Anti-UBE2T antibody: a novel biomarker of progressive-fibrosing interstitial lung disease

Mari Hikichi  
Nihon University: Nihon Daigaku  
https://orcid.org/0000-0002-7193-9758

Yasuhiro Gon  
Nihon University: Nihon Daigaku

Kenji Mizumura  
Nihon University: Nihon Daigaku

Shu Hashimoto  
Nihon University: Nihon Daigaku

Shuichiro Ph.D., M.D. Maruoka (maruoka.shuichiro@nihon-u.ac.jp)  
University School of Medicine  
https://orcid.org/0000-0003-0118-342X

Research

Keywords: Idiopathic interstitial pneumonia, Pulmonary fibrosis, autoantibody, Ubiquitin-conjugating enzyme E2T

Posted Date: October 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-961630/v1

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background

Antifibrotic therapy has demonstrated efficacy against progressive-fibrosing interstitial lung disease (PF-ILD); therefore, it has become a priority to identify disease behavior before disease presentation. As autoimmunity is implicated in the pathogenesis of various ILDs, we explored the possibility of a circulating biomarker that can predict the chronic progressive behavior of ILDs.

Methods

A single-center retrospective cohort study was conducted to investigate a biomarker of PF-ILD. Circulating autoantibodies against 9,483 purified full-length human recombinant proteins of patients with interstitial pneumonia were screened by microarray analysis. The candidate auto-antibodies were verified their existence by multiples solution assay. In addition, enzyme-linked immunosorbent assay (ELISA) was performed in larger sample sets to evaluate accurate sensitivity, specificity and clinical significance in ILDs.

Results

In total, 61 healthy subjects and 87 patients with various ILDs enrolled in this study. Anti-UBE2T antibody was discovered by performing protein microarray and multiplex solution assay as a candidate biomarker of ILDs. By measuring its concentration by ELISA, anti-ubiquitin-conjugating enzyme E2T (UBE2T) antibody levels were significantly higher in patients with idiopathic interstitial pneumonias (IIPs), especially in those with PF-ILDs, than in healthy participants. The receiver operating characteristic analysis of anti-UBE2T antibody in diagnosing PF-ILD was calculated. The area under the curve was 0.85 and yielded a cut-off value of 238.1 ng/mL. Anti-UBE2T antibody-positive IIP patients demonstrated significantly higher ILD-gender age physiology scores, PF-ILD diagnosis rates and were more likely to develop honeycomb structures than anti-UBE2T-negative IIP patients after two years of follow up. The anti-UBE2T antibody positivity did not correlate with other commercial biomarkers such as KL-6 and commercial autoantibodies, suggesting the presence of anti-UBE2T antibody was independent of the others. Immunohistochemical staining of UBE2T in normal lungs was observed sparsely in the bronchiole epithelium and macrophages. Controversially, idiopathic pulmonary fibrosis lung tissue showed robust expression of the UBE2T protein in the lining epithelium of honeycomb structures.

Conclusion

This is the first report to describe anti-UBE2T antibody, a new biomarker that is significantly elevated in idiopathic PF-ILDs. This new antibody may constitute a sensitive biomarker to detect cases of PF-ILDs.
that are not currently detected by commercially available biomarkers.

**Background**

Idiopathic interstitial pneumonia (IIP) refers to a heterogeneous group of idiopathic interstitial lung disorders with varying degrees of inflammation and fibrosis. Idiopathic pulmonary fibrosis (IPF) and non-specific pulmonary fibrosis (NSIP) are the two major IIPs. Given the efficiency of antifibrotic therapies in the treatment of progressive interstitial lung diseases (ILDs), including IIPs,(1, 2), it is crucial to predict the clinical course of IIPs at an early stage.

Autoimmunity has been implicated in the pathogenesis of IIPs. Kinder et al.(3) classified idiopathic NSIP as an autoimmune disease because, in most cases, it satisfies the definition of undifferentiated connective tissue disease. In contrast, IPF is clinically classified as a non-autoimmune disease, with the exception of cases that meet the criteria for interstitial pneumonia with autoimmune features (IPAF) or connective-tissue disease (CTD). Although IPF pathogenesis is likely to be based on wound healing rather than on autoimmune processes, there is still significant evidence demonstrating background immune abnormalities in IPF. For example, IPF lung tissues exhibit aggregation of mature dendritic cells, lymphocytes, and IgG in the active fibrotic site.(4–6) IgG autoantibodies against cellular antigens are found in sera from IPF patients.(6)

Considering the autoimmune pathogenesis observed in IIPs, we hypothesized that unknown autoantibodies could have a bearing in terms of clinical outcomes. In this study, we present the discovery of a new circulating autoantibody in progressive IIPs.

