RESEARCH ARTICLE

IFNΛ3/4 locus polymorphisms and IFNΛ3 circulating levels are associated with COPD severity and outcomes

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Abstract

Background: Interferon lambda (IFNLs) have important anti-viral/bacterial and immunomodulatory functions in the respiratory tract. How do IFNLs impact COPD and its exacerbations?

Methods: Five hundred twenty eight patients were recruited in a prospective observational multicentre cohort (PROMISE) study. The genetic polymorphisms (rs8099917 and rs12979860) within the IFNL3/4 gene region and circulating levels of IFNL3 in COPD patients were determined and associated with disease activity and outcome during a median follow-up of 24 months.

Results: The GG genotype significantly influenced severe exacerbation rate (42 vs. 23%; p = 0.032) and time to severe exacerbation (HR = 2.260; p = 0.012). Compared to the TT or TG genotypes, the GG genotype was associated with severe dyspnoea (modified medical research council score ≥ median 3; 22 vs 42%, p = 0.030). The CC genotype of the rs12979860 SNP was associated with a poorer prognosis (body mass index, airflow obstruction, dyspnea and exercise capacity index ≥ median 4; 46 vs. 36% TC vs. 20.5% TT; p = 0.031). Patients with stable COPD and at exacerbation had significantly lower circulating IFNL3 compared to healthy controls (p < 0.001 and p< 0.001, respectively). Circulating IFNL3 correlated to post-bronchodilator FEV1%predicted and the tissue maturation biomarker Pro-collagen 3.

Conclusion: IFNL3/4 polymorphisms and circulating IFNL3 may be associated with disease activity and outcomes in COPD.

Trial registration: Clinical Trial registration http://www.isrctn.com/ identifier ISRCTN99586989 on 16 April 2008.

Keywords: Interleukin 28B, Cohort, Mortality, Biomarker, Single nucleotide polymorphisms

Background

Interferons (IFN) are known to have important direct anti-viral and anti-bacterial effects, as well as potent modulatory effects on the adaptive immune response via the induction of hundreds of IFN-stimulated genes (ISGs) [1, 2] The newest discovered class of IFN, the IFN lambda (IFNL) family, has four members: IFNL1–4 [3]. The IFNL receptor consists of a heterodimer with an alpha subunit (IL28RA) and a beta subunit (IL10RB). IL10RB is ubiquitously expressed, whereas IL28RA expression is restricted and interestingly, it is highly expressed on lung epithelial cells [4] and alveolar macrophages [5]. When a virus is seen by the pattern recognition receptors which are found on macrophages and epithelial cells, IFNL gene expression is stimulated via various signalling pathways [6]. This leads to increased circulating IFNL3 which interacts with the IFNL receptor expressed on lung, intestinal and liver cells, and via the JAK-STAT signalling cascade induces interferon stimulated genes which in turn influence viral
replication [6]. A series of single nucleotide polymorphisms (SNPs) in the IFNL3/4 gene region have been described [7–9] and associated with variable IFNL3/4 gene expression [10–13]. The variability of IFNL3 during viral or bacterial infections may lead to significant differences in the subsequent immune response and thus variable clinical outcomes [3].

IFNL3 has immune-modulatory and anti-tumorigenic effects and is induced by viral infections [6, 14]. Viral infections play an important role in the exacerbation of asthma [15–17] and COPD [18–20]. Reduced interferon activity during a respiratory syncytial virus infection has been linked to the later development of asthma in children [20, 21]. The mechanism of virus-induced exacerbations of COPD is not well-defined. Recently, the role of IFNLs in the exacerbation of asthma has been explored [22–24]. However, no data are available regarding the effect of IFNL3 or its polymorphisms on the exacerbation and further clinical outcomes of COPD. COPD patients exposed to rhinovirus consistently showed a trend towards less IFNL expression in bronchoalveolar lavage fluid [25] and in animal models it has been shown that IFNL plays a role in viral (influenza A, coronavirus and rotavirus) modulation [6].

We hypothesize, that in patients with COPD, SNPs in the IFNL3/4 gene will impact clinical outcomes such as exacerbation and that they might be associated with circulating markers of inflammation and tissue remodelling. Therefore, we aimed to explore the IFNL3/4 polymorphisms (rs8099917 and rs12979860) and circulating IFNL3 in association with the occurrence of exacerbation of COPD and all-cause mortality in a multinational, multicenter, prospective, longitudinal, observational cohort study of patients with clinically stable and exacerbated COPD.

