Risk factors associated with the development of interstitial lung abnormalities

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Shareable abstract (@ERSpublications)
Some individuals older than 50 years may present interstitial lung abnormalities which are associated with a greater risk of all-cause mortality. This study provides demographic and molecular factors which may help to identify individuals at higher risk. https://bit.ly/2Lc4y88

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Abstract

Background Around 8–10% of individuals over 50 years of age present interstitial lung abnormalities (ILAs), but their risk factors are uncertain.

Methods From 817 individuals recruited in our lung ageing programme at the Mexican National Institute of Respiratory Diseases, 80 (9.7%) showed ILAs and were compared with 564 individuals of the same cohort with normal high-resolution computed tomography to evaluate demographic and functional differences, and with 80 individuals randomly selected from the same cohort for biomarkers. We evaluated *MUC5B* variant rs35705950, telomere length, and serum levels of matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, interleukin (IL)-6, surfactant protein (SP)-D, α-Klotho and resistin.

Results Individuals with ILAs were usually males ($p<0.005$), older than controls ($p<0.0001$), smokers ($p=0.01$), with a greater frequency of *MUC5B* rs35705950 (OR 3.5, 95% CI 1.3–9.4; $p=0.01$), and reduced diffusing capacity of the lung for carbon monoxide and oxygen saturation. Resistin, IL-6, SP-D, MMP-1, MMP-7 and MMP-13 were significantly increased in individuals with ILAs. Resistin (12±5 *versus* 9±4 $ng \cdot mL^{-1}$; $p=0.0005$) and MMP-13 (357±143 *versus* 298±116 $pg \cdot mL^{-1}$; $p=0.004$) were the most increased biomarkers. On follow-up (24±18 months), 18 individuals showed progression which was associated with gastro-oesophageal reflux disease (OR 4.1, 95% CI 1.2–12.9; $p=0.02$) and in females with diabetes mellitus (OR 5.3, 95% CI 1.0–27.4; $p=0.01$).

Conclusions Around 10% of respiratory asymptomatic individuals enrolled in our lung ageing programme show ILAs. Increased serum concentrations of pro-inflammatory molecules and MMPs are associated with ILAs.

Introduction

Several studies including large populations, mostly non-Hispanic White, have identified ~7–9% of individuals showing subclinical forms of interstitial lung abnormalities (ILAs) as detected by chest high-resolution computed tomography (HRCT) [1–3]. An ILA is defined as the presence of ground-glass opacities, reticular abnormalities, diffuse centrilobular nodules, honeycombing, traction bronchiectasis, nonemphysematous cysts or architectural distortion involving at least 5% of nondependent portions of the lung [4].

Recently, it was proposed that ILAs can be subclassified as nonsubpleural nonfibrotic, subpleural nonfibrotic and subpleural fibrotic, with prognostic consequences [5].

Individuals with ILAs may be asymptomatic, although they are more likely to report chronic cough or shortness of breath and may display reduced lung function compared with subjects without ILAs [3]. Recent reports on longitudinal studies have shown that ∼40% of ILAs progress over ∼5 years of follow-up, displaying a higher rate of all-cause mortality, more likely associated with a respiratory cause when compared with those who did not have ILAs [6, 7].

Some risk factors have been recognised so far, including occupational exposures to vapours, gas, dust and fumes, smoking, higher serum matrix metalloproteinase (MMP)-7 and interleukin (IL)-6, higher plasma concentrations of galectin-3, and the common promoter polymorphism (rs35705950) in *MUC5B* [1–3, 8, 9].

At the National Institute of Respiratory Diseases (Mexico City, Mexico), we have an ongoing lung ageing programme that includes respiratory asymptomatic subjects over 60 years old (smokers and nonsmokers); from 817 individuals enrolled up to now, 80 (9.7%) showed ILAs.

This study aimed to identify genetic, molecular and environmental risk factors or comorbidities likely associated with the development of ILAs in respiratory asymptomatic individuals and their clinical behaviour.

Material and methods

Study population

Respiratory asymptomatic volunteers aged over 60 years were invited to participate in our "lung ageing programme", initiated in the National Institute of Respiratory Diseases in Mexico City in March 2015. The present study included 817 adults that were recruited from 2015 until July 2019. From them, in addition to the 80 individuals with ILAs, this cohort includes 45 subjects with emphysematous lesions, 48 with bronchiectasis, 49 with air trapping on expiratory phase and 31 with miscellaneous changes (atelectasis, pleural thickening, *etc.*). Only 564 had completely normal HRCT, and were included as controls for demographic and functional comparisons.

