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3 **Alteration of primary cilia in chronic obstructive pulmonary disease**
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39 23 **Key words:** Airway epithelial cells; Chronic Obstructive Respiratory Disease; Cilia;
40 24 Histopathology
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3 1 To the Editor,
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6 3 Chronic obstructive pulmonary disease (COPD) is a major economic and social concern
7 4 worldwide because of its impact on mortality and morbidity [1]. COPD is characterized by
8 5 airway epithelium remodelling, a hallmark of dysregulated airway epithelium plasticity [2].
9 6 There are currently no available therapeutics to restore the integrity and functionality of the
10 7 epithelium. Therefore, novel sources of investigation are becoming crucial to understand the
11 8 alterations at the root of COPD initiation. Non-motile primary cilium (PC) is a solitary sensor
12 9 organelle playing a critical role in cell cycle control, proliferation, polarity and differentiation,
13 10 particularly of ciliated cells possessing motile cilia [3-4]. PC are assembled on different types
14 11 of human cells depending on their state and activities in response to cellular quiescence where
15 12 they relay extracellular signals and retract upon cell cycle re-entry [5]. Alterations of PC
16 13 structure and function are responsible for ciliopathies [6-7]. PC may be crucial in determining
17 14 outcomes during airway epithelial cell differentiation thus we hypothesized that PC are
18 15 present in adult epithelial cells and may play a key role in airway plasticity. First, we
19 16 investigated the presence and localization of PC in the bronchial epithelium. Secondly, we
20 17 analysed the relationships between PC and clinical, functional and histological characteristics
21 18 of non-COPD and COPD patients.

22 19 Patients scheduled for lung resection for cancer (University Hospital of Reims, France) were
23 20 prospectively recruited following standards approved by the institutional review board (IRB
24 21 Reims-CHU-20110612). Informed consent was obtained from all the patients. Clinical
25 22 assessment and pulmonary function tests were performed. Emphysema quantification on the
26 23 resected lobe was performed visually on thoracic CT-scan by two independent investigators
27 24 as previously described [8-9]. Formalin-fixed paraffin-embedded (FFPE) lung tissues distant
28 25 from the tumour were stained with hematoxylin and eosin for bronchial epithelium analysis.
29 26 Immunofluorescence was performed on FFPE lung tissues with the following primary
30 27 antibodies: anti-Arl13b (ProteinTech, 1:200); anti- γ -tubulin (Sigma, 1:200); anti-acetylated- α -
31 28 tubulin (Sigma, 1:1000), anti-GT335 (AdipoGen 1:500), and anti-p63 (R&D systems, 1:100).
32 29 Images were taken by Confocal Zeiss LSM710 microscope (63xDIC/1.40oil). Primary cilia
33 30 were analysed on 10 random fields per stained slide. Differences between groups were
34 31 determined using the Student *t* test or Fisher exact test and associations between variables
35 32 were analysed using the Spearman rank correlation test. A p-value <0.05 was considered
36 33 significant.

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

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

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

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

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3 1 FEV₁ (%) (Figure 1e, the differences between elevations of linear regressions were extremely
4 2 significant for each parameter in non-COPD vs COPD groups, p<0.0001).

5 3 Localization of PC in the lung is consistent with their known functions including acquisition
6 4 of polarity, migration, differentiation and cell cycle control [14]. PC were rare in epithelia
7 5 from non-COPD patients suggesting that either PC did not stabilize long enough to be
8 6 observed or only a few cells were quiescent. The increase of PC among COPD patients may
9 7 pave the way to a novel understanding of cell plasticity in the context of this disease: (i) if PC
10 8 are cell cycle progression indicators, are the basal cells no longer able to divide to renew the
11 9 epithelium? (ii) Is the onset of COPD responsible for the apparition of PC or vice versa? (iii)
12 10 Can an increase in PC be considered as a marker of abnormal and dysfunctional bronchial
13 11 epithelium that would be involved in the development of airway obstruction and/or
14 12 emphysema, especially regarding to airway regeneration anomalies?

15 13 Interestingly, PC numbers were altered between a normal and a remodelled area indicating
16 14 that PC may appear during the renewal or the repair of the epithelium [10]. This is also
17 15 consistent with the known role of PC in cell migration and tissue homeostasis [15].
18 16 Abnormalities of bronchial epithelial wound closure have been shown in severe COPD, and
19 17 were associated with the severity of airway obstruction and emphysema [8]. Likewise,
20 18 anomalies of PC could be involved in this phenomenon.