**Methods**

**Study design and participants**

This was a single-center retrospective cohort study conducted at Nihon University Itabashi Hospital in Tokyo, Japan. The institutional ethics committee approved all protocols. Patients (≥20 years of age) who visited Nihon University Itabashi Hospital for regular follow-up were asked to participate. In total, 61 healthy subjects and 87 patients with ILDs enrolled between October 2010 to December 2017, and all participants provided written informed consent for analyzing blood samples and clinical data. Blood samples were centrifuged at 1,800 g (at 4 °C for 10 min). The sera were aliquoted and immediately stored at -80 °C until use. Lung tissues analyzed in this study were collected between April 1996 and September 2013. Normal lung tissue specimens were obtained from the uninvolved areas of surgically removed lung cancer tissues. IPF lung tissue specimens were obtained during surgical biopsies for the purpose of IPF diagnosis. All samples and clinical data were analyzed from December 2010 to April 2021 for this study.

Interstitial pneumonia was classified according to the official American Thoracic Society and European Respiratory Society joint statement.(7) Hypersensitivity pneumonitis (HP) and IPF were diagnosed
according to the official guidelines. (8, 9) Expert rheumatologists diagnosed CTDs according to the American College of Rheumatology criteria. All diagnoses were screened and confirmed by the consensus of two expert respiratory physicians.

To analyze progressive IIPs, IPF and NSIP were reclassified into progressive (PF-ILD) and non-progressive-fibrosing ILD (non-PF-ILD). No official definition exists for PF-ILD. In this study, the criteria for PF-ILD were defined according to the methods of INBUILD trial (1, 10): decline in forced vital capacity (FVC) of at least 10% of the predicted value, relative decline in the FVC (5-10% of the predicted value) and worsening of respiratory symptoms or increased extent of fibrosis on high-resolution computed tomography (HRCT), or worsening of respiratory symptoms and an increased extent of fibrosis.

**Protein microarray**

ProtoArray Human Protein Microarray v5.0® (Invitrogen, Carlsbad, CA, USA) contains 9,483 purified full-length glutathione-s-transferase (GST)-tagged human recombinant proteins immobilized on the microarray.

The serum samples were diluted to 1:500. Alexa Fluor®647-conjugated goat anti-human IgG antibody was used as a secondary antibody to detect IgG levels of associated autoantibodies. Arrays were scanned using the Axon GenePix 4000B fluorescent microarray scanner (Molecular Devices, LLC., San Jose, CA, USA). GenePix 6.0 software (Molecular Devices, LLC., San Jose, CA, USA) was used for data acquisition. Invitrogen's proprietary ProtoArray® Prospector software (Invitrogen, Carlsbad, CA) was used for analyzing images. This assay was performed by Invitrogen Corporation (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

**Multiplex Solution Assay**

ProtoPlex™ (Invitrogen, Carlsbad, CA, USA) is a multiplex solution assay that validates candidate autoantibodies using fluorescent bead-conjugated human recombinant proteins. (11)

Diluted serum samples (1:200) were incubated with the bead-antigen complex. Biotinylated anti-human IgG was used as a secondary antibody. After incubation with streptavidin R-phycoerythrin, the beads were analyzed using a Luminex® 200™ system (Invitrogen, Carlsbad, CA, USA). By monitoring the spectrum of the beads and the amount of associated R-phycoerythrin fluorescence, the presence of autoantibodies against particular antigens was assessed. This assay was performed by the Invitrogen Corporation (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

**Enzyme-linked immunosorbent assay**

Enzyme-linked immunosorbent assay (ELISA) was performed to measure the concentration of anti-ubiquitin-conjugating enzyme E2T (UBE2T) antibodies following a standard protocol. Histidine-tagged human recombinant UBE2T protein was incubated overnight on a 96-well plate. Diluted serum samples (1:200) and anti-histidine-tag antibody (diluted gradually from 0 to 10 ng/ml for calibration curve, GeneTex, Los Angeles, CA, USA) were applied to the wells, respectively. Biotin-tagged anti-human IgG Fc
antibody was used as a secondary antibody. After being incubated with streptavidin-horseradish peroxidase (Biolegend, Inc., San Diego, CA, USA), KPL SureBlue™ TMB Microwell Peroxidase Substrate (Kirkegaard and Perry Laboratories, Inc. Gaithersburg, MD, USA) was added for coloring reaction. The reaction was stopped with hydrochloric acid. The absorbance at 450 nm was measured, and the calibration curve was obtained using the concentration of the anti-histidine antibody and its absorbance.