The project was approved by the Scientific and Ethics Committee of the National Institute of Respiratory Diseases (approval C39-14), and all individuals signed a consent letter.

A modified questionnaire used in the PLATINO study [10] was applied. PLATINO is a composite instrument that includes sections of the following questionnaires: American Thoracic Society-Division of Lung Disease respiratory questionnaire, European Community Respiratory Survey II, Lung Health Study, and some questions about place and time of residence, and has been validated in Spanish [10]. From this questionnaire, we obtained the information on smoking, occupational exposure, comorbidities, economic impact and exposure to intra-household pollutants. Regarding smoking, subjects were categorised as never-smokers, ex-smokers or current smokers.

Body mass index (BMI) was calculated from weight and height squared, and underweight (BMI $<18.5\ kg\cdot m^{-2}$) and obese (BMI $>40\ kg\cdot m^{-2}$) individuals were not included. Other exclusion criteria included inability to complete the walking functional tests and spirometry, presence of chronic nonrespiratory diseases without medical control (diabetes mellitus, systemic arterial hypertension, hypothyroidism, epilepsy, *etc.*), or ever treated with chemotherapy or radiotherapy.

Routine laboratory studies including blood biometrics, blood chemistry, lipid profile and acute-phase reactants were registered.

Pulmonary function tests

All individuals underwent pulmonary function tests. Forced vital capacity (FVC) and forced expiratory volume in 1 s were obtained by spirometry, and diffusing capacity of the lung for carbon monoxide (D_{LCO}) was measured using the EasyOne Pro LAB (NDD Medical Technologies, Andover, MA, USA). D_{LCO} % pred was adjusted for altitude according to American Thoracic Society/European Respiratory Society guidelines [11]. All subjects also performed the 6-min walk test (6MWT).

High-resolution computed tomography

HRCT analysis was done with helicoidal tomography (Somatom Definition AS, 128 detectors, dual energy; Siemens, Malvern, PA, USA), and individuals were scanned in supine and prone positions. The prone position was included because dependent abnormalities may be misleading unless persistent in the prone position. HRCT scans were prospectively and independently reviewed blindly by two radiologists experienced in interstitial lung diseases (ILDs), as defined elsewhere [1–4]. The κ-value for interobserver

variability for ILA diagnosis was 0.61. In discordant cases, the final diagnosis was performed by consensus.

HRCT findings were retrospectively classified as subpleural fibrotic, subpleural nonfibrotic and nonsubpleural nonfibrotic ILAs [5]. Subpleural fibrotic ILAs included predominantly reticular abnormalities of subpleural localisation with or without architectural distortion with traction bronchiectasis or honeycombing.

Serum biomarkers

Serum was obtained from peripheral blood samples by centrifugation at 4000 rpm at 4°C for 20 min. The serum was collected and stored at −80°C until use.

Serum levels of MMP-7, MMP-1, IL-6, surfactant protein (SP)-D, α-Klotho and resistin were quantified by ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols. α-Klotho was also measured by ELISA (Immuno-Biological Laboratories, Minneapolis, MN, USA).

Serum concentrations of MMP-2, MMP-3, MMP-8, MMP-9, MMP-12 and MMP-13 were determined by Luminex assay. For each sample, 50 µL serum was used to measure MMP levels using a Human Premixed Multi-Analyte Kit for simultaneous detection of multiple human biomarkers (LXSAHM; R&D Systems) using a Bio-Plex 200 array reader (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Bio-Plex Manager version 4 (Bio-Rad) was used for data acquisition.

Genotyping

Genotyping was performed on genomic DNA extracted from 10 mL peripheral blood using a commercial extraction kit (BD Tract Isolation Kit; Maxim Biotech, San Francisco, CA, USA). DNA was quantified by absorption spectrophotometry at 260 nm wavelength. The minor allele of the single nucleotide polymorphism (SNP) rs35705950 from the *MUC5B* promoter and the SNP rs2736100 in the telomerase reverse transcriptase (*TERT*) gene was determined using TaqMan-specific probes for allelic determination.

