Abstract S17 Table 1. Cox regression modelling of clotting defects and survival in IPF

Clotting defect	Number of deaths	Person-years	Mortality rate per 1000 person years (95% CI)	Hazard Ratio (95% CI)*	Hazard Ratio (95% CI)#	p value
No clotting defects	8	86.7	92.3 (46.2–184.6)	1.00	1.00	0.0013
Prothrombotic state	78	298.9	257.6 (206.1–322.1)	2.85 (1.37–5.93)	2.77 (1.28–5.99)	0.0039

*Hazard Ratioadjusted for age and sex
#Hazard Ratio adjusted for age, sex, smoking habit,hsCRP, baseline FVC and Dlco

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Background Our previous case-control study demonstrated that a prothrombotic state was almost five times more common in people with idiopathic pulmonary fibrosis (IPF) than general population controls. We followed up the incident cases of IPF to determine if a prothrombotic state altered prognosis in terms of survival.

Methods People with IPF were recruited from five teaching and eight district general hospitals in England and Wales. All participants were asked for details of medical history, medication and smoking habit. Venous blood samples were taken to test for inherited and acquired clotting defects. We also collected high resolution computed tomography (HRCT) scans and pulmonary function tests done as part of routine care. All HRCT scans were reviewed by two thoracic radiologists to confirm the diagnosis of IPF. A prothrombotic state was defined as having at least one of the inherited or acquired clotting defects tested. Cox regression modelling was used to investigate the association between a prothrombotic state and survival amongst people with IPF, adjusting for age, sex, highly sensitive C Reactive Protein (hsCRP), smoking habit and baseline pulmonary function indices.

Results There were 211 incident cases of definite or probable IPF with a median follow up of 2 years (IQR: 1.2 to 2.5). During this time, 86 out of the 211 (40.8%) cases died, which equates to an overall mortality rate of 220.5 per 1000 person years (95% CI: 178.2 to 272.7). 78 out of the 86 deaths (90.7%) were in individuals with a prothrombotic state (see Table 1). The proportion of the IPF cohort surviving at 2 years was 84.7% in patients without any clotting defects and 61.1% in patients with a prothrombotic state (p = 0.0035).

Conclusions A prothrombotic state is associated with increased mortality amongst people with IPF. Further research into manipulation of the clotting cascade to improve the outlook of people with IPF is warranted.

Asthma biology

S18 ACTIVATION OF NOCICEPTIN ORPHANIN FQ (N/OFQ) – N/OFQ PEPTIDE (NOP) RECEPTOR SYSTEM PLAYS A KEY IMMUNOMODULATORY ROLE IN ASTHMA

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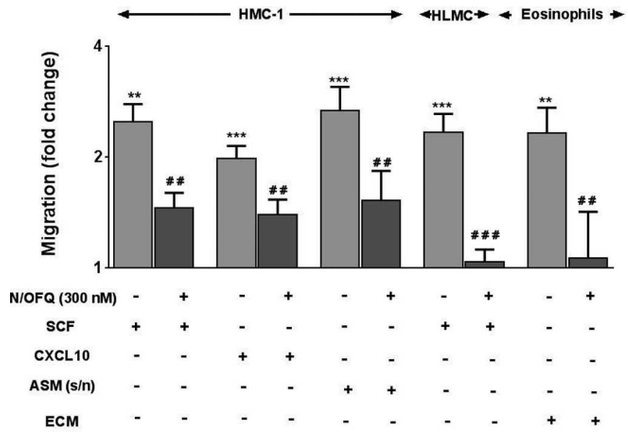
Asthma is a complex heterogeneous disease characterised by variable airflow obstruction, bronchial hyper-responsiveness, airway inflammation and remodelling. The heptadecapeptide nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand for the N/OFQ peptide (NOP) receptor, a non-opioid member of the opioid receptor family. The role of N/OFQ-NOP system in asthma is uncertain. We sought to evaluate N/OFQ-NOP expression in healthy and asthmatic human airway tissues and relate this to an established animal model of asthma.