Immunohistochemistry

Formalin-fixed, paraffin-embedded lung tissues were used for UBE2T protein immunohistochemistry following a standard protocol. Staining with primary antibody against UBE2T (Novus Biological, Inc. Littleton, CO) diluted at 1:100 and with the secondary antibody using Histofine Simplestain Max-PO® (Nichirei Bioscience Inc. Tokyo, Japan) was performed.

Statistical analysis

M-statistics were performed to analyze data from the ProtoArray® and ProtoPlex™ platforms provided by Invitrogen Corporation (Life Technologies, Carlsbad, CA, USA). Otherwise, data were analyzed using GraphPad Prism version 5.04 (GraphPad Software Inc., La Jolla, CA, USA). Comparisons of continuous variables were conducted using Mann-Whitney and Kruskal-Wallis tests. The Fisher's exact test was used to compare categorical variables. Spearman's product-moment correlation was used to investigate correlations between variables. P-values <0.05 were considered significant. Data are presented as means ± standard errors.

Results

Discovery and verification of the circulating autoantibodies

The workflow of identifying circulating biomarkers in this study falls into three broad categories; discovery method using protein microarray, verification by multiplex solution assay, and validation by ELISA.

The discovery method by protein microarray was performed to identify the profiles of circulating autoantibodies. The goal of this method was to select several candidate autoantigens that react with the ILD serum autoantibodies for further study.

We collected serum from healthy participants (n=5), patients with IPF (n=7), and patients with CTD-ILD (n=2). CTD-ILDs included systemic scleroderma (n=1) and rheumatoid arthritis (n=1).

Signals arising from the serum-loaded microarrays were evaluated for changes in fluorescence intensity relative to each other and to the control array. In total, 127 proteins exhibited elevated interactions with serum autoantibodies in the ILD group. The top 50 proteins were ranked by the ratio of the average signal value for the ILD group divided by the average signal value for the control group (Supplemental Table 1). Among the 50 candidate autoantigens, UBE2T had the highest average signal ratio in the ILD group.
The second step was the verification study using the multiplex solution assay. In this assay, we screened ten potential autoantibodies identified during the discovery method using the same serum samples to ensure that the candidate autoantibody could be moved forward into a larger validation study. We examined serum samples from healthy control participants (n=12) and patients with IPF (n=13), NSIP (n=7), and CTD-ILDs (n=3). CTD-ILDs included systemic scleroderma (n=1), rheumatoid arthritis (n=1), and systemic lupus erythematosus (n=1). These samples include the same samples used in the protein microarray method.

UBE2T had the highest prevalence among ILD patients, with the lowest prevalence observed in the control group (Table 1). This verification assay demonstrated that anti-UBE2T antibodies constitute potentially useful biomarker.
Table 1
Results of interstitial pneumonia-specific serum autoantibodies in multiplex solution assay

| Autoantigen                                                                 | Prevalence in controls | Prevalence in patients | p-value  | Control (average signal) | Patient (average signal) | Signal ratio (patients/controls) |
|----------------------------------------------------------------------------|------------------------|------------------------|----------|--------------------------|--------------------------|----------------------------------|
| Ubiquitin-conjugating enzyme E2T (putative) (UBE2T)                        | 7%                     | 68%                    | 6.04E-05 | 305                      | 1764                     | 5.8                              |
| Hexokinase 1                                                               | 7%                     | 36%                    | 2.08E-02 | 168                      | 3907                     | 23.2                             |
| Proteasome activator subunit 1, transcript variant 2                       | 7%                     | 36%                    | 2.08E-02 | 289                      | 1380                     | 4.8                              |
| USO1 homolog, vesicle docking protein (yeast)                              | 7%                     | 28%                    | 6.22E-02 | 176                      | 1489                     | 8.4                              |
| Gamma-interferon-inducible protein Ifi-16                                  | 14%                    | 36%                    | 9.49E-02 | 4161                     | 6015                     | 1.4                              |
| Glycolipid transfer protein                                                | 14%                    | 36%                    | 9.49E-02 | 508                      | 1306                     | 2.6                              |
| Protein regulator of cytokinesis 1, transcript variant 1                   | 21%                    | 40%                    | 1.65E-01 | 3686                     | 3837                     | 1.0                              |
| Transcription factor binding to IGHM enhancer 3                           | 14%                    | 40%                    | 1.65E-01 | 507                      | 975                      | 1.9                              |
| Wolf-Hirschhorn syndrome candidate 1, transcript variant 9                | 14%                    | 28%                    | 2.17E-01 | 2521                     | 3098                     | 1.2                              |
| DNA repair protein XRCC4                                                   | 7%                     | 12%                    | 4.25E-01 | 235                      | 403                      | 1.7                              |

