



FAM13A polymorphism as a prognostic factor in patients with idiopathic pulmonary fibrosis[☆]



Chihiro Hirano^a, Shinichiro Ohshimo^{a,b,*}, Yasushi Horimasu^a, Hiroshi Iwamoto^a, Kazunori Fujitaka^a, Hironobu Hamada^c, Noboru Hattori^a, Nobuaki Shime^b, Francesco Bonella^d, Josune Guzman^e, Ulrich Costabel^d, Nobuoki Kohno^{a,f}

^a Department of Molecular and Internal Medicine, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan

^b Department of Emergency and Critical Care Medicine, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan

^c Department of Physical Analysis and Therapeutic Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

^d Interstitial and Rare Lung Disease Unit, Ruhrlandklinik, University of Duisburg-Essen, Essen, Germany

^e General and Experimental Pathology, Ruhr University Bochum, Germany

^f Hiroshima Cosmopolitan University, Hiroshima, Japan

ARTICLE INFO

Article history:

Received 23 August 2016

Received in revised form

10 December 2016

Accepted 13 December 2016

Available online 22 December 2016

Keywords:

Idiopathic pulmonary fibrosis
Single nucleotide polymorphism
Family with sequence similarity 13
Member A (*FAM13A*)

ABSTRACT

Background: Family with sequence similarity 13, member A (*FAM13A*) variants have been associated with susceptibility to chronic lung diseases. A recent genome-wide association study has shown an association between a polymorphism in *FAM13A* rs2609255 and idiopathic interstitial pneumonias in a Caucasian population. However, the relationship between rs2609255 polymorphism and prognosis in idiopathic interstitial pneumonias has not been investigated.

Methods: Sixty-five patients with idiopathic pulmonary fibrosis (IPF) and 310 Japanese healthy volunteers were enrolled in this study. Genomic DNA was extracted from all subjects. rs2609255 was genotyped by a commercially available assay. The correlations between rs2609255 polymorphism and survival and the occurrence of acute exacerbation were evaluated.

Results: The frequency of the minor G allele was significantly higher in IPF patients (59.2%) than in controls (41.9%; OR = 1.78, 95% CI: 1.29–2.44, $p < 0.001$). The rs2609255 major T allele was associated with lower diffusing capacity of carbon monoxide values and higher composite physiologic index after adjustment for age, sex and smoking ($\beta = -7.20$, $p = 0.005$ and $\beta = 5.59$, $p = 0.009$, respectively). In the Kaplan-Meier analysis, the T allele carriers showed a significantly increased mortality compared to the non-carriers ($p < 0.05$). In the multivariate Cox-proportional hazards analysis, the T allele of rs2609255 was independently associated with poor survival (hazard ratio, 5.37; $p = 0.031$; 95% confidence interval, 1.16–24.82).

Conclusions: *FAM13A* gene polymorphism showed a significant association with the susceptibility to IPF, with severity of lung function impairment and with poor prognosis.

© 2016 Elsevier Ltd. All rights reserved.

[☆] Notation of prior abstract presentation: Some of the results have been presented at the American Thoracic Society 2016 International Conference.

* Corresponding author. Department of Molecular and Internal Medicine/Department of Emergency and Critical Care Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.

E-mail addresses: cyamaoka62@hiroshima-u.ac.jp (C. Hirano), yasushi17@hiroshima-u.ac.jp (Y. Horimasu), hir@hiroshima-u.ac.jp (H. Iwamoto), fujikazu@hiroshima-u.ac.jp (K. Fujitaka), hirohamada@hiroshima-u.ac.jp (H. Hamada), nhattori@hiroshima-u.ac.jp (N. Hattori), nshime@hiroshima-u.ac.jp (N. Shime), francesco.bonella@ruhrlandklinik.uk-essen.de (F. Bonella), josune.guzman@ruhr-uni-bochum.de (J. Guzman), ulrich.costabel@ruhrlandklinik.uk-essen.de (U. Costabel), nokohno@hiroshima-u.ac.jp (N. Kohno).

