Interaction between the promoter *MUC5B* polymorphism and mucin expression: is there a difference according to ILD subtype?

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ABSTRACT

The *MUC5B* promoter variant rs35705950 is associated with idiopathic pulmonary fibrosis (IPF). MUC5B glycoprotein is overexpressed in IPF lungs. We examined immunohistochemical expression of MUC5B in different interstitial lung disease patterns according to rs35705950 T-allele carriage. We observed increased expression of MUC5B in T-allele carriers in both distal airways and honeycomb cysts in patients with IPF (n=23), but no difference in MUC5B expression according to T-carrier status in the distal airways of patients with idiopathic non-specific interstitial pneumonitis (n=17), in scleroderma-associated non-specific interstitial pneumonitis (n=15) or in control lungs (n=20), suggesting that tissue overexpression in *MUC5B* rs35705950 T-carriers is specific to IPF.

The *MUC5B* promoter variant rs35705950 is the common genetic variant which confers the greatest risk for idiopathic pulmonary fibrosis (IPF) and may also be associated with a less rapid disease course,[1 2] although this is disputed.[3] How *MUC5B* overexpression affects development of fibrosis remains poorly understood. We previously described overexpression of MUC5B in the small airways and honeycomb cysts of patients with IPF compared with controls. We sought to establish whether the overexpression seen in IPF was linked to the *MUC5B* allele and whether differences in the relationship between allele and lung MUC5B expression were observed among different interstitial lung disease (ILD) patterns.

The current study uses MUC5B immunohistochemical expression data, analysed as previously described.[4] Briefly, quantification of MUC5B expression was evaluated in available sequential histology blocks from 2003 to 2010 of surgical lung biopsies from 23 patients with IPF/usual interstitial pneumonia (UIP), 17 with idiopathic non-specific interstitial pneumonitis (I-NSIP), 15 with NSIP-associated with scleroderma (NSIP-SSc) and 20 normal lung tissue peripheral to resected cancer. In each biopsy, three distal airways were evaluated, and in UIP, three honeycomb cysts were evaluated. In each area, quantification of the proportion of MUC5B+ cells was evaluated in six randomly selected fields, each containing 100 airway epithelial cells (or honeycomb cysts).[4] DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue using the QIAamp DNA FFPE tissue kit (Qiagen). Genotyping was performed using a commercially available TaqMan assay (Applied Biosystems).

Since very few TT genotypes were observed, comparison of the genotype distribution between disease groups was performed by logistic regression using the binary variable *MUC5B* rs35705950 T-allele carriage (hereafter T carrier) as the dependent variable, and expressed as OR±SE. To compare MUC5B immunohistochemical expression in the airways between groups, multilevel mixed-effects linear regression was performed, in which patients were analysed as random effect variables, microscopic fields nested into patients, and diagnostic group, and their interaction as fixed effect variables. Results are expressed as mean % MUC5B+ cells±SE. The difference in the expression of MUC5B between airways and honeycomb cysts was evaluated only in patients with IPF with a similar model, including zone (airways or cysts), T-carrier status and their interaction as fixed-effect variables. Analyses of differences between pairs of groups, using reduced models, were only performed when a significant difference was observed in the complete model. The Impact of the T-carrier and the percentage of MUC5B+ cells on the Composite Physiological Index (CPI=91.0−(0.65×DLCO%predicted)−(0.53×FVC%)+(0.34×FEV1%)) as a measure of disease severity[5] was analysed using generalised linear models with CPI as the dependent variable and T-carrier and/or mean percentage of MUC5B+ cells per patient as independent variables. As the distribution of CPI was normal with no significant skewness or kurtosis, a Gaussian distribution was assumed. A p value of ≤0.05 was considered significant (STATA V.16 for Windows).