**Methods**

**Study overview**

Patients in stable state COPD with GOLD II to IV were enrolled for an observational prospective trial (PROMISE-COPD; www.controlled-trials.com identifier ISRCTN9 9586989). The study details have been published previously [26]. For the current nested biomarker study, 638 patients were consecutively recruited and followed at 11 European hospital pneumology departments from November 2008 to October 2011 (Fig. 1). Details of inclusion and exclusion criteria were previously published [27]. We analysed data from 528 patients, who completed the first 6 month-follow-up of the study and for whom serum samples from visit 1 were available. We used serum samples at the stable phase and during the first episode of exacerbation of COPD.

For each patient, a physical examination was performed, vital signs were registered, and a detailed history...
obtained. Spirometry and 6-min walk tests (6MWTs) were performed following American Thoracic Society guidelines [24, 25]. One- and two-year follow-up in a stable phase, including a series of outcome markers were determined as previously published [26]. Additionally, patients treated for infection-triggered exacerbation of COPD had a scheduled follow-up four weeks post-exacerbation onset. Acute exacerbations were defined as an acute sustained worsening of dyspnoea, cough and/or sputum beyond normal day-to-day variations in a patient with underlying COPD; severe exacerbations were defined as an exacerbation requiring hospitalisation [28]. A total of 30 age and gender unmatched healthy controls were included in the study.

Ethics
The study was an observational study without specific intervention. The IFNL3 genotypes and serum levels were retrospectively determined and had no impact on the treatment decisions. The study was approved by the respective participating local IRBs in all centers (Ethikkommission beider Basel EKBB/295707, Medical Ethical Committee Amphia Ziekenhuis 958, Medical Ethical Committee North Holland M08–016, Klinicki Centar Srbije Eticki Odbor, Clinical Research Ethics Committee Germans Trias I Pujol Hospital, Medizinische Hochschule Hannover Ethikkommission 5071, Ethics committee of the Hospital Clinic of Barcelona, Ethics committee of the Policlinico of Milan, Ethics committee in Greece). All patients provided a written informed consent.

IFNL3 ELISA assay
IFNL3 serum levels were determined in the stable phase and during exacerbation of COPD. A commercially available sandwich immunoassay ELISA kit that showed no cross-reactivity with IFNL2 (IL28A) or IFNL4 (IL29; Human IL-28B/IFN-lambda 3 DuoSet ELISA, DY5259, R&D Systems Minneapolis, MN, USA) was used, according to the manufacturer’s instructions. The IFNL3 assay had a linear range of detection from 31.20–2000 pg/ml.

IFNL genotyping
Two common SNPs, rs8099917 and rs12979860, in the IFNL3/L4 gene regions were determined as previously described [11]. The distribution of minor and major allele genotypes is comparable to previous publications in European populations [29, 30].

Collagen markers
As previously described [31], serum levels of fragments of collagen type III (C3M), fragments of collagen type VI (C6M), pro-form of collagen type III (Pro-C3) and pro-form of collagen type VI (Pro-C6) were measured with Nordic Bioscience assays according to the manufacturer’s instructions.

Inflammatory markers
Procalcitonin, Copeptin, pro-adrenomedullin (ADM), and atrial natriuretic polypeptide (ANP) were measured as previously described [32].

Statistics
Differences in dichotomous variables were evaluated using the Chi-square test or Fischer’s exact test, as appropriate. Normally distributed parameters were analyzed using the Student’s t-test for equality of means. All other continuously non-normally distributed parameters were evaluated using the non-parametric Mann-Whitney U test or Kruskal-Wallis test, as appropriate. If the IFNL3 was below the detection level, the sample was assigned the value 31.20 pg/ml which is the lowest detectable value with the assay used in this study. Kaplan Meier curves were created to determine survival within 2 years and overall survival, occurrence of exacerbation and occurrence of severe exacerbation. The log-rank test was used to compare differences between survival curves. The Statistical Package for Social Sciences Program (SSPS Inc., version 22 for Windows) was used. All tests are two-tailed; a p-value of < 0.05 was considered significant. Results are expressed as mean (standard deviation) or median (interquartile range), unless otherwise stated.