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is characterized by a progressive fibrosis and irreversible destruction of the lung architecture with a five-year survival rate of less than 50% [1,2,3]. Genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) in genes including *TOLLIP* and *MUC5B* which were associated with the susceptibility to IPF [4,5]. A recent Genome-wide association study has shown that a SNP in *FAM13A* (rs2609255) is associated with the susceptibility to idiopathic interstitial pneumonias in a non-Hispanic white population [6],

List of abbreviations

AE-IPF	acute exacerbation of idiopathic pulmonary fibrosis
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CPI	composite physiologic index
DLco	diffusing capacity of carbon monoxide
FAM13A	Family with sequence similarity 13, member A
FEV1	forced expiratory volume in 1 s
FVC	forced vital capacity
HR	hazard ratio
HV	healthy volunteers
IPF	idiopathic pulmonary fibrosis
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism

which was validated in a Mexican population [7]. However, the correlation between rs2609255 and clinical outcomes of IPF has not been investigated before. The aim of our study was to investigate the correlations between rs2609255, a SNP in *FAM13A*, and the susceptibility to IPF, the occurrence of acute exacerbation and survival in Japanese patients with IPF.

2. Methods

2.1. Subject

A total of 65 consecutive Japanese patients with IPF newly diagnosed at Hiroshima university hospital between 2003 and 2013 and 310 healthy volunteers (HV) were enrolled in this study. All patients and healthy volunteers completely consisted of the Japanese population and were of similar ethnic background. All patients were diagnosed as having IPF in accordance with the criteria of the American Thoracic Society/European Respiratory Society [8]. Each HV underwent pulmonary function tests and chest X-ray studies, which excluded apparent lung diseases, such as interstitial lung diseases or chronic obstructive pulmonary disease (COPD). Diagnosis of acute exacerbation of IPF (AE-IPF) was made according to the criteria defined by Collard et al. [9]: (1) Unexplained worsening or development of dyspnea within 1 month, (2) HRCT with new bilateral ground-glass abnormality and/or consolidation superimposed on a background reticular or honeycomb pattern, (3) Exclusion of alternative causes, including infection, left heart failure and identifiable cause of acute lung injury. We excluded patients who presented AE-IPF at the time of diagnosis, or developed AE-IPF within 1 month from the time of diagnosis. This study was approved by the Ethics Committees of Hiroshima University Hospital (IRB33) and conducted in accordance with the ethical standards established in the Helsinki Declaration of 1975. All patients and HV gave informed consents in writing and permission to use their samples.

2.2. Pulmonary function tests

Physiologic assessment included measurements of thoracic gas volume, forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), and single breath diffusing capacity of the lung for carbon monoxide (DLco) according to the American Thoracic Society/European Respiratory Society guidelines [10]. The composite physiologic index (CPI) was calculated as follows; $91.0 - (0.65 \times \%DLco) - (0.53 \times \%FVC) + (0.34 \times \%FEV1)$ [11]. We evaluated gender, age, and

physiology (GAP) staging system by using %FVC and %DLco values [12].

2.3. DNA preparation and genotype analyses

We extracted DNA from peripheral whole venous blood samples using the phenol-chloroform extraction and ethanol precipitation methods, as previously described [13]. The rs2609255 genotype was determined by using a real-time polymerase chain reaction (PCR) method. PCR mixture was prepared with genomic DNA (2.0 µg/mL), TaqMan SNP Genotyping Assay (C15906608-10; Life Technologies Corp. Carlsbad, CA, USA) and TaqMan Fast Universal PCR Master Mix (Life Technologies Corp.). Real-time PCR analysis was conducted with Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies Corp.).