MUC5B rs35705950 genotype distribution was as follows IPF: GG=8, GT=13, TT=2; I-NSIP: GG=12, GT=4, TT=1; NSIP-SSc: GG=13, GT=2, TT=0; controls: GG=14, GT=6, TT=0. Allele carriage revealed, as expected, a significantly higher proportion of T-carriers in IPF patients compared with controls (OR: 4.4±2.9, p=0.024) but no difference between the other groups and controls (I-NSIP: OR: 0.97±0.70,

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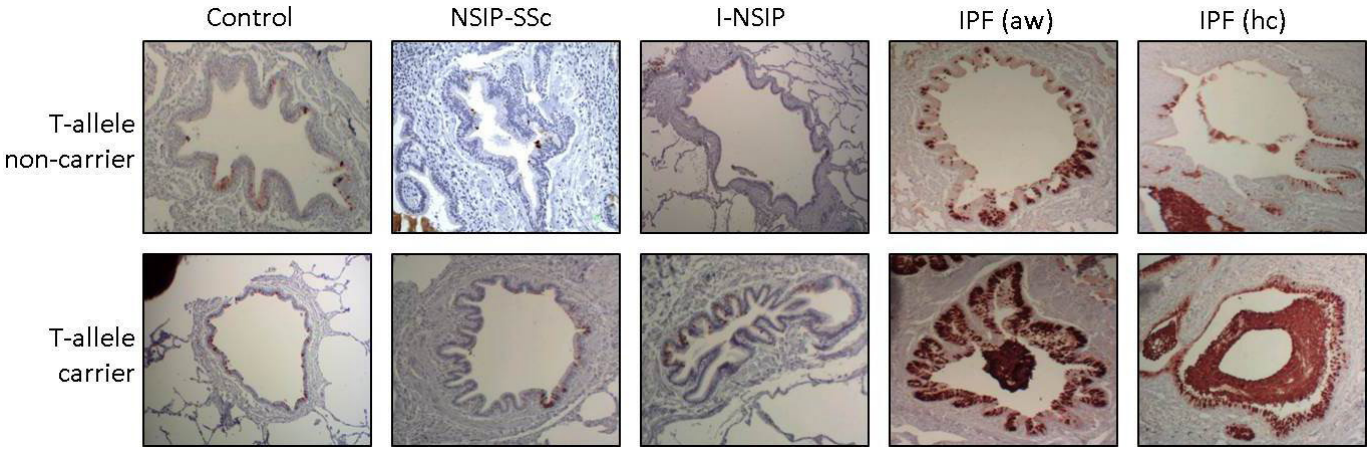

Figure 1 Photomicrographs showing mucin MUC5B immunohistochemical staining in the distal aw of rs35705950 T-allele non-carriers and T-allele carriers in control lungs, NSIP-SSc, I-NSIP and IPF. In IPF, both distal aw and hc were evaluated. MUC5B is stained in brown and counterstained with haematoxylin (×100 magnification). aw, airways; hc, honeycomb cysts; I-NSIP, idiopathic non-specific interstitial pneumonitis; IPF, idiopathic pulmonary fibrosis; NSIP-SSc, non-specific interstitial pneumonitis-associated with scleroderma.

p=0.97; NSIP-SSc: OR: 0.36±0.32, p=0.2). To assess the effect of T-carrier on MUC5B tissue expression in the different diagnostic groups (figure 1), the differences in the mean percentage of MUC5B+ cells in the distal airways of the four groups (and for IPF also honeycomb cysts) according to T-carrier status were assessed. A significantly increased expression of MUC5B in distal airways compared with controls was observed only in patients with IPF, regardless of T-carrier positivity (figure 2). Furthermore, an increase in MUC5B expression in distal airways was associated with T-carrier positivity only in IPF (difference in MUC5B expression: 12.5%±5.3%, p=0.02 vs T-carrier negative), resulting in a greater difference with controls in T-carrier-positive than in T-carrier-negative