Results
Five hundred twenty eight patients were included in this nested study (Fig. 1). The majority of the patients were male (71%) and the average age was 66.9 years (Table 1). 70% of the patients were past smokers and 50% of the patients were classified as GOLD II.

IFNL3 genotyping
The distribution of both rs8099917 and rs12979860 SNPs adhered to the Hardy Weinberg Equilibrium with the χ2 test for deviation equalling 2.21 for rs8099917 and 0.781 for rs12979860. Both values were less than 3.84 which represents the 5% significance level for 1 degree of freedom and therefore the null hypothesis that the population is in the Hardy-Weinberg frequencies is not rejected [33]. The most common genotype rs8099917 TT (65%) was followed by TG (30%) and GG (5%; Table 1).

Patients with the rs8099917 GG genotype had a significantly shorter time to severe exacerbation than patients with the TT or TG genotype (Fig. 2; p = 0.037). Significantly more severe exacerbations occurred in patients with rs8099917 GG genotype compared to patients with rs8099917 TT or rs8099917 TG genotype (42
Table 1 Baseline characteristics of patients included in the study (Continued)

| Mean (SD), n (%)                  | Gender: Male 377 (71) |
|-----------------------------------|-----------------------|
| Age, years                        | 66.9 (9.3)            |
| Current smoker, n (%)             | 157 (30)              |
| BMI (kg/m2)                       | 26.00 (5.13)          |
| Unadjusted Charlson Score (n = 528)| 1.82 (1–16)          |
| BODE index (median; IQR)          | 3 (1–4)               |
| 6MWT (m)                          | 380.61 (104.69)       |
| Exacerbation rate                 | 2.05 (0–15)           |
| Severe exacerbation rate          | 0.39 (0–8)            |
| Lung function (post-brd)          |                       |
| FEV₁, in L                        | 1.40 (0.71)           |
| FVC, in L                         | 2.81 (0.89)           |
| FEV₁/FVC%                         | 47.87 (13.97)         |
| FEV₁ % predicted                  | 49.89 (16.8)          |
| FVC % predicted                   | 80.70 (21.21)         |
| Collagen markers [ng/ml]          |                       |
| C3M                               | 30.54 (12.61)         |
| C6M                               | 15.25 (8.62)          |
| Pro-C3                            | 13.29 (10.03)         |
| Pro-C6                            | 8.76 (4.31)           |
| EL-NE                             | 7.78 (6.78)           |
| GOLD Gradea                       |                       |
| GOLD II                           | 262 (50)              |
| GOLD III                          | 180 (35)              |
| GOLD IV                           | 80 (15)               |
| rs8099917 genotypes               |                       |
| TT                                | 339 (65)              |
| GG                                | 26 (5)                |
| TG                                | 155 (30)              |
| rs12979860 genotypes              |                       |
| CC                                | 76 (30)               |
| TT                                | 45 (18)               |
| TC                                | 131 (52)              |
| MMRC score (median; IQR)          | 2 (1–2)               |
| Inflammation markers at baseline  |                       |
| Copeptin, pMol/L                  | 12.57 (16.66)         |
| Adrenomedullin, nMol/L            | 0.69 (0.38)           |
| Atrial Natriuretic Peptide, pMol/L| 113.67 (101.03)       |
| Procalcitonin, μg/l               | 0.09 (0.14)           |
| SF-36                             |                       |
| Physical function                 | 51.54 (25.94)         |
| Role physical                     | 51.05 (43.48)         |
| Role emotional                    | 66.26 (43.52)         |
| Social Functioning                | 69.49 (28.50)         |

Continuous data are shown as mean (SD) or median (interquartile range) and categorical variables as No. (%). BMI = body mass index; brd = bronchodilator; BODE = BMI, airflow obstruction, dyspnea and exercise capacity; 6MWD = 6-min walk distance; C3M = fragments of collagen type III; C6M = fragments of collagen type VI; Pro-C3 = pro-forms of collagen type III; Pro-C6 = pro-forms of collagen type VI; EL-NE = neutrophil elastase-generated fragments of elastin; GOLD = Gold Initiative for Chronic Obstructive Lung Disease; MMRC = modified Medical Research Council; SF-36 = 36-item Short-Form Health Survey; SGRQ = St. George’s Respiratory Questionnaire