2.4. Statistical analysis

Individual variables for two groups were analyzed by the Mann-Whitney *U* test or chi-square test as appropriate. To avoid the assumptions of genetic models, we analyzed by the dominant model (heterozygotes; G/T plus minor allele homozygotes; G/G versus major allele homozygotes; T/T), the recessive model (minor allele homozygotes; G/G versus major allele homozygotes; T/T plus heterozygotes; G/T) and the additive model (minor allele homozygotes; G/G versus heterozygotes; G/T versus major allele homozygotes; T/T) for rs2609255 in association with IPF. The associations between the case-control status and rs2609255 genotypes were estimated using logistic regression analysis. Linear regression analysis was conducted to study the independent effect of rs2609255 allele on lung function values. The survival analysis was estimated with the Kaplan-Meier method, and the differences in mortality rates were evaluated by log-rank test. Multivariate analysis of predictive factors for mortality was done using the Cox regression hazard model. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University) [14], which is a graphical user interface for R (the R Foundation for Statistical Computing, version 3.1.1). More precisely, it is a modified version of R commander (version 1.25) that includes statistical functions that are frequently used in biostatistics.

3. Results

3.1. Demographic characteristics

Table 1 shows the clinical characteristics of the patients studied. A total of 53 (81.5%) patients had usual interstitial pneumonia

Table 1
Clinical characteristics of the patients studied.

	IPF	HV
Number of subjects	65	310
Age (years)	65.8 ± 9.9	50.6 ± 7.8
Gender (male/female)	55/10	255/55
Smoking (Cu or Ex/Non)	53/12	170/140
FVC (percent predicted)	79.7 ± 19.1	100.0 ± 12.9
FEV ₁ (percent predicted)	82.3 ± 19.0	95.9 ± 13.4
FEV ₁ /FVC (%)	82.5 ± 9.3	81.1 ± 5.7
DLco (percent predicted)	50.5 ± 15.0	ND
CPI	44.0 ± 12.5	ND
GAP score	3.45 ± 1.37	ND

Data are shown as mean ± SEM. IPF, idiopathic pulmonary fibrosis; HV, healthy volunteers; Cu, current smoker; Ex, ex-smoker; Non, non-smoker; ND, no data; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; DLco, diffusing capacity of the lung for carbon monoxide; CPI, composite physiologic index; GAP score, gender, age, physiology score.

pattern by HRCT and 12 (18.5%) patients had the histopathological diagnosis of usual interstitial pneumonia. Eleven (16.9%) patients had emphysematous changes in more than 10% area of the whole lung. The mean follow-up period was 44.7 ± 16.9 months. During follow-up period, 20 patients (30.8%) died and 17 patients (26.2%) experienced AE-IPF.

3.2. Allele frequencies of rs2609255

DNA extraction and genotyping was successful in all subjects. Table 2 shows the genotype distributions of rs2609255, which were consistent with Hardy-Weinberg equilibrium both in patients with IPF ($p = 0.86$) and in HV ($p = 0.85$). The genotype distributions of rs2609255 in patients with IPF were significantly different from those in HV ($p = 0.0012$); the G/G genotype was more frequent in patients with IPF than in HV, and the T/T genotype was more frequent in HV than in patients with IPF. The calculated frequency of the minor G allele was 59.2% in patients with IPF compared to 41.9% in HV, which was a significant difference between the groups (OR = 1.78, 95% CI: 1.29–2.44, $p < 0.001$). The results of the association analysis with three different genetic models (additive, dominant, and recessive) are shown in Table 3. With the additive model, the G allele of rs2609255 increased the risk of developing IPF (OR = 1.93, 95% CI = 1.32–2.82, $p < 0.001$). Similar associations were found with the dominant model (OR = 2.14, 95% CI = 1.12–4.10, $p = 0.022$) and with the recessive model (OR = 2.72, 95% CI = 1.53–4.83, $p < 0.001$).

3.3. rs2609255 and pulmonary function

Table 4 shows the comparison of the FAM13A genotypes with pulmonary function variables. In the univariate linear regression analysis, the carriage of the T allele of rs2609255 was significantly associated with decreased %DL_{CO} and higher CPI ($\beta = -6.34$, $p = 0.014$; $\beta = 4.81$, $p = 0.024$; respectively; Table 4). These associations remained significant after adjustment for age, sex and pack-years of smoking ($\beta = -7.20$, $p = 0.005$; $\beta = 5.59$, $p = 0.009$; respectively; Table 4).