Validation study of anti-UBE2T antibody by ELISA

A validation against anti-UBE2T antibodies among various ILDs was performed using ELISA (Figure 1) as the final step of this study. This method aimed to test the accurate sensitivity, specificity and
quantitatively of this novel autoantibody and evaluate the clinical significance in PF-ILDs.

Healthy participants (n=61) and patients with IIPs (IPF, n=43; NSIP, n=21), sarcoidosis (n=14), and HP (n=6) were registered. Two IPF patients were excluded due to the lack of follow-up PFT and HRCT data, incomplete medical records regarding respiratory symptoms, and unconfirmed PF-ILD diagnosis. Finally, 62 IIP patients were eligible for analysis (IPF, n=41; NSIP, n=21). Overall, 73% of IPF patients (n=30) and 29% of NSIP patients (n=6) were classified as having PF-ILD.

The mean anti-UBE2T antibody concentrations were 196 ± 19 ng/mL, 462 ± 56 ng/mL, 338 ± 60 ng/mL, 246 ± 22 ng/mL, and 182 ± 25 ng/mL for healthy participants, patients with PF-ILD, non-PF-ILD, sarcoidosis, and HP, respectively. The levels of anti-UBE2T antibodies were significantly higher in PF-ILD patients (p<0.01) than in healthy participants. There was no significant difference in anti-UBE2T antibody levels between healthy participants and patients with non-PF-ILD, sarcoidosis, and HP.

**Clinical characteristics of anti-UBE2T antibody**

We calculated the accuracy of anti-UBE2T antibody to diagnose PF-ILD using a receiver operating characteristic analysis. The area under the curve was 0.85 (95% confidence interval 0.76-0.93, p<0.01) and yielded a cut-off value of 238.1 ng/mL. Using this cut-off value, the sensitivity was 83.3% and specificity was 85.3%. To compare the clinical characteristics, IIP patients were categorized into anti-UBE2T-positive or anti-UBE2T-negative groups using the cut-off value (Table 2).
Table 2
Baseline characteristics of IIP patients according to anti-UBE2T antibody status at study enrolment

|                               | Anti-UBE2T antibody negative (n=21) | Anti-UBE2T antibody positive (n=41) | p-value |
|-------------------------------|-------------------------------------|-------------------------------------|---------|
| Age, y                        | 69±2                                | 72±2                                | 0.31    |
| Female sex, %                 | 52                                  | 46                                  | 0.79    |
| History of malignancy, n (%)  | 3 (14)                              | 7 (19)                              | 0.73    |
| Use of glucocorticoid or immunomodulator, n (%) | 5 (26)                              | 8 (23)                              | 1.00    |
| Honeycomb structure on HRCT, n (%) | 8 (38)                              | 22 (54)                             | 0.29    |
| Pulmonary function test       |                                     |                                     |         |
| Predicted FVC, %              | 81±4                                | 80±3                                | 0.75    |
| Predicted FEV₁, %             | 91±5                                | 93±4                                | 0.89    |
| Predicted DLCO, %             | 65±7                                | 65±6                                | 0.52    |
| ILD-GAP score                 | 1.7±0.4                             | 2.9±0.3                             | 0.01    |
| UBE2T level, ng/mL            | 154±11                              | 541±52                              | <0.01   |
| KL-6 level, U/mL              | 1063±188                            | 1209±155                            | 0.38    |
| CRP, ng/mL                    | 0.6±0.3                             | 2.0±0.8                             | 0.58    |
| IPAF serological domain prevalence, n (%) | 8 (38)                              | 16 (39)                             | 1.00    |

IIP, idiopathic interstitial pneumonia; HRCT, high-resolution computed tomography; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; ILD-GAP, interstitial lung disease-gender age physiology; UBE2T, ubiquitin-conjugating enzyme E2T; KL-6, Krebs von den Lungen-6; CRP, C-reactive protein; IPAF, interstitial pneumonia with autoimmune features. Data are presented as means ± standard errors.