The PCRs were carried out in a 25 µL mixture, which contained 2 µL DNA (10 ng) and 12.5 µL Master Mix 2× PCR (Applied Biosystems, Waltham, MA, USA). The conditions of the PCRs were 2 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

Analysis of telomere length by quantitative real-time PCR

Relative telomere length was measured by quantitative PCR (qPCR) as previously described [12]. Genomic DNA was extracted from blood samples and reactions were performed with Power SYBR Green PCR Master Mix (Life Technologies, Loughborough, UK), RNase-free water (Sigma, Gillingham, UK), primer single gene (S) forward (36B4d F-300 nM) (CCCATTCTATCATCAACGGGTACAA)/single copy gene (S) reverse (36B4u R-300 nM) (CAGCAAGTGGGAAGGTGTAATCC) and primer Tel (T) forward (900 nM) (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT)/Tel (T) reverse (900 nM) (GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCC). The cycling profile was 95°C for 10 min; 95°C for 15 s, 58°C for 1 min, 72°C for 30 s×40 cycles; 95°C for 15 s, 55°C for 15 s and 95°C for 15 s. Outlier values were excluded. The relative value for telomere length (telomere repeat copy number (T) to a single copy gene (S) (T/S) ratio) was determined by comparison with control calibration curves and was graphed as natural logarithm *versus* age.

Follow-up

Individuals with ILAs were followed every 6 months and examined with the same pulmonary function tests. Progression was determined when the individual presented two of the following conditions: 1) decline of pulmonary function tests of either FVC >10%, D_{LCO} >15% or reduction of 50 m in the 6MWT, 2) initiation or worsening of respiratory symptoms, or 3) an increase of >30% of lesions compared with the previous HRCT or the appearance of new lesions such as honeycombing or traction bronchiectasis, regardless of the percentage change of the lesions on the first scan [6]. Briefly, the total lung area was divided into three zones: 1) upper zone (above the level of the carina), 2) middle zone (between the level of the carina and the level of the inferior pulmonary veins) and 3) lower zone (under the level of the inferior pulmonary veins). The extent of abnormalities was evaluated visually for each lung zone and was scored based on a semiquantitative estimate as a percentage. The final percentage of involvement was obtained by averaging the three zones in the initial and follow-up HRCT scans. All paired HRCT evaluations were examined by two radiologists and progression was determined by consensus.

Statistical analysis

Descriptive data are presented as frequency with percentage or as mean and standard deviation. Univariate analyses of baseline characteristics were performed with the t-test or Chi-squared test as appropriate for the data. We performed a normality test with the Kolmogorov–Smirnov test. p-values <0.05 were considered significant. Bonferroni's correction was used to adjust for multiple testing and corrected p-values were considered where applicable. We used a logistic regression model to estimate odds ratios with 95% confidence intervals. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the biomarkers for detecting the presence of ILAs. All analyses were performed using Stata version 15.0 (StataCorp, College Station, TX, USA) and SPSS Statistics for Windows version 24.0 (IBM, Armonk, NY, USA).

Results

In our ongoing longitudinal ageing lung programme involving respiratory asymptomatic individuals over 60 years old, we found 80 individuals (9.7%) presenting ILAs characterised by visual assessments of chest HRCT scans (figure 1). The demographic and baseline characteristics of the 80 subjects with ILAs were compared with 564 individuals of the same cohort with normal HRCT scans (non-ILA control group). The results are summarised in table 1. Individuals with ILAs were significantly older (72±8 *versus* 69±8 years; p<0.0001) with a male predominance (43% *versus* 26%; p=0.005) and ex-/current smokers (p=0.01). No differences were found in the presence of comorbidities usually associated with ageing (*e.g.* hypertension or diabetes mellitus). Likewise, no differences were found regarding environmental or occupational exposure, or in routine laboratory studies.

At baseline, compared with the 564 controls, individuals with ILAs displayed reduced pulmonary function tests associated with gas exchange, while no differences were found in FVC (table 2).