3.4. rs2609255 and prognosis

In the Kaplan-Meier analysis, the overall survival of patients with IPF differed significantly among the three genotypes of rs2609255 ($p = 0.021$, Fig. 1A). No significant difference was observed in the occurrence of AE-IPF between the groups ($p = 0.37$, Fig. 1B). The average time to first AE-IPF among the three genotypes are G/G genotype; 33.6 ± 25.3 months, G/T genotype; 21.7 ± 17.5 months, and T/T genotype; 24.2 ± 24.5 months. We divided patients according to the median value of lung function tests for analysis. In the univariate Cox-proportional hazards analysis, %FVC lower than 80% and T allele carrier of rs2609255 were associated with poor

Table 2
Genotype distributions and allele frequency of rs2609255 in patients with IPF and healthy volunteers.

Genotype	IPF (n = 65) N (%)	HV (n = 310) N (%)	OR (95%CI)	p-value
TT	13 (20.0%)	108 (34.8%)	Ref	
GT	27 (41.5%)	144 (46.5%)	1.47 (0.79–2.73)	0.29
GG	25 (38.5%)	58 (18.7%)	2.80 (1.53–5.16)	<0.001
HWE p-value	0.86	0.85		
MAF(G)	59.2%	41.9%	1.78 (1.29–2.44)	<0.001

Statistical significance was tested by chi-square test. HWE, Hardy-Weinberg equilibrium; IPF, idiopathic pulmonary fibrosis; HV, healthy volunteers; OR, odds ratio; CI, confidence interval; MAF, minor allele frequency.

Table 3
Analysis of rs2609255 based on three genetic models.

Model	OR	95%CI	p-value
Dominant model	2.14	1.12–4.10	0.022
TT vs. GG + GT			
Recessive model	2.72	1.53–4.83	<0.001
TT + GT vs. GG			
Additive model	1.93	1.32–2.82	<0.001

Statistical significance was tested by logistic regression model. OR, odds ratio; CI, confidence interval.

Table 4
Correlation between the number of T alleles of rs2609255 and lung function variables in patients with IPF.

	β	SE	p-value
Unadjusted model			
FVC (percent predicted)	-4.44	3.23	0.17
DL _{CO} (percent predicted)	-6.34	2.50	0.014
CPI	4.81	2.08	0.024
Adjusted model			
FVC (percent predicted)	-5.14	3.22	0.12
DL _{CO} (percent predicted)	-7.20	2.46	0.005
CPI	5.59	2.06	0.009

Statistical significance was tested by linear regression model. Covariates included age, sex and smoking history. SE, standard error; FVC, forced vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; CPI, composite physiologic index.

overall survival (Table 5). In the multivariate analysis, T allele carrier of rs2609255 was independently associated with poor survival after adjustment for GAP score (HR 5.37, 95%CI 1.16–24.81, $p = 0.031$; Table 5).

4. Discussion

This study shows that the G allele in rs2609255 of FAM13A is significantly associated with the susceptibility to IPF in the Japanese population. The major T allele of rs2609255 was associated with decreased %DL_{CO} and higher CPI. Moreover, the T allele of rs2609255 appears to be an independent risk factor for poor overall survival in IPF.

FAM13A is expressed in airway and alveolar type II epithelial cells and macrophages in human lungs [15]. While little is known about its biological function, FAM13A has been shown to contain a Rho GTPase activating proteins domain. Li et al. [16] demonstrated that Fam13a activates the GTPase by interacting with AnexinA2 and promote autophagosome in the *P. aeruginosa* infected mouse model. Recently two studies revealed that FAM13A is associated with the activation of Wnt signaling pathway [15,17]. Jin et al. [17] showed that suppression of FAM13A in human lung cancer cells induces an obvious reduction in Wnt signaling activity. Conversely, Jiang et al. [15] found that FAM13A promotes the degradation of β -catenin and decreases the Wnt signal in a COPD model. They discussed that the discrepancy between these two studies could be explained by the different pathological conditions, namely lung cancer and COPD. It is still unclear how FAM13A interacts with the Wnt signal in a fibrosis model. However, the Wnt signaling pathway was demonstrated to be significantly activated in the lung tissue of patients with IPF, and activated Wnt signaling caused increased fibroblast migration [18]. In addition, inhibition of Wnt signaling attenuated bleomycin-induced pulmonary fibrosis in the mouse model [19]. Based on these previous findings, we speculate that the interaction of FAM13A with the Wnt signaling pathway may play a role in the development and progression of pulmonary fibrosis.