21 19 Some limitations must be pointed out in our study. Despite the novelty of the findings, these
22 20 analyses are exploratory and conducted in a monocentric study including a relatively low
23 21 number of patients. Thus sample selection and cohort's size represent biases, as well as the
24 22 lack of "normal controls" which cannot be circumvent in studies conducted from lung
25 23 resection tissues. Moreover, further investigations are clearly needed to precise the underlying
26 24 mechanisms of PC alteration.

27 25 In conclusion, we have shown an increase of PC on bronchial epithelia associated with
28 26 clinical, morphological, functional and histological parameters defining COPD. As the
29 27 presence of PC correlates with clinical features representative of COPD, understanding the
30 28 dysregulation of PC expression and function would provide additional clues in this complex
31 29 pathology where the role of the epithelium appears increasingly important. To our knowledge,
32 30 it is the first observation of non-motile PC in human adult airway epithelia. Whether an
33 31 accumulation of primary ciliated cells is a disease-driving process or a consequence of
34 32 epithelium alterations will require further investigations.

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3 **1 FOOTNOTES**
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6 **3 Contributors:** Study concept: VD; study design: JMP and VD; acquisition data: JMP, EL,
7 GoD, GaD and VD; analysis and data interpretation: JMP, GaD, MP and VD; revision of
8 manuscript: CC, PB, MP and GaD; manuscript writing: JMP and VD
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10 **6**

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14 **9**

15 **10 Competing interests:** None declared
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3 **1 FIGURE LEGENDS**

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5 **2 Figure 1: Primary cilia are present in adult bronchial epithelia and altered in COPD**
6 **3 patients.**

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9 4 a) Examples of maximum intensity z-stack projection showing the presence of PC on
10 5 confocal acquisition of the bronchial epithelia for two smoker COPD patients: DIC (grey),
11 6 Arl13b (red) and γ -tubulin (up, green) or acetylated tubulin (down, green). Dashed lines
12 7 indicate basal lamina. Boxed areas are shown as magnifications. Nuclei are stained with
13 8 DAPI (blue).

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17 9 b) Representative maximum intensity z-stack projections comparing PC on non-remodelled
18 10 and remodelled areas for non-COPD and COPD patients on confocal acquisition for DIC
19 11 (grey), Arl13b (red) and p63 (green). Arrowheads show PC localization. Dashed lines indicate
20 12 basal lamina. Boxed areas are shown as magnifications. Nuclei are stained with DAPI (blue).

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25 13 c) Box and whiskers plot showing means with IQR of the numbers of primary cilia per
26 14 epithelium types in non-COPD patients and COPD patients on the epithelium as a whole
27 15 (total), normal epithelium (normal), remodelled areas (remodelling), basal cell hyperplasia
28 16 (BCH), mucous cell hyperplasia (MCH) and non-differentiated epithelium (NDE).

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32 17 d) Graph depicting the repartition of non-COPD (white dots) and COPD (grey dots) patients
33 18 according to the number of primary cilia per mm of normal (x axis) and remodelled (y axis)
34 19 epithelium;

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37 20 e) Graphs depicting the repartition of non-COPD (white dots) and COPD (grey dots) patients
38 21 according to FEV₁ (%) (x axis) and the number of primary cilia per mm of epithelium (y axis)
39 22 as a whole (left graph), normal (middle graph) or remodelled (right graph) areas. Lines
40 23 indicate linear regressions.