High-resolution computed tomography

Of the 80 subjects with ILAs, 24 (30%) exhibited a predominance of subpleural reticular opacities and 56 (70%) exhibited a predominance of subpleural or central ground-glass attenuation. In all of the cases the abnormalities were early/mild or moderate. According to the recent classification proposed by the Fleischner Society, 38 subjects (48%) showed subpleural nonfibrotic abnormalities, 24 (30%) showed subpleural fibrotic abnormalities and 18 (22%) showed nonsubpleural nonfibrotic abnormalities [5].

Gene polymorphisms and biomarkers

We examined the common gene variant of *TERT* (rs2736100), the gain-of-function *MUC5B* promoter variant (rs35705950) and biomarkers in the ILA group (n=80) and 80 randomly selected subjects of the non-ILA control group. The minor allele frequency of the *MUC5B* promoter polymorphism was found to

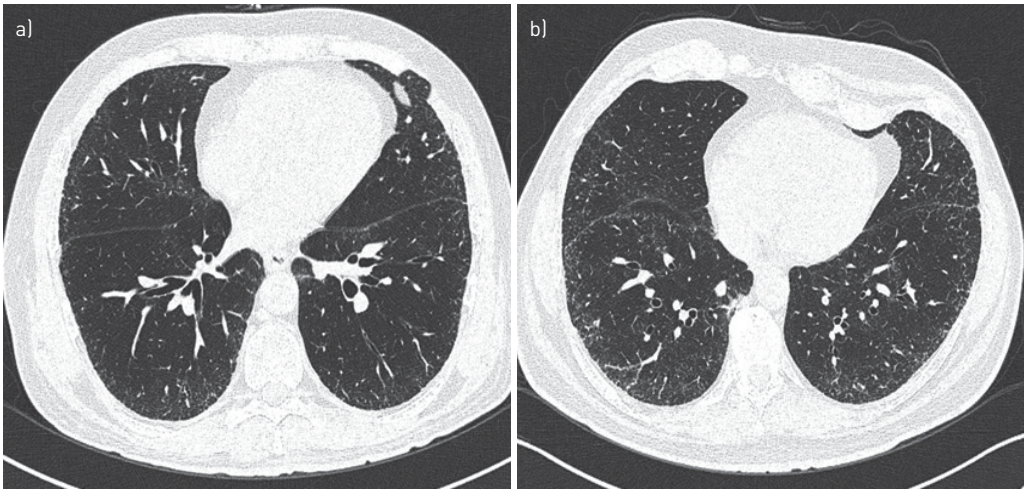

FIGURE 1 High-resolution computed tomography images showing interstitial lung abnormalities in two respiratory asymptomatic subjects from the lung ageing programme. a) Central and subpleural reticular lesions in a male, 67 years old, nonsmoker, without occupational or home exposures. b) Predominant subpleural reticular abnormalities in a male, 63 years old, ex-smoker, without other exposures.

TABLE 1 Demographic and clinical characteristics of the study population

	ILA	Non-ILA control	p-value
Subjects	80	564	
Age years	72±8	69±8	<0.0001
Male	34 (43)	149 (26)	0.005
Smoking status			
Never-smoker	31 (39)	302 (54)	0.01
Ex-smoker	40 (50)	161 (29)	0.3
Current smoker	9 (11)	101 (18)	0.08
Systemic hypertension	28 (35)	224 (40)	0.5
Diabetes mellitus	15 (19)	127 (23)	0.5
Gastro-oesophageal reflux	22 (28)	197 (35)	0.2

Data are presented as n, mean±SD or n (%), unless otherwise stated; percentages may not total 100% due to rounding. ILA: interstitial lung abnormality.

be significantly associated with ILAs (OR 3.5, 95% CI 1.3–9.4; p=0.01). No differences were observed with the common *TERT* variant or in telomere length.

As putative biomarkers, we measured the serum concentrations of several MMPs, as well as markers of inflammation, ageing and alveolar epithelial cell integrity. From all of them, we found that individuals with ILAs displayed a significant increase in MMP-1, MMP-7, MMP-13, IL-6, SP-D and resistin (table 3). After Bonferroni correction to adjust for multiple testing, only MMP-13 and resistin remained statistically associated with the risk of ILAs.

ROC curves were constructed and the area under the curve (AUC), cut-off, sensitivity and specificity were determined to analyse the diagnostic performance of resistin and MMP-13. After adjustment by age, sex and D_{LCO}, only resistin was associated with ILAs (AUC 0.74; OR 1.1, 95% CI 1.0–1.2; p=0.01).