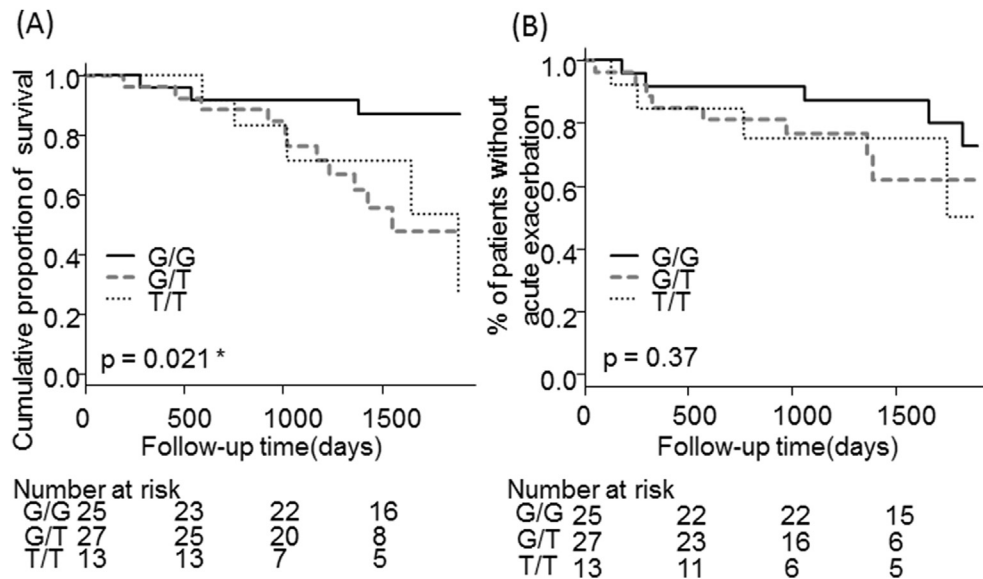


Fig. 1. Kaplan-Meier curves showing overall survival (Figure 1A) and acute exacerbation of IPF (Figure 1B). The heavy line indicates the G/G genotype of rs2609255 of *FAM13A*. The heavy dotted line indicates the G/T genotype of rs2609255. The fine dotted line indicates the T/T genotypes of rs2609255. * Log rank test.

Table 5

Univariate and multivariate Cox-proportional hazards analyses for predicting overall survival in patients with IPF.

Variables	HR	95%CI	p-value
Univariate analysis			
Age (continuous)	0.99	0.95–1.03	0.53
Male sex	1.07	0.31–3.68	0.91
Positive smoking history	0.76	0.25–2.30	0.63
%FVC<80%(median)	6.35	1.79–22.52	0.0042
%DL _{CO} <50%(median)	2.70	0.83–8.83	0.10
GAP score (continuous)	1.46	0.95–2.25	0.088
rs2609255 T allele carrier	4.94	1.42–17.16	0.012
Multivariate analysis ^a			
rs2609255 T allele carrier	5.37	1.16–24.82	0.031

HR, hazard ratio; FVC, forced vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; CI, confidence interval; GAP score, gender, age, physiology score.

^a Adjusted for GAP score.

We observed that the T allele of rs2609255 in *FAM13A* was associated with reduced susceptibility to IPF, but also with poor pulmonary function and with poor prognosis. These observations seem to be conflicting. However, similar conflicting data have been previously reported in studies investigating the *MUC5B* gene polymorphism in patients with IPF. Peljto et al. [20] demonstrated that the minor T allele of rs35705950 in the *MUC5B* gene which was associated with increased susceptibility to IPF, was associated with improved survival. Stock et al. [21] observed a tendency of an association between the T allele of rs35705950 and a slower decline in FVC in patients with IPF. The association between the T allele of rs35705950 and less severe pathological changes was also reported in familial interstitial pneumonia [22]. The specific mechanisms of the T allele of rs35705950 implicated in the survival advantage are not yet clarified in IPF.