*GOLD grades are based on FEV1% predicted: 50% ≤ II ≤ 80%; 30% ≤ III ≤ 50%; and IV ≤ 30%

Fig. 2 Kaplan Meier curve showing the significant effect of the rs8099917 GG genotype on time to severe exacerbation; p = 0.037. GG p = 26, 11 events; TT n = 335, events = 73; TG n = 154, events = 39

The rs8099917 TT and TG genotypes had no significant effect on mortality (p = 0.032). There was a significant association between MMRC and the rs8099917 GG genotype with 42% (11/26) of patients with the GG genotype having an MMRC more than the median compared to 22% (107/479) of patients with rs8099917 TT or rs8099917 TG
genotype (Table 2; chi-squared, \( p = 0.030 \)). Although there was no difference in the unadjusted Charlson score \( (p = 0.705) \), suggesting a similar distribution of comorbidities and life expectancy between the groups, five times the number of patients with rs8099917 GG genotype had been diagnosed with a malignancy at the start of the study compared to patients with rs8099917 TT or rs8099917 TG genotype (chi-squared, \( p = 0.014 \)). The association between rs8099917 GG genotype and having a malignancy remained after adjusting for age and smoking (OR = 6.726, \( p = 0.003 \)). Mann-Whitney-U-Test showed a significant difference in C6M between rs8099917 GG genotype and rs8099917 TT/TG genotype (Table 2).

The rs12979860 genotypes had no effect on mortality \( (p = 0.703) \), exacerbation rate \( (p = 0.946) \) or time to exacerbation \( (p = 0.324) \). The rs12979860 CC genotype was associated with BODE index with 46% \( (30/65) \) of the patients with a BODE index more than the median compared to 36% \( (44/122) \) of patients with the rs12979860 TC and 20.5% \( (8/39) \) of patients with the rs12979860 TT genotype \( (p = 0.031) \).

**Circulating IFNL3 in serum**

Circulating IFNL3 was detectable in 3.6% \( (19/528) \) of the COPD patients during stable phase and in 7.2% \( (32/446) \) of the COPD patients during the exacerbation phase whereas it was detectable in 80% \( (24/30) \) of the blood samples from healthy controls. The level of IFNL3 was higher in the controls compared to the COPD patients during stable phase, exacerbation phase and follow-up to the exacerbation (Fig. 3).

There was no association between the rs8099917 (chi-square test; \( p = 0.392 \)) or rs12979860 genotypes (chi-square test; \( p = 0.733 \)) and whether IFNL3 was detectable or not during the stable phase.

**Circulating IFNL3 during stable COPD phase**

None of the patients with a GOLD IV classification had detectable circulating IFNL3 during stable phase. 3% \( (5/180) \) of the patients classified as GOLD III and 5% \( (14/262) \) of the patients classified as GOLD II had detectable circulating IFNL3 levels. GOLD II \( (31.2 \text{ pg/ml}; \text{Range} = \text{undetectable} – 661.83) \) and GOLD III \( (31.2 \text{ pg/ml}; \text{Range} = \text{undetectable} – 122.26) \) patients had similar levels of circulating IFNL3, which was less than in healthy controls \( (82.7 \text{ pg/ml}; \text{IQR} = 81.9–83.4) \). Using a Mann-Whitney-U-test, patients with detectable circulating IFNL3 had significantly better post-bronchodilator FEV\(_1\)%predicted than patients with undetectable IFNL3 \( (57.97 \text{ vs. } 49.62; \ p = 0.035) \). We found a significant correlation between circulating IFNL3 and post bronchodilator FEV\(_1\)%predicted (Spearman Rho = 0.098; \( p = 0.034 \)). There was no significant difference in other variables, including quality of life, between patients with detectable circulating IFNL3 and patients with non-detectable circulating IFNL3.

**Circulating IFNL3 and collagen biomarkers**

There was a significant association between circulating IFNL3 and Pro-C3 (Linear regression, Beta = 0.099 95% CI 0.037–0.634; \( p = 0.028 \)) but no association with C3M, C6Mor Pro-C6. Patients with detectable circulating IFNL3 had significantly more Pro-C3 than patients with undetectable levels of IFNL3 \( \text{Mann-Whitney U-Test, median } 16.1 \text{ [IQR } = 8.0] \text{ vs } 10.7 \text{ [IQR } = 5.6] \text{ ng/ml, respectively; } p = 0.003 \). There was no significant difference in the other collagen biomarkers between the two groups of patients.