Follow-up

On follow-up (24±18 months), 18 individuals (23%) showed progression revealed by a decline in pulmonary function tests and increased HRCT opacities in the whole-lung visual ILA score or initiation of symptoms (figure 2). Lung functional deterioration of progressor individuals is shown in supplementary table S1.

Three of these progressors showed changes suggestive of a usual interstitial pneumonia-like pattern and therefore were included in our idiopathic pulmonary fibrosis (IPF) programme. In five that showed progression, the presence of some autoantibodies was revealed and the progressors were included in the autoimmune-associated ILD programme. Finally, three of the subjects showing progression died from nonrespiratory causes.

We evaluated whether there were differences at baseline between progressive and nonprogressive ILAs. No association was found with pulmonary function and type of opacities on HRCT (*e.g.* subpleural fibrotic

TABLE 2 Comparison of baseline pulmonary function tests between individuals with and without interstitial lung abnormalities (ILAs)

	ILA	Non-ILA control	p-value
Subjects	80	564	
FVC % pred	93±17	95±15	0.19
D_{LCO} % pred[#]	87±18	102±19	<0.0001
D_{LCO}/V_A index	95±29	109±20	<0.0001
S_{PO_2} at rest %	94±2	95±2	0.0003
S_{PO_2} post-exercise %	87±9	91±5	<0.0001
6MWD m	407±144	460±112	0.0001

Data are presented as n or mean±SD, unless otherwise stated. FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; S_{PO_2}: arterial oxygen saturation measured by pulse oximetry; 6MWD: 6-min walk distance. [#]: adjusted for altitude according to American Thoracic Society/European Respiratory Society guidelines.

TABLE 3 Basal serum concentrations of biomarkers between groups

	ILA	Non-ILA control	p-value	Corrected p-value[#]
Subjects	80	80		
MMP-1 ng·mL^{-1}	7±4	6±3	0.02	0.2
MMP-2 ng·mL^{-1}	38±4	37±2	0.53	1.0
MMP-3 ng·mL^{-1}	19±11	17±10	0.28	1.0
MMP-7 µg·mL^{-1}	6±4	4±2	0.008	0.09
MMP-8 ng·mL^{-1}	4±4	3±3	0.28	1.0
MMP-9 ng·mL^{-1}	14±9	12±8	0.32	1.0
MMP-12 pg·mL^{-1}	30±12	27±10	0.16	1.0
MMP-13 pg·mL^{-1}	357±143	298±116	0.004	0.04
IL-6 ng·mL^{-1}	16±21	11±15	0.04	0.4
SP-D ng·mL^{-1}	10±11	8±6	0.04	0.4
α-Klotho pg·mL^{-1}	735±462	519±133	0.99	1.0
Resistin ng·mL^{-1}	12±5	9±4	0.0005	0.006

Data are presented as n or mean±SD, unless otherwise stated. ILA: interstitial lung abnormality; MMP: matrix metalloproteinase; IL: interleukin; SP: surfactant protein. [#]: corrected by Bonferroni adjustment.