Noth et al. [5] suggested the concept of a “protective allele”. They demonstrated that individuals who developed IPF despite having the minor allele of rs5743890 in *TOLLIP* showed an increased mortality. This minor allele of rs5743890 in *TOLLIP* is associated with reduced susceptibility to IPF and was named a protective allele. Although our findings have not yet been well validated, the T allele of rs2609255 in *FAM13A* is also likely a protective allele

against IPF. Those individuals who develop IPF despite having this allele may show more severe lung function impairment and poorer prognosis.

Our study has several potential limitations. First, the number of the patients included in the study was not large. We need further investigations with larger sample size and in a prospective manner to confirm the role of rs2609255 in IPF. Second, there were differences in age and smoking history between patients with IPF and controls. Because we could not match these variables between the cohorts due to the limited sample size, we adjusted these variables using multivariate analysis to minimize the potential bias introduced by these variables. Third, our cohort included patients with IPF concurrent with emphysematous changes, which could have affected the pulmonary function variables.

In conclusion, the results from our study suggest that *FAM13A* polymorphism is significantly associated with disease susceptibility, impaired pulmonary function and poor survival in IPF.

Authors' contributions

CH drafted and finalized the manuscript, performed part of the extraction of DNA, genotyping, and statistical analyses. SO, NS, NK and UC conceived the study, and participated in its design and coordination and helped to draft and finalize the manuscript. YH performed part of the extraction of DNA and genotyping. FB, JG, NH, HI, KF, HH recruited the study subjects and ascertained diagnosis.

Financial/nonfinancial disclosures

This study was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant (Number JP 16K09541) and Arbeitsgemeinschaft zur Förderung der Pneumologie an der Ruhrlandklinik (AFPR).

Summary conflict of interest statements

All authors have nothing to disclose in this study.

Acknowledgements

Guarantor statement: SO takes responsibility for and is the guarantor of the content of the manuscript, including the data and analysis.