NOP expression in human airway cells was investigated predominantly by qRT-PCR. The functional role of N/OFQ on human airway structural and immune cells was then interrogated using a range of functional assays including proliferation,migration,collagen gel contraction and wound healing. We further investigated the functional role of N/OFQ *in vivo* using ovalbumin-sensitised mice.

NOP expression was detected in human airway smooth muscle cells (HASM; mean?C? = 11 ± 0.7,n = 13), bronchial epithelial cells (HBEC;mean?C? = 10 ± 0.49,n = 12), lung mast cells (mean?C? = 7 ± 0.64,n = 5) and peripheral blood eosinophils (mean?C? = 10.4 ± 1.2,n = 16). N/OFQ inhibited chemoattractant-induced migration of mast cells and eosinophils (see Figure). N/OFQ stimulated significant HBEC wound closure with 49.62 ± 3.58% (p < 0.001, n = 8) of the wound area



Abstract S18 Figure 1. N/OFQ inhibits chemoattractant-induced migration of lung mast cells and eosinophils. Migration of HMC-l cells (through an 8- m pore-size transwell membrane) was enhanced following stimulation With SCF (p < 0.01, n = 5), CXCL10 (p < 0.001, n = 5) and TNF- stimulated ASM s/n (p < 0.001, n = 8) and this was significantly inhibited by N/OFQ (p < 0.01). HLMC migration induced by SCF (p < 0.001, n = 7) was significantly attenuated by N/OFQ (p < 0.001, n = 7). Migration of peripheral blood eosinophils (n = 6) stimulated by epithelial conditioned media (p < 0.01) was significantly inhibited by N/OFQ (p < 0.01). Cell counts were performed by a blinded observer and data was represented as fold change over control in log (2) scale (mean ± SEM). Statistical analysis was performed by one-Way ANOVA With Bonferroni's multiple comparison test. (*: p value vs control; #: p value vs different relevant stimulation).

healed relative to the control (30.88 ± 4.13%, n = 8)18 h post-wound. Similarly N/OFQ significantly promoted HASM wound closure ($p < 0.01$). Our findings showed that SCF-stimulated IL-8 release (4.43 ± 0.69 fold over control, $p < 0.01$,n = 7) was significantly inhibited by N/OFQ (3.32 ± 0.56 fold over control, $p < 0.01$, n = 7). *Ex vivo* human studies demonstrate significantly ($p < 0.01$) increased endogenous N/OFQ in asthmatic airways relative (sputum N/OFQ:59.02 ± 2.57 pg/ml,n = 26) to healthy airways (sputum N/OFQ:44.69 ± 0.43,n = 10) and identifies eosinophils as a potential source for these. Pre-treatment with N/OFQ was shown to significantly reduce agonist-induced bronchial hyper-responsiveness using *in vitro* ($p < 0.01$) and *in vivo* models ($p < 0.001$). *Ex vivo* animal studies show that N/OFQ significantly inhibits release of inflammatory mediators including IL4, IL5, IL12, IL13 and inflammatory cell recruitment including mast cells and eosinophils within the airways. Further mucus hyper-secretion was also reduced following N/OFQ pre-treatment in these models.

This is the first study to perform a comprehensive and complementary *in vivo* and *in vitro* study of the expression and actions of the N/OFQ-NOP system in the airways and provide evidence for a role of NOP activation in the management of asthma.

S19 INCREASED CRTH2 EXPRESSION IN ASTHMATIC BRONCHIAL EPITHELIUM

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Introduction and Objectives Prostaglandin-D2 (PGD2) mediates chemotaxis of Th2 cells, basophils and eosinophils through the chemoattractant receptor homologous-molecule expressed on T-helper-type-2 cells (CRTh2). Pulmonary PGD2 levels are increased in patients with more severe asthma and higher levels of Th2 inflammation. CRTh2 antagonists, designed to block PDG2 signalling, are in clinical development for the treatment of asthma. We investigated whether pulmonary CRTh2 expression is upregulated in patients with asthma compared to controls; we focused on the bronchial epithelium and cells within the submucosa.