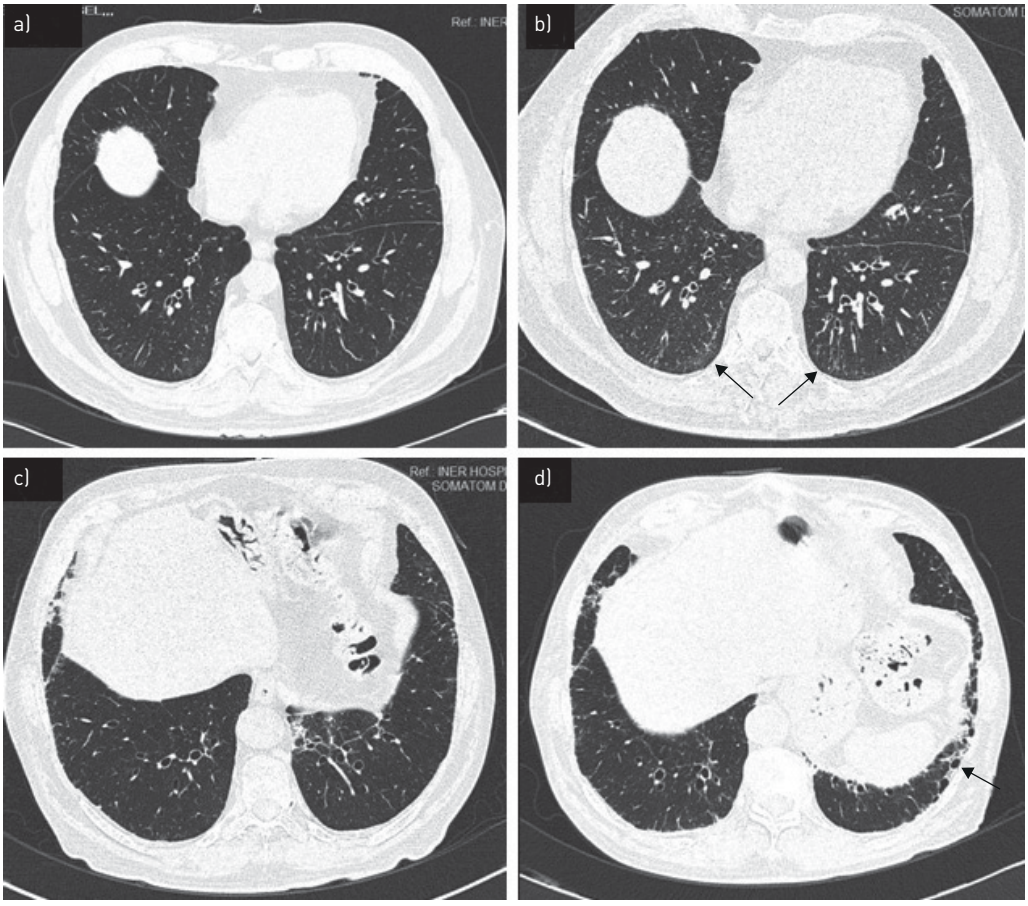

FIGURE 2 Representative high-resolution computed tomography images of progressing interstitial lung abnormalities. Images are from a single subject at the time of enrolment (male, 79 years old, ex-smoker and welder, working in casting for 12 years, knife sharpener) and at 2-year follow-up. a) Baseline and b) follow-up showing an increase of subpleural ground-glass and reticular opacities (arrows). c) Baseline and d) follow-up showing an increase of subpleural cystic lesions, reticular pattern and traction bronchiectasis (arrow).

versus subpleural nonfibrotic or nonsubpleural nonfibrotic). Likewise, none of the biomarkers, *MUC5B* polymorphism or demographic data showed an association with progression. Interestingly, progression was associated with gastro-oesophageal reflux (OR 4.1, 95% CI 1.2–12.9; p=0.02) and in females with diabetes mellitus (OR 5.3, 95% CI 1.0–27.4; p=0.01).

Discussion

Several population-based studies have demonstrated that ∼8% of individuals aged over 50 years, mostly smokers, show ILAs on HRCT [3, 4]. However, the mechanisms implicated in the development of ILAs remain unclear. Since different radiological features and diverse localisation of the lung abnormalities are labelled as ILAs, they likely represent diverse biopathological processes that will require longitudinal long-term observation of large cohorts at risk to determine the aetiology. In our study, for example, after follow-up, three patients were diagnosed as IPF and five as suffering from an autoimmune disease. Although studies on pathological correlates are scarce, a recent report showed that individuals with ILA were more likely to have subpleural fibrosis, fibroblastic foci and atypical adenomatous hyperplasia compared with those without ILAs, suggesting that ILAs, in some cases, represent an early stage and/or mild form of pulmonary fibrosis [13]. Certainly, these changes are not specific and this retrospective study was performed in the context of lung nodule resections.

An important finding that emerges from the analysis of four large cohorts is that ILAs are associated with a greater risk of all-cause mortality, although the reasons are uncertain [7].