There were no statistically significant differences in age, sex, history of malignancy, or glucocorticoid or immunomodulator use between these two groups. Additionally, anti-UBE2T status did not correlate with positive results of commercial biomarkers such as C-reactive protein, KL-6, or IPAF. No IIP patients developed CTD within two years of registration. Pulmonary function parameters such as predicted FVC, predicted diffusing capacity for carbon monoxide (DLCO), and predicted forced expiratory volume in 1 second were similar between groups. The ILD-gender age physiology (ILD-GAP) score(12) was significantly higher in the anti-UBE2T-positive group than in the anti-UBE2T-negative group (2.9 ± 0.3 vs. 1.7 ± 0.4, p = 0.01).
To assess the progression of IIPs objectively, we analyzed HRCT scans taken on registration and on the second-year follow-up visit. Since a second-year HRCT scan was not available in two patients, we screened 60 HRCT scans (anti-UBE2T-negative group, n=20; anti-UBE2T-positive group, n=40). At registration, there was no difference in the prevalence of honeycomb structures between the two groups (p=0.29). However, after two years of follow-up, the prevalence of honeycomb structures was significantly higher in the anti-UBE2T-positive group (p=0.04) (Table 3). Additionally, the prevalence of PF-ILD was higher in the anti-UBE2T-positive group than in the anti-UBE2T-negative group (Table 3).

### Table 3
Clinical characteristics of IIP patients after two years of follow-up

|                        | Anti-UBE2T antibody negative (n=21) | Anti-UBE2T antibody positive (n=41) | p-value |
|------------------------|-------------------------------------|-------------------------------------|---------|
| Honeycomb structure in HRCT, n (%) | 10 (50)*                           | 31 (78)*                            | 0.04    |
| PF-ILD, n (%)           | 6 (29)                              | 30 (73)                             | <0.01   |

IIP, idiopathic interstitial pneumonia; UBE2T, ubiquitin-conjugating enzyme E2T; HRCT, high-resolution computed tomography; PF-ILD, progressive-fibrosing interstitial lung disease.

* Since the 2-year follow-up HRCT images of two patients were not available, statistics were calculated with 20 patients in the anti-UBE2T antibody-negative group and 40 patients in the anti-UBE2T antibody-positive group.

**Comparing anti-UBE2T antibody and commercial biomarkers**

IPAF is a concept wherein ILDs display specific symptoms and serological and morphological signs of CTDs without meeting sufficient diagnostic findings for a CTD diagnosis. Among the IIP patients, 39% (n=14) of patients with PF-ILD and 38% (n=10) of patients with non-PF-ILD were positive for the IPAF serological domain. The prevalence of these commercial autoantibodies did not show any relation with the levels of anti-UBE2T antibody (p=0.60). No IIP patients developed CTD within two years of registration.

Among the 62 IIP patients, clinical information for acute or subacute exacerbations was available in 55 patients. At registration, 13 patients had acute or subacute exacerbations, as judged by their treating physician. The average concentration of anti-UBE2T antibodies was higher in exacerbated patients than in stable patients (668±133 ng/mL vs. 308±30 ng/mL, p<0.01); the same result was observed for KL-6 levels (1556±193 U/mL vs. 1098±161 U/mL, p<0.01). Although both anti-UBE2T and KL-6 levels were increased in patients with acute or subacute exacerbations, these two variables did not correlate with each other (Spearman's r=0.19, p=0.17), suggesting their presence was independent of the other.

**Immunohistochemical staining of UBE2T**
We conducted immunohistochemical staining of UBE2T in the lung tissues of IPF patients and control participants. Twelve normal tissues and nine tissues with usual interstitial pneumonia (UIP) patterns were analyzed. In the normal lung biopsies, expression of UBE2T was observed sparsely in the bronchiole epithelium and macrophages (Figures 2A and 2B). Conversely, all lungs with IPF showed a strong expression of UBE2T, not only on the bronchiole epithelium but also on the regenerated type II alveolar epithelium lining inside the honeycomb structure (Figures 2C and 2D).

**Discussion**

This study is the first to describe the existence of anti-UBE2T antibodies and their potential role as biomarkers to predict progression in IIP patients.

We found that anti-UBE2T antibody levels measured using ELISA were increased only in patients with idiopathic PF-ILD and not in those with non-PF-ILD or other ILDs, suggesting that this tool may predict the future progression of IIPs.