**Discussion**

Viral infections are thought to play a role in the exacerbation of COPD [18, 19]. IFNL3, a member of the interferon lambda family, has immune-modulatory and antitumorigenic effects and is induced by viral infections [14]. This is the first study evaluating the association between IFNL circulating levels and its polymorphisms in patients with COPD.

The distribution of both rs8099917 and rs12979860 SNPs adhered to the Hardy Weinberg Equilibrium. Ethnic background strongly impacts SNP distribution, however, people with Caucasian ancestry (the main ethnic background of the present cohort) have a more balanced distribution of genetic polymorphisms [9, 34]. In our COPD cohort, the SNP rs8099917 GG genotype significantly influenced the severe exacerbation rate, and the time to severe exacerbation and it was associated with a higher MMRC score. Conversely, SNP rs12979860 had no effect on exacerbations or death. This is in line with what is known for hepatitis and diabetes, where the SNP rs8099917 GG genotype is considered to be the risk genotype and the SNP rs12979860 CC genotype the protective genotype [35–37]. The IFNL3 polymorphisms predict response to treatment in patients with hepatitis C [27, 30, 31]. We found that the prevalence of malignancy was increased among patients with the rs8099917 GG genotype. This was also seen in patients with chronic hepatitis C, where more patients with the rs8099917 GG or non-TT genotype had hepatocellular carcinoma [38]. In antiretroviral-treated HIV-infected patients, however, the SNP rs12979860 CC genotype was associated with higher mortality and thus it was not protective [39]. In COPD patients, we found that the SNP rs12979860 CC genotype was associated with a higher BODE index, and thus possibly a higher risk of mortality, though the rs12979860 CC genotype did not associate with mortality directly in this study.
Table 2 A comparison of the patient baseline characteristics according to their rs8099917 genotype