Since 2015, we have been recruiting respiratory asymptomatic individuals aged over 60 years, all residents of Mexico City (2240 m altitude above sea level) and exposed to variable levels of air pollution. We found, similar to other populations with a different genetic background (*e.g.* non-Hispanic White participants from the general population, African American participants from COPDGene, and in a geographically and genetically isolated population from Iceland [1, 7]) and diverse environmental conditions, that ∼10% of them presented ILAs (compared with none out of 61 young individuals; data not shown), supporting that age is likely one of the driving forces of these ILAs. In general, our results support the universality of this clinical problem and that screening of ageing populations may help to detect early subclinical stages of ILDs, as has been suggested in other studies [14]. Our findings also confirm that ILA is more frequent in older males, smokers and those carrying the common *MUC5B* variant (rs35705950). This gain-of-function polymorphism has been associated with ILAs and undiagnosed HRCT findings that are consistent with an early stage of pulmonary fibrosis [3, 15]. More recently, genome-wide association studies of ILAs in six different cohorts also confirmed the association with this *MUC5B* promoter variant, and described novel genome-wide associations near *IPO11* (rs6886640) and *FCF1P3* (rs73199442) with ILA and near *HTRE1* (rs7744971) with subpleural-predominant ILA [16].

In contrast, we did not find significant differences regarding the common gene variant of *TERT* that has been associated with an increased risk of sporadic IPF [17].

As putative biomarkers, we selected several MMPs, IL-6, SP-D, α-Klotho and resistin. MMP-7 has been found to increase in ILAs, as well as in several ILDs, and together with MMP-1 has been proposed as a biomarker of IPF [18, 19].

Likewise, SP-D, likely reflecting alveolar epithelial injury, is increased in several ILDs [20, 21] and IL-6 is regarded as one of the main components of the so-called “inflamm-ageing” process that may result in age-associated pathologies [22–24]. Finally, α-Klotho was selected because is an anti-ageing molecule that decreases progressively with age and has been implicated in some ageing-associated lung disorders [25, 26].

We corroborated that MMP-7 is significantly increased in ILA subjects and found a marked increase in some new biomarkers, such as MMP-1, MMP-13, SP-D and resistin. After Bonferroni correction for multiple testing, MMP-13 and resistin remained markedly increased in ILA subjects. In a recent report, it was also found that higher serum resistin levels were associated with greater high-attenuation areas on CT imaging [27]. Resistin is a small, cysteine-rich secretory protein that among other functions is an inflammatory regulator [28].

MMP-13 plays important roles not only in extracellular matrix remodelling but also in the processing of numerous bioactive mediators, such as growth factors, cytokines and chemokines, modulating their activity or releasing them from extracellular matrix-bound stocks [29]. Interestingly, higher levels of collagen biomarkers have been associated with ILAs independently of sex, race and smoking status [30]. Together,

these results suggest that extracellular matrix remodelling may occur early and even before the onset of clinically evident disease.

Several studies have demonstrated rates of imaging progression oscillating between 20% and 40% [31]. In our study, a longitudinal follow-up of the subjects with ILAs showed progression in ∼20% in a period of 2–3 years characterised by impaired lung function and increased radiological abnormalities. Compared with those without progression, progressors showed a high prevalence of gastro-oesophageal reflux and in females progression was associated with diabetes mellitus. None of the genetic or molecular markers studied in our study showed an association with progression. This finding suggests that the biomarkers confirmed or revealed in our study may be useful as diagnostic biomarkers for early detection of ILAs in respiratory asymptomatic subjects.

In other studies, it has been demonstrated that a definite pattern of fibrosis by HRCT, characterised by traction bronchiectasis and honeycombing, is associated with an increased risk of progression [32]. However, older individuals with HRCT abnormalities suspected of usual interstitial pneumonia, mainly if they are symptomatic, are diagnosed in our institution as "probably early IPF in study" and are excluded from the ILA group.

Our study has several limitations. First, the small number of patients with ILAs compromises the statistical power for some measurements, *e.g.* the relationship with telomere length. Likewise, the absence of differences in the *TERT* variant (rs2736100) between groups may likely reflect the limited number of subjects in the groups and the relatively high frequency of this variant in controls. Another limitation is the time of follow-up. The rate of imaging progression of ILAs ranges from 20% over 2 years [33] but may be >50% over 5 years as reported in the AGES-Reykjavik study [32]. Therefore, these results should be corroborated in larger samples and greater follow-up periods.

In summary, our findings suggest that a significant percentage of respiratory asymptomatic individuals aged over 60 years develop ILAs. Increased serum concentrations of pro-inflammatory molecules such as resistin and MMPs (*e.g.* MMP-13) may play a pathogenic role and could help to identify individuals who may be at higher risk of developing ILAs.

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