In this study, most patients presenting with PF-ILD had IPF (n=30; 73%). Most theories on IPF pathogenesis are based on mechanisms relating to epithelial cell apoptosis, tissue repair, and regeneration,(14) acknowledging little participation of autoimmune inflammatory responses. IPAF criteria do not include UIP patterns in the morphological domain, implying that IPF is far from having an autoimmune nature. However, replicated observations support an abnormal immune response and autoantibody production in IPF. CD3+ T cells, mature dendritic cells, and CD20+ B cells near the fibrotic foci with collagen deposition have been recognized repeatedly by many researchers(4, 5, 15). Abnormal CD4+ T cells expressing MHC class II and CD154+ proteins show clonal expansions in the sera of patients with IPF, with increased secretion of transforming growth factor-β1, interleukin-10, and tumor necrosis factor-α; 82% of these patients had circulating antibodies against cellular antigens.(6)

Regulatory T cells in IPF bronchoalveolar lavage fluid and blood are numerically and functionally impaired, indicating a deficiency of immunological self-tolerance.(16) T-cell activation against accessible self-antigens does not occur in healthy people.(17) Given that IPF is more frequent in smokers and in elderly people, the abundant autoantibodies against cellular autoantigens may be due to injury and senescence of the epithelial cells. The anti-UBE2T antibody was found in 76% (n=31) of IPF patients; to our knowledge, this prevalence is higher than that of every commercial autoantibody used in IPF.(18–20)

Ubiquitination plays an essential role in proteasome-mediated protein degradation and DNA repair, affecting the cell cycle and regulating signaling pathways.(21, 22) The process of ubiquitination involves the sequential action of activating, conjugating, and ligating enzymes that introduce an isopeptide link between the C-terminus of ubiquitin and target proteins.(22) UBE2T is one among the 35 types of ubiquitin-conjugating enzymes identified in humans.(22) UBE2T was initially identified as the possible cause of Fanconi anemia because it impairs DNA repair.(23) Overexpression of UBE2T has been observed in carcinomas in the lung, prostate, nasopharyngeal tissue, breast, and stomach.(24–28) UBE2T upregulation promotes epithelial cell proliferation and epithelial-mesenchymal transition *in vitro,*
whereas knockdown of UBE2T attenuates this process. (25, 26, 29) However, it remains unclear whether the UBE2T antibody has an effect on the epithelial cells, and further investigation is thus required.

In this study, the prevalence of HRCT-diagnosed honeycomb structure increased over time in the anti-UBE2T-positive group. We found that UBE2T was highly expressed in the lining epithelium of the honeycomb structure, where repeated DNA damage(30) and repair is expected to occur. The higher prevalence of IPF in the anti-UBE2T antibody-positive group may have affected the honeycomb structure frequency observed in the second-year follow-up data. However, considering that early stages of IPF lack the specific honeycomb structure and are often indistinguishable from those of other IIPs, the relation between elevated anti-UBE2T antibody levels and tissue remodeling may represent a breakthrough in identifying the future clinical course.

Older age, male sex, low baseline FVC or DLCO, and UIP patterns on HRCT are risk factors for mortality in ILDs.(12, 31) Although these parameters have been used to define PF-ILD in previous studies, a standardized definition of PF-ILD has not yet been established. One of the reasons why diagnosing PF-ILDs has been challenging is because the overtime trajectories of FVC and DLCO vary among patients and are often unpredictable. The INSIGHT-IPF registry(32) demonstrated that the FVC after 2 years of follow-up was similar between patients with or without antifibrotic therapy, despite mortality being higher in those who did not receive antifibrotic therapy. Conversely, in the INPULSIS study, IPF patients demonstrated a continuous decline in FVC. (33, 34) In our study, although the average change in FVC after 2 years remained small in both anti-UBE2T-positive and -negative patients (data not shown), PF-ILD was much more prevalent in those with a positive UBE2T status due to worsening of irreversible respiratory symptoms and continuous disruptions of the lung structure as seen on HRCT scans. Considering that predicting disease progression requires a combination of several parameters such as PFTs, HRCTs, or even 6-minute walking tests (which can be too strenuous for patients with exertional dyspnea), measuring circulating anti-UBE2T antibody levels can be an alternative, convenient tool.

Some of the past studies of IPAF have reported that the positivity of IPAF serological domain correlates with better prognosis in IIPs(31, 35). However, IPAF serological domain positivity in our data did not correlate with fewer PF-ILD development.