References

- [1] E.R. Fernández Pérez, C.E. Daniels, J. St Sauver, T.E. Hartman, B.J. Bartholmai, E.S. Yi, J.H. Ryu, D.R. Schroeder, Incidence, prevalence, and clinical course of idiopathic pulmonary fibrosis: scoring system and survival model, *Chest* 137 (1) (Jan. 2010) 129–137.
- [2] M. Natsuzaka, H. Chiba, K. Kurohama, M. Otsuka, K. Kudo, M. Mori, M. Bando, Y. Sugiyama, H. Takahashi, Epidemiologic survey of Japanese patients with idiopathic pulmonary fibrosis and investigation of ethnic differences, *Am. J. Respir. Crit. Care Med.* 190 (7) (2014) 773–779.
- [3] T.E. King, J.a. Tooze, M.I. Schwarz, K.R. Brown, R.M. Cherniack, Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model, *Am. J. Respir. Crit. Care Med.* 164 (7) (2001) 1171–1181.
- [4] M.A. Seibold, A.L. Wise, M.C. Speer, M.P. Steele, K.K. Brown, J.E. Loyd, T.E. Fingerlin, W. Zhang, G. Gudmundsson, S.D. Groshong, C.M. Evans, S. Garantziotis, K.B. Adler, B.F. Dickey, R.M. du Bois, I.V. Yang, A. Herron, D. Kervitsky, J.L. Talbert, C. Markin, J. Park, A.L. Crews, S.H. Slifer, S. Auerbach, M.G. Roy, J. Lin, C.E. Hennessy, M.I. Schwarz, D.a. Schwartz, A common MUC5B promoter polymorphism and pulmonary fibrosis, *N. Engl. J. Med.* 364 (16) (Apr. 2011) 1503–1512.
- [5] I. Noth, Y. Zhang, S. Ma, C. Flores, M. Barber, Y. Huang, S.M. Broderick, M.S. Wade, P. Hysi, J. Scuirba, T.J. Richards, B.M. Juan-Guardela, R. Vij, M.K. Han, F.J. Martinez, K. Kossen, S.D. Seiwert, J.D. Christie, D. Nicolae, N. Kaminski, J.G.N. Garcia, Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study, *Lancet. Respir. Med.* 1 (4) (Jun. 2013) 309–317.
- [6] T.E. Fingerlin, E. Murphy, W. Zhang, A.L. Peljto, K.K. Brown, M.P. Steele, J.E. Loyd, G.P. Cosgrove, D. Lynch, S. Groshong, H.R. Collard, P.J. Wolters, W.Z. Bradford, K. Kossen, S.D. Seiwert, R.M. du Bois, C.K. Garcia, M.S. Devine, G. Gudmundsson, H.J. Isaksson, N. Kaminski, Y. Zhang, K.F. Gibson, L.H. Lancaster, J.D. Cogan, W.R. Mason, T.M. Maher, P.L. Molyneaux, A.U. Wells, M.F. Moffatt, M. Selman, A. Pardo, D.S. Kim, J.D. Crapo, B.J. Make, E.A. Regan, D.S. Walek, J.J. Daniel, Y. Kamatani, D. Zelenika, K. Smith, D. McKean, B.S. Pedersen, J. Talbert, R.N. Kidd, C.R. Markin, K.B. Beckman, M. Lathrop, M.I. Schwarz, D.A. Schwartz, Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis, *Nat. Genet.* 45 (6) (2013) 613–620.
- [7] A.L. Peljto, M. Selman, D.S. Kim, E. Murphy, L. Tucker, A. Pardo, J.S. Lee, W. Ji, M.I. Schwarz, I.V. Yang, D.A. Schwartz, T.E. Fingerlin, The MUC5B promoter polymorphism is associated with idiopathic pulmonary fibrosis in a Mexican cohort but is rare among Asian ancestries, *Chest* 147 (2) (Feb. 2015) 460–464.
- [8] S. International, M. Consensus, American Thoracic Society American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias, vol. 165, 2002, pp. 277–304.
- [9] H.R. Collard, B.B. Moore, K.R. Flaherty, K.K. Brown, R.J. Kaner, T.E. King, J.A. Lasky, J.E. Loyd, I. Noth, M.A. Olman, G. Raghun, J. Roman, J.H. Ryu, D.A. Zisman, G.W. Hunninghake, T.V. Colby, J.J. Egan, D.M. Hansell, T. Johkoh, N. Kaminski, S.K. Dong, Y. Kondoh, D.A. Lynch, J. Müller-Quernheim, J.L. Myers, A.G. Nicholson, M. Selman, G.B. Toews, A.U. Wells, F.J. Martinez, Acute exacerbations of idiopathic pulmonary fibrosis, *Am. J. Respir. Crit. Care Med.* 176 (7) (2007) 636–643.
- [10] M.R. Miller, R. Crapo, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, P. Enright, C.P.M. van der Grinten, P. Gustafsson, R. Jensen, D.C. Johnson, N. MacIntyre, R. McKay, D. Navajas, O.F. Pedersen, R. Pellegrino, G. Viegi, J. Wanger, A.T.S./E.R.S. Task Force, General considerations for lung function testing, *Eur. Respir. J. Off. J. Eur. Soc. Clin. Respir. Physiol.* 26 (1) (2005) 153–161.