|                              | GG (median, IQR) | TT/TG (median, IQR) | p-value |
|------------------------------|------------------|---------------------|---------|
| Gender: Male (n,%)           | 16 (61.5)        | 355 (71.9)          | 0.269   |
| Age, years                   | 70.50 (14.50)    | 67.00 (13)          | 0.139   |
| Current smoker (n, %)        | 4 (15.4)         | 149 (30.3)          | 0.125   |
| PY, months                   | 40.00 (36.75)    | 45.00 (35)          | 0.261   |
| BMI, kg/m2                   | 27.29 (7.83)     | 25.86 (6.14)        | 0.339   |
| Unadjusted Charlson Score    | 1.00 (2.00)      | 1.00 (1.00)         | 0.305   |
| 6MWT, m                      | 375 (161.25)     | 395 (120)           | 0.362   |
| Exacerbation rate (number of exacerbations/year) | 2.00 (3.00) | 1.00 (3.00) | 0.935   |
| Severe exacerbation rate (number of severe exacerbations/year) | 0.58 (0–3) | 0.37 (0–8) | 0.033   |
| Lung function (post-brd)     |                  |                     |         |
| FEV₁, in L                   | 1.31 (0.8)       | 1.32 (0.74)         | 0.749   |
| FVC, in L                    | 2.53 (1.95)      | 2.69 (1.07)         | 0.688   |
| FEV₁/FVC%                    | 49.38 (27.47)    | 47.00 (22.91)       | 0.723   |
| FEV₁, % predicted            | 56.50 (28.17)    | 49.50 (25.15)       | 0.314   |
| FVC, % predicted             | 82.40 (42.75)    | 80.00 (26.22)       | 0.349   |
| BODE index                   |                  |                     |         |
| ≤ median of 3                | 15 (62.5)        | 302 (67.3)          | 0.659   |
| > median of 3                | 9 (37.5)         | 147 (32.7)          |         |
| Collagen markers [ng/ml]     |                  |                     |         |
| C3M                          | 25.3 (14.5)      | 28.6 (11.6)         | 0.302   |
| C6M                          | 11.0 (9.7)       | 13.3 (8.1)          | 0.032   |
| Pro-C3                       | 12.1 (6.1)       | 10.8 (5.6)          | 0.684   |
| Pro-C6                       | 8.1 (3.7)        | 8.0 (0.535)         | 1.000   |
| GOLD Grade³ (n,%)            |                  |                     | 0.699   |
| GOLD II                      | 15 (57.7)        | 242 (49.6)          |         |
| GOLD III                     | 8 (30.8)         | 169 (34.6)          |         |
| GOLD IV                      | 3 (11.5)         | 77 (15.8)           |         |
| MMRC Test (n,%)              |                  |                     | 0.030   |
| ≤ median of 2                | 15 (57.7)        | 372 (77.7)          |         |
| > median of 2                | 11 (42.3)        | 107 (22.3)          |         |
| Inflammation markers         |                  |                     |         |
| Copeptin, pMol/l             | 8.24 (10.45)     | 8.57 (12.53)        | 0.465   |
| Adrenomedullin, nMol/l       | 0.60 (0.28)      | 0.60 (0.3)          | 0.929   |
| ANP, pMol/l                  | 91.38 (52.43)    | 83.47 (83.18)       | 0.408   |
| Procalcitonin, μg/l          | 0.08 (0.03)      | 0.08 (0.03)         | 0.862   |
| SF-36                        |                  |                     |         |
| Physical function            | 45 (47.5)        | 50 (45)             | 0.346   |
| Role physical                | 50 (100)         | 50 (100)            | 0.742   |
| Role emotional               | 100 (75)         | 100 (100)           | 0.518   |
| Social Functioning           | 75 (65.6)        | 75 (50)             | 0.852   |
| Mental Health                | 67.50 (36.25)    | 65 (27.50)          | 0.780   |
| Body Pain                    | 80 (58)          | 80 (48)             | 0.720   |
| Vitality                     | 46.88 (32.81)    | 50 (31.25)          | 0.403   |
| General Health               | 37.50 (42.5)     | 50 (36.69)          | 0.570   |
We found no association between the genotype and the circulating IFNL3 levels. In the literature, the association between genotype and circulating IFNL3 levels varies according to illness and group. Arpaci et al. [40] found no association between genotype and circulating IFNL3 in patients with Hashimoto’s Thyroiditis. Langhans et al. [41] found that hepatitis C patients with the protective SNP rs12979860 CC genotype had more circulating IFNL3 compared to patients with the SNP rs12979860 TT genotype whereas Alborzi A., et al. [42] found no association between circulating IFNL3 and genotype. Less IFNL3 is secreted by primary cells from asthmatic patients compared to cells from healthy controls infected with a virus but the basal levels are similar between the two groups [22]. Bullens et al. [43] found increased basal IFNL3 mRNA in the sputum of asthmatic patients compared to controls. Thus far there is no literature regarding basal serum IFNL3 levels in asthma patients compared to controls. Circulating IFNL3 levels were similar between hepatitis C patients and healthy controls [41] and Arpaci et al. [40] found increased basal circulating IFNL3 in patients with Hashimoto’s thyroiditis compared to healthy controls. In our cohort of COPD patients, basal circulating IFNL3 levels were significantly less compared to controls. The circulating IFNL3 levels increased during an exacerbation, as was also seen in vitro in the cells from asthmatic patients that were infected with virus [22], and then returned to basal levels after the patient had recovered from the exacerbation. In COPD, the basal circulating IFNL3 levels were associated with the severity of airflow limitation. We hypothesise that the difference in circulating IFNL3 between healthy donors, GOLD II, GOLD III and GOLD IV patients may be due not only to the association between IFNL3 and FEV1, but also due to remodelling of the extracellular matrix in the lungs. This hypothesis is in part corroborated by the fact that there is a strong association between circulating IFNL3 and Pro-C3. Pro-C3 is the N-protease cleavage site of type III collagen and is a marker of tissue formation [44]. Low levels of Pro-C3 is associated with worse lung function [31, 45] and with a shorter time to severe exacerbation [31]. It is therefore possible that changes in the cell structure of the lung results in decreased secreted IFNL3 which causes an impaired immune response to infection. More exacerbations occur resulting in more remodelling of the cells and less IFNL3 secretion, both of which are associated with impaired lung function, and a vicious cycle is continued. It is also possible that the decreased circulating IFNL3 facilitates viral infection leading to less Pro-C3 which results in worse lung function and shorter time to exacerbation. We are unable to determine which element is the catalyst therefore further studies are required to explore the association between IFNL3 polymorphisms and the remodelling of the extra-cellular matrix in stable and exacerbated COPD.