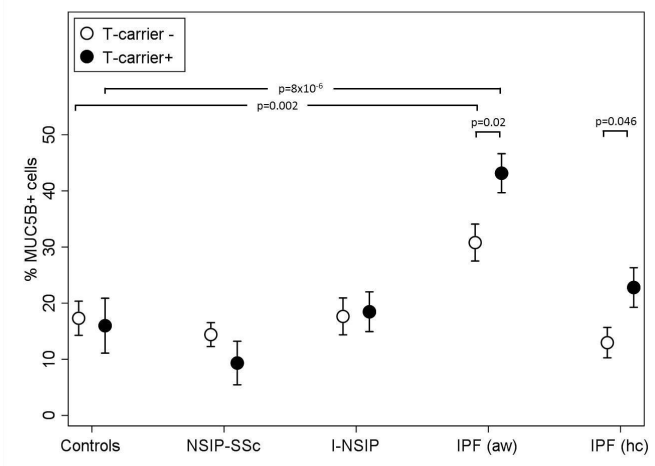

Figure 2 Comparison of distal airway MUC5B expression between control lungs, NSIP-SSc, I-NSIP and IPF. In IPF, both distal aw and hc were evaluated. MUC5B expression was significantly higher in IPF distal aw, but not in hc compared with controls, while no difference was observed between either I-NSIP or SSc-NSIP and controls. T-allele carriage was associated with signifcantly higher MUC5B expression both in IPF aw and hc, while no difference was observed in the other groups (Scheffe's post hoc analysis). aw, airways; hc, honeycomb cysts; I-NSIP, idiopathic non-specific interstitial pneumonitis; IPF, idiopathic pulmonary fibrosis; NSIP-SSc, non-specific interstitial pneumonitis-associated with scleroderma.

patients ($p=8\times10^{-6}$ between T-carrier positive, and p=0.002 between T-carrier-negative patients and controls). An increased expression according to T-carrier positivity in patients with IPF was also seen in honeycomb cysts (difference in % MUC5B+ cells: 9.8%±4.9% p=0.047). There was instead no significant difference in percentage MUC5B+ cells according to T-carrier status, in I-NSIP (difference: 0.4%±5.3%, p=0.94), NSIP-SSc (difference: −5.1±6.5%, p=0.43) or controls (difference: −1.3±5.8%, p=0.82).

We then focused on assessing the relationship of MUC5B and T-carrier status with disease severity in patients with IPF. The CPI score was higher (more severe disease) in patients with T-carrier-negative IPF (mean±SE: 48.1±2.8), compared with patients with T-carrier-positive IPF (38.2±2.7, p<0.001). Similarly, FVC % predicted was significantly lower in patients with T-carrier-negative IPF (64%±4%) than patients with T-carrier-positive IPF (89±7, p=0.009), and a similar trend, bordering on significance, was observed for DLCO (T-carrier negative: 47±3% vs 54±2%, p=0.059). The CPI score was inversely correlated to the percentage of MUC5B+ cells (p=0.011). When both variables were included in the multivariable model, only the T-carrier effect remained significant (p=0.005), while the effect of the MUC5B+ cells was no longer significant (p=0.08).

We conclude that *MUC5B* rs35705950 T-carrier status is associated with increased expression of MUC5B, but only in IPF. We also find that the increase in MUC5B+ cells in IPF is not solely related to the higher frequency of T-carriers but also occurs independently of allele carriage, as described for mRNA expression by Seibold *et al*.[1] Interestingly, Seibold *et al* reported significantly higher mRNA expression in T-carriers in both control and IPF lungs. Nakano *et al* reported a strong correlation between MUC5B promoter activity and MUC5B+ epithelial cells in IPF lungs but had not investigated other ILD patterns.[6] An explanation for the specificity of the increased MUC5B staining in T carriers observed only for IPF lungs in this study will require further investigation. Downstream 32 bp of rs35705950 is a highly conserved FOXA2 binding motif. This region is hypermethylated in the presence of IPF, increased lung tissue MUC5B expression and the rs35705950 risk allele. This hypermethylation may result in increased occupancy of FOXA2 in the binding motif, leading to increased MUC5B expression.[7]

Although analysis of mRNA expression would have been desirable, mRNA in FFPE tissue can be heavily degraded, resulting in severe limitations in the reliability of the relative mRNA expression in these samples.[8–10] Fresh frozen samples, known to provide a better preservation of mRNA, were not available. The *MUC5B* rs35705950 T allele also seems to be associated with less severe disease. Although this may be related to a separate mechanism than increased MUC5B expression, further larger studies are needed to confirm this finding. Further research is also needed to assess relative contributions of the rs35705950 T-allele and MUC5B glycoprotein expression in relation to IPF severity, and to explore the mechanisms underlying this association, if confirmed. Our study did not have sufficient power to reliably assess links between the T-allele and MUC5B tissue expression and prognosis.

In conclusion, we find that a positive relationship between the *MUC5B* risk allele and expression of MUC5B glycoprotein in the lungs is specific to IPF. Further studies are needed to confirm this observation and to verify whether it is also observed in non-idiopathic UIP, including rheumatoid arthritis and fibrotic hypersensitivity pneumonitis-associated UIP.

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