- [11] A.U. Wells, S.R. Desai, M.B. Rubens, N.S.L. Goh, D. Cramer, A.G. Nicholson, T.V. Colby, R.M. Du Bois, D.M. Hansell, Idiopathic pulmonary fibrosis: A composite physiologic index derived from disease extent observed by computed tomography, *Am. J. Respir. Crit. Care Med.* 167 (7) (2003) 962–969.
- [12] B. Ley, C.J. Ryerson, E. Vittinghoff, J.H. Ryu, S. Tomassetti, J.S. Lee, V. Poletti, M. Bucciolli, B.M. Elicker, K.D. Jones, T.E. King, H.R. Collard, A multidimensional index and staging system for idiopathic pulmonary fibrosis, *Ann. Intern. Med.* 156 (10) (2012) 684–695.
- [13] Y. Horimasu, N. Hattori, N. Ishikawa, S. Kawase, S. Tanaka, K. Yoshioka, A. Yokoyama, N. Kohno, F. Bonella, J. Guzman, S. Ohshimo, U. Costabel, Different MUC1 gene polymorphisms in German and Japanese ethnicities affect serum KL-6 levels, *Respir. Med.* 106 (12) (2012) 1756–1764.
- [14] Y. Kanda, Investigation of the freely available easy-to-use software 'EZ' for medical statistics, *Bone Marrow Transpl.* 48 (3) (2013) 452–458.
- [15] Z. Jiang, T. Lao, W. Qiu, F. Polverino, K. Gupta, F. Guo, J.D. Mancini, Z.Z.C. Naing, M.H. Cho, P.J. Castaldi, Y. Sun, J. Yu, M.E. Laicho-Contreras, L. Kobzik, B.A. Raby, A.M.K. Choi, M.A. Perrella, C.A. Owen, E.K. Silverman, X. Zhou, A chronic obstructive pulmonary disease susceptibility gene, FAM13A, regulates protein stability of β -catenin, *Am. J. Respir. Crit. Care Med.* 194 (2) (Jul. 2016) 185–197.
- [16] R. Li, S. Tan, M. Yu, M.C. Jundt, R. Li, S. Tan, M. Yu, M.C. Jundt, S. Zhang, Annexin A2 regulates autophagy in *Pseudomonas aeruginosa* infection through the Akt1 – mTOR – ULK1/2 signaling pathway, *J. Immunol.* 195 (2015) 3901–3911.
- [17] Z. Jin, J.W. Chung, W. Mei, S. Strack, C. He, G.W. Lau, J. Yang, Regulation of nuclear-cytoplasmic shuttling and function of Family with sequence similarity 13, member A (Fam13a), by B56-containing PP2As and Akt, *Mol. Biol. Cell* 26 (6) (2015) 1160–1173.
- [18] M. Chilosi, V. Poletti, A. Zamò, M. Lestani, L. Montagna, P. Piccoli, S. Pedron, M. Bertaso, A. Scarpa, B. Murer, A. Cancellieri, R. Maestro, G. Semenzato, C. Dogliani, Aberrant Wnt/ β -catenin pathway activation in idiopathic pulmonary fibrosis, *Am. J. Pathol.* 162 (5) (2003) 1495–1502.
- [19] W.R. Henderson, E.Y. Chi, X. Ye, C. Nguyen, Y. Tien, B. Zhou, Z. Borok, D.a. Knight, M. Kahn, Inhibition of Wnt/ β -catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis, *Proc. Natl. Acad. Sci. U. S. A.* 107 (32) (2010) 14309–14314.
- [20] A.L. Peljto, Y. Zhang, T.E. Fingerlin, S.-F. Ma, J.G.N. Garcia, T.J. Richards, L.J. Silveira, K.O. Lindell, M.P. Steele, J.E. Loyd, K.F. Gibson, M.A. Seibold, K.K. Brown, J.L. Talbert, C. Markin, K. Kossen, S.D. Seiwert, E. Murphy, I. Noth, M.I. Schwarz, N. Kaminski, D.A. Schwartz, Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis, *Jama* 309 (21) (2013) 2232–2239.
- [21] C.J. Stock, H. Sato, C. Fonseca, W.a.S. Banya, P.L. Molyneaux, H. Adamali, A.-M. Russell, C.P. Denton, D.J. Abraham, D.M. Hansell, A.G. Nicholson, T.M. Maher, A.U. Wells, G.E. Lindahl, E.a. Renzoni, Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis, *Thorax* 68 (5) (2013) 436–441.
- [22] G.P. Cosgrove, S.D. Groshong, A.L. Peljto, J. Talbert, D. McKean, C. Markin, R. Kidd, C.D. Cool, M. Seibold, E. Murphy, M.P. Steele, J.E. Loyd, K.K. Brown, T. Fingerlin, D.A. Schwartz, M.I. Schwarz, The MUC5B promoter polymorphism is associated with a less severe pathological form of familial interstitial pneumonia (FIP)[abstract], *Am. J. Respir. Crit. Care Med.* (2012) 185. A6865.