Multiple studies have supported the utility of serum KL-6 as a diagnostic, prognostic, active disease marker for ILDs.(36–38) In our study, KL-6 was elevated in patients with exacerbations and chronic progression, which is consistent with the findings of past reports. (36–38) High anti-UBE2T antibody levels were associated with the appearance of honeycomb structures that ultimately develop into PF-ILD. Although anti-UBE2T antibody and KL-6 both predict progression, the values of these two biomarkers did not correlate. Even though the function and origin of anti-UBE2T antibodies remain unclear, anti-UBE2T antibodies and KL-6 may be reflecting different pathological states. Thus, the anti-UBE2T antibody may accurately predict progressions that KL-6 or IPAF serological domain cannot detect.

This study had several limitations. First, clinical data were collected and analyzed retrospectively. Second, clinical data, such as information regarding exacerbations, PFT, HRCT, and treatments were limited in
some patients. Third, due to the small sample size, we combined IPF and NSIP cases into the IIP group, which increased the variability of clinical characteristics and prognoses. Fourth, the single-center nature of our study carries a risk of institutional bias. Currently, the concept of PF-ILD includes various lung disorders and pathophysiological processes. In this study, we mainly focused on analyzing limited target groups, especially IIPs. The study of a broader range of participants, such as CTD-ILD patients, is required for future research.

Conclusions

This is the first report to describe anti-UBE2T antibody, a new biomarker that is significantly elevated in progressive IIPs. This new antibody may constitute a sensitive biomarker to detect cases of progressive IIPs that are not currently detected by commercially available biomarkers.

Abbreviations

HP  
hypersensitivity pneumonitis
CTD  
connective-tissue disease
DLCO  
diffusing capacity for carbon monoxide
FVC  
forced vital capacity
GST  
glutathione-s-transferase
HRCT  
high-resolution computed tomography
IIP  
idiopathic interstitial pneumonia
ILDs  
interstitial lung diseases
IPAF  
interstitial pneumonia with autoimmune features
IPF  
idiopathic pulmonary fibrosis
non-PF-ILD  
non-progressive-fibrosing interstitial lung disease
NSIP  
non-specific pulmonary fibrosis
PF-ILD
progressive-fibrosing interstitial lung disease
PFT
pulmonary function tests
UBE2T
ubiquitin-conjugating enzyme E2T
UIP
usual interstitial pneumonia

Declarations

Ethics approval and consent to participate: The study protocol was approved by the institutional review board of Nihon University Itabashi Hospital (Institutional Review Board No. RK-150908-13, RK-151013-04, RK-180710-15). All patients gave written informed consent.

Consent for publications: Not applicable.

Availability of data and materials: applicable: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests: None.

Funding: This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2015–2019 (Project No. S1511014, awarded to YG).

Author's contributions: MH and YG contributed equally to this work. MH, YG, TO, TM, KM, SH, and SM had the idea for the study. MH and YG drafted the manuscript. All authors took part in recruiting patients and clinical management. MH, YG, KM, and SM were responsible for PF-ILD diagnosis and radiographic interpretation. MH, YG, TO, TM, KM, YK, NY, HH, TS, SH, and SM participated in analyzing the data regarding protein microarray, multiplex system, and ELISA assays. All authors contributed to and approved the final version of the manuscript.

Acknowledgements: The authors would like to thank the people with ILDs and care providers at Nihon University Itabashi Hospital for their contributions to this study.

References

1. Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, Richeldi L, Kolb M, Tetzlaff K, Stowasser S, Coeck C, Clerisme-Beaty E, Rosenstock B, Quaresma M, Haeufel T, et al. Nintedanib in Progressive Fibrosing Interstitial Lung Diseases. N Engl J Med. 2019;381(18):1718–27.
2. Maher TM, Corte TJ, Fischer A, Kreuter M, Lederer DJ, Molina-Molina M, Axmann J, Kirchgaessler KU, Samara K, Gilberg F, Cottin V. Pirfenidone in patients with unclassifiable progressive fibrosing
interstitial lung disease: a double-blind, randomised, placebo-controlled, phase 2 trial. The Lancet Respiratory medicine. 2020;8(2):147–57.

3. Kinder BW, Collard HR, Koth L, Daikh DI, Wolters PJ, Elicker B, Jones KD, King TE. Jr. Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease? Am J Respir Crit Care Med. 2007;176(7):691–7.

4. Todd NW, Scheraga RG, Galvin JR, Iacono AT, Britt EJ, Luzina IG, Burke AP, Atamas SP. Lymphocyte aggregates persist and accumulate in the lungs of patients with idiopathic pulmonary fibrosis. Journal of inflammation research. 2013;6:63–70.