Methods Bronchial biopsies were obtained from asthmatic patients (n = 20) and healthy subjects (n = 10). Sections were probed with an anti-human CRTh2 antibody, a secondary biotinylated goat anti-rabbit antibody and the position of the CRTh2 receptors were visualised with 3,3'-Diaminobenzidine (DAB) and counter-staining with haemotoxylin. Digital micrographs were taken and analysed using the ImageProPlus 6.0 software. The percentage positive area of epithelium was calculated along with the intensity of staining. These values were multiplied together to give an overall immunohistochemical (IHC) score for each subject. The number of positive cells/mm^2 within the submucosa was also counted.

Results CRTh2 expression was predominantly within the epithelium rather than the submucosa. There was a statistically significant (p = 0.04) increase the immunohistochemical score in the epithelium of asthmatics (74.7) compared to healthy non-smokers (38.8). There was no significant difference (p = 0.64) in the number of positive cells expressing CRTh2 within the submucosa between asthma patients (473/mm^2) and healthy controls (353/mm^2).

Conclusion CRTh2 expression is upregulated in the epithelium of asthmatic patients compared to healthy controls. This is a novel finding, suggesting that CRTh2 antagonists may exert therapeutic effects on the bronchial epithelium as well as blocking inflammatory cell chemotaxis in asthma.

Abstract S19 Table 1.

	% +ve Area of Epithelium	Intensity of Epithelial Staining	Overall IHC Score	% Positive Cells in Submucosa/mm2
Asthma	30.3* (12.6)	2.3 (4.4)	74.7* (45.9)	473.1 (509.0)
Healthy	16.6 (16.3)	1.7 (4.1)	38.8 (47.7)	353.3 (296.0

*Statistical analysis by unpaired T-test between Asthma and Healthy controls *p<0.05.*

S20 CRTH2 IS EXPRESSED BY THE BRONCHIAL EPITHELIUM AND ITS ACTIVATION DRIVES EPITHELIAL DIFFERENTIATION

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Background The chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2) is implicated in the pathogenesis of asthma, but its expression in the bronchial epithelium and potential role in airway remodelling is unknown.

Methods CRTh2 protein expression was assessed in bronchial biopsies (n = 24) and primary epithelial cells (n = 16) using immunohistochemistry, and using flow cytometry, immunofluorescence, and quantitative RT-PCR (QT-PCR) respectively. The effects of 13, 14-dihydro-15-keto Prostaglandin D2 (DK-PGD2) on epithelial cell migration and differentiation was determined.

Results The number of submucosal CRTh2 positive inflammatory cells was increased in asthma compared to healthy controls 27.57 per mm^2 sub-mucosal area (9.82) versus 48.17 (14.04) (p = 0.0049). CRTh2 expression was identified on normal and asthmatic epithelial cells, but its expression was decreased in bronchial biopsies from asthmatics 21.43 per 10mm^2 epithelial area (7.85) versus healthy controls 62.34 (36.41) (p = 0.0071) and similar findings were observed in primary epithelial cells. Squamous metaplasia of the bronchial epithelium was increased in asthma and related to decreased CRTh2 expression. DK-PGD2 promoted epithelial cell migration 12-fold increase (p = <0.0001) and in air-liquid interface cultures increased the number of MUC5AC$^+$ and involucrin$^+$ cells, which were blocked with a CRTh2 specific antagonist.

Conclusions CRTh2 is expressed by the bronchial epithelium and its activation drives epithelial differentiation suggesting that in addition to its well characterised role on inflammatory cell migration CRTh2 might contribute to airway remodelling in asthma.

S21 TYPE-2 INNATE LYMPHOID CELLS INDUCE CD4 T HELPER CELL TYPE-2 IMMUNE FUNCTIONS

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Introduction Type-2 innate lymphoid cells (ILC2) are a novel subset of immune cells characterised by their responsiveness to