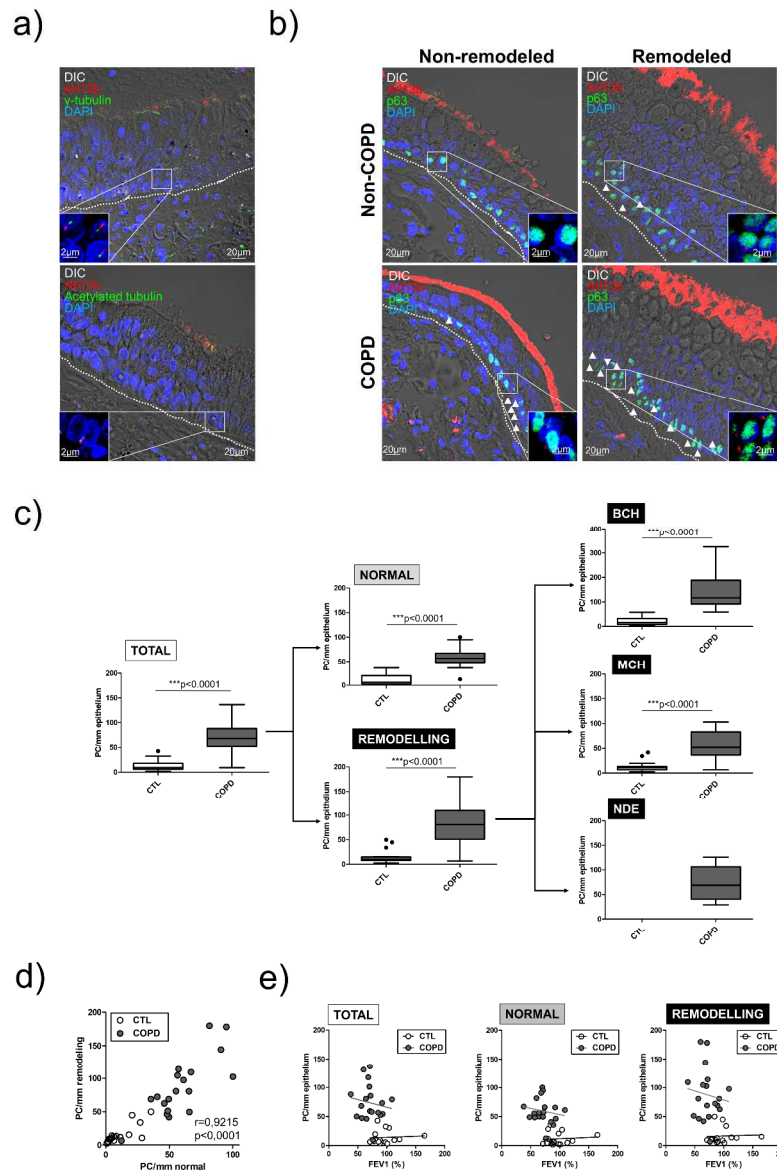
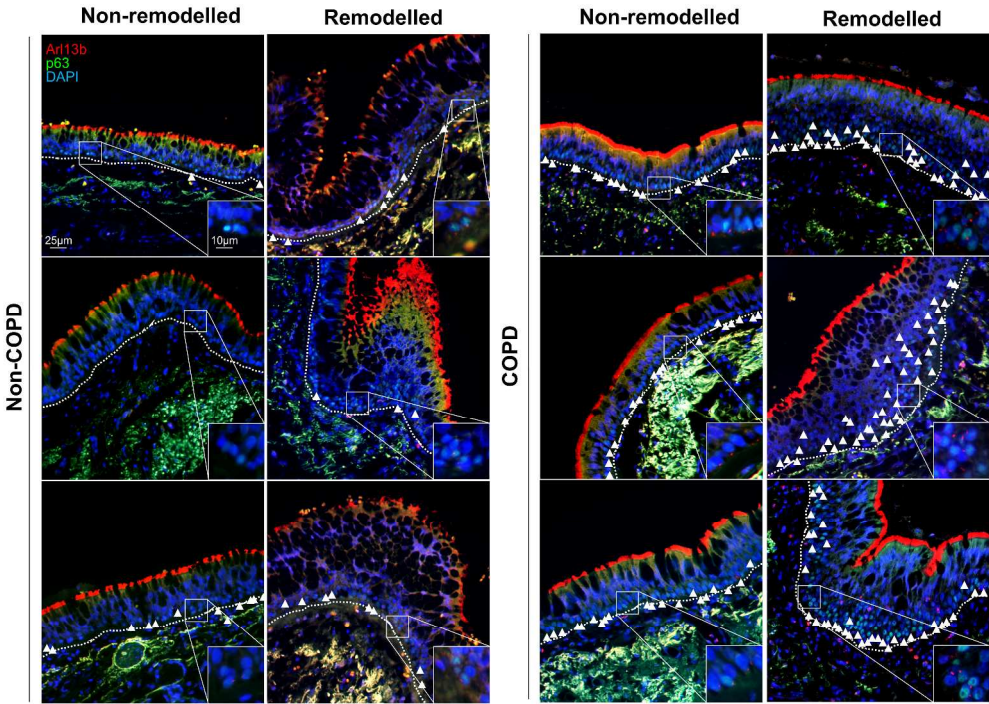


Figure 1: Primary cilia are present in adult bronchial epithelia and altered in COPD patients.

408x615mm (300 x 300 DPI)



450x329mm (300 x 300 DPI)