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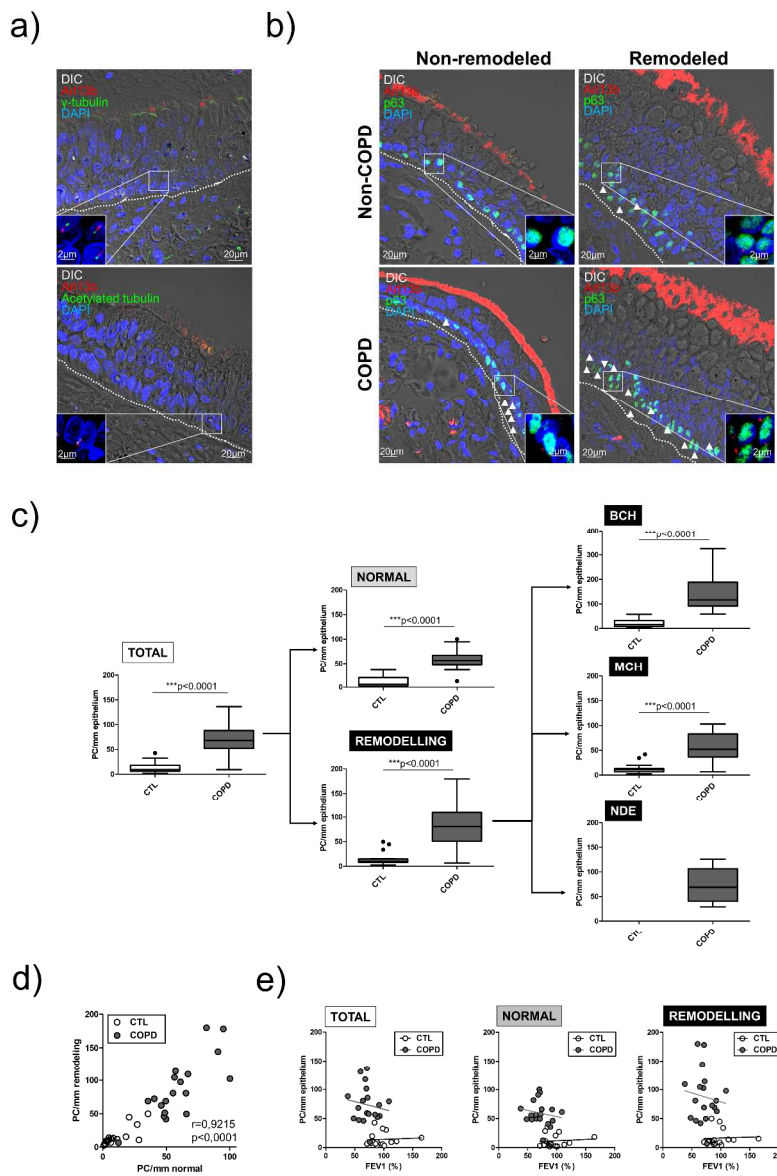
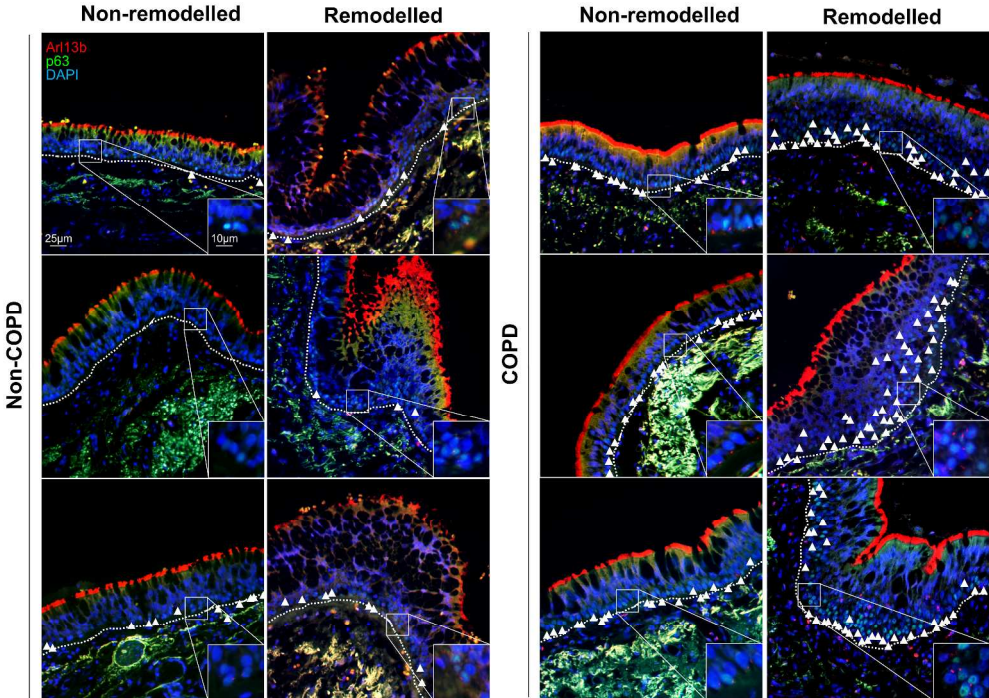


Figure 1: Primary cilia are present in adult bronchial epithelia and altered in COPD patients.

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