We found no association between circulating IFNL3 levels and disease outcome as is also evident in patients with hepatitis C [41].

The main limitation to this study is that there are no genotyping results for healthy controls and the SNP data was not validated in a separate cohort. We only investigated two SNPs, further studies are needed to investigate

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Table 2 A comparison of the patient baseline characteristics according to their rs8099917 genotype (Continued)

|                      | GG (median, IQR) | TT/TG (median, IQR) | p-value |
|----------------------|------------------|--------------------|---------|
| Symptoms score       | 46.60 (26.87)    | 49.72 (34.44)      | 0.356   |
| Activity score       | 66.19 (31.99)    | 54.43 (31.81)      | 0.607   |
| Impact score         | 29.16 (25.68)    | 29.39 (26.46)      | 0.802   |
| Total score          | 44.15 (31.27)    | 39.03 (27.54)      | 0.892   |

Continuous data are shown as median (interquartile range) and categorical variables as No. (%). Italicized p-values are statistically significant, i.e. p < 0.05. BMI body mass index, brd bronchodilator, BODE BMI, airflow obstruction, dyspnea and exercise capacity; 6MW WD 6-min walk distance, CJM fragments of collagen type III, C6M fragments of collagen type VI, Pro-C3 pro-forms of collagen type III, Pro-C6 pro-forms of collagen type VI, GOLD Gold Initiative for Chronic Obstructive Lung Disease, MMRC modified Medical Research Council, ANP Atrial Natriuretic Peptide, SF-36 36-item Short-Form Health Survey, SGRQ St. George’s Respiratory Questionnaire

*GOLD grades are based on FEV1% predicted: 50% ≤ II ≤ 80%; 30% ≤ III ≤ 50%; and IV ≤ 30%*
other SNPs related to IFNL3. In addition, the circulating IFNL3 was measured from unmatched blood donor samples. However, we found no association between circulating IFNL3 levels and gender or age, so the differences seen between the healthy controls and the COPD patients probably are not due to gender or age differences. The clinical value of IFNL3 alone or in combination with other biomarkers has to be assessed in conformational and randomized clinical trials.

Strengths of the study include the originality, longitudinal design assessing clinically relevant end-points and the fact that both genotypes and circulating IFNL3 were determined in a large multicentric cohort.

Conclusions
IFNL3 polymorphisms may play a role in disease activity and outcomes in COPD and circulating IFNL3 may be associated with disease severity and stability. Further investigations are required to determine the underlying mechanisms.

Abbreviations
6MWT: 6Minute walk test; ADM: Adrenomedullin; ANP: Atrial natriuretic polypeptide; BMI: Body mass index; BODE: Body mass index, airflow obstruction, dyspnea and exercise capacity; C3M: Collagen type III; C6M: Collagen type VI; GOLD: Global Initiative for Chronic Obstructive Lung Disease; IFNL: Interferon lambda; MMRC: Modified medical research council; Pro-C3: Pro-form of collagen type III; Pro-C6: Pro-form of collagen type VI; QoL: Quality of life; SNPs: Single nucleotide polymorphisms

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
Data collection, accuracy of data, contributed to discussion of results, statistical analysis, writing of the manuscript, finalization of the manuscript, and approval of the submitted article: AE, JM, DMS, MR. Data collection, statistical analysis and accuracy of accuracy of data, revision and approval of the submitted article: BT, DLT, KK, FB, WB, BM, AL, KK, GR, RL, JA, TW, AT, MT. Conceived the research project, contributed in clinical work, integrity and accuracy of data, preparing, and approval of the submitted article: D.S. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the respective participating local IRBs in all centers (Ethikkommission beider Basel EK88/295707, Medical Ethical Committee Amphia Ziekenhuis 958, Medical Ethical Committee North Holland M08–016, Klinikkent Centar Srbije Eticki Odbor, Clinical Research Ethics Committee Germans Trias i Pujol Hospital, Medizinische Hochschule Hannover Ethikkommission 5071, Ethics committee of the Hospital Clinic of Barcelona, Ethics committee of the Policlinico of Milan, Ethics committee in Greece). All patients provided a written informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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