5. Marchal-Somme J, Uzunhan Y, Marchand-Adam S, Kambouchner M, Valerye D, Crestani B, Soler P. Dendritic cells accumulate in human fibrotic interstitial lung disease. Am J Respir Crit Care Med. 2007;176(10):1007–14.

6. Feghali-Bostwick CA, Tsai CG, Valentine VG, Kantrow S, Stoner MW, Pilewski JM, Gadgil A, George MP, Gibson KF, Choi AM, Kaminski N, Zhang Y, Duncan SR. Cellular and humoral autoreactivity in idiopathic pulmonary fibrosis. Journal of immunology (Baltimore, Md: 1950). 2007;179(4):2592-9.

7. Travis WD, Costabel U, Hansell DM, King TE Jr, Lynch DA, Nicholson AG, Ryerson CJ, Ryu JH, Selman M, Wells AU, Behr J, Bouros D, Brown KK, Colby TV, Collard HR, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188(6):733–48.

8. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, Behr J, Cottin V, Danoff SK, Morell F, Flaherty KR, Wells A, Martinez FJ, Azuma A, Bice TJ, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(5):e44–68.

9. Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vasakova M, Bargagli E, Chung JH, Collins BF, Bendstrup E, Chami HA, Chua AT, Corte TJ, Dalphin JC, Danoff SK, et al. Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2020;202(3):e36–69.

10. Nasser M, Larrieu S, Si-Mohamed S, Ahmad K, Boussel L, Brevet M, Chalabreysse L, Fabre C, Marque S, Revel D, Thivolet-Bejui F, Traclet J, Zeghmar S, Maucort-Boulch D, Cottin V. Progressive fibrosing interstitial lung disease: a clinical cohort (the PROGRESS study). Eur Respir J. 2021;57(2):2002718.

11. Krumkamp R, Struck NS, Lorenz E, Zimmermann M, Boahen KG, Sarpong N, Owusu-Dabo E, Pak GD, Jeon HJ, Marks F, Jacobs T, May J, Eibach D. Classification of invasive bloodstream infections and Plasmodium falciparum malaria using autoantibodies as biomarkers. Scientific reports. 2020;10(1):21168.

12. Ryerson CJ, Vittinghoff E, Ley B, Lee JS, Mooney JJ, Jones KD, Elicker BM, Wolters PJ, Koth LL, King TE Jr, Collard HR. Predicting survival across chronic interstitial lung disease: the ILD-GAP model. Chest. 2014;145(4):723–8.
13. Fischer A, Antoniou KM, Brown KK, Cadranel J, Corte TJ, du Bois RM, Lee JS, Leslie KO, Lynch DA, Matteson EL, Mosca M, Noth I, Richeldi L, Strek ME, Swigris JJ, et al. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur Respir J. 2015;46(4):976–87.

14. Phan THG, Paliogiannis P, Nasrallah GK, Giordo R, Eid AH, Fois AG, Zinellu A, Mangoni AA, Pintus G. Emerging cellular and molecular determinants of idiopathic pulmonary fibrosis. Cell Mol Life Sci. 2021;78(5):2031–57.

15. DePianto DJ, Chandriani S, Abbas AR, Jia G, N'Diaye EN, Caplazi P, Kauder SE, Biswas S, Karnik SK, Ha C, Modrusan Z, Matthay MA, Kukreja J, Collard HR, Egen JG, et al. Heterogeneous gene expression signatures correspond to distinct lung pathologies and biomarkers of disease severity in idiopathic pulmonary fibrosis. Thorax. 2015;70(1):48–56.

16. Kotsianidis I, Nakou E, Bouchliou I, Tzouvelekis A, Spanoudakis E, Steiropoulos P, Sotiriou I, Aidinis V, Margaritis D, Tsatalas C, Bouros D. Global impairment of CD4+CD25+FOXP3+ regulatory T cells in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009;179(12):1121–30.

17. Monaco C, Andreakos E, Kiriakidis S, Feldmann M, Paleolog E. T-cell-mediated signalling in immune, inflammatory and angiogenic processes: the cascade of events leading to inflammatory diseases. Current drug targets Inflammation allergy. 2004;3(1):35–42.

18. Lee JS, Kim EJ, Lynch KL, Elicker B, Ryerson CJ, Katsumoto TR, Shum AK, Wolters PJ, Cerri S, Richeldi L, Jones KD, King TE Jr, Collard HR. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. Respiratory medicine. 2013;107(2):249–55.

19. Kang BH, Park JK, Roh JH, Song JW, Lee CK, Kim M, Jang SJ, Colby TV, Kim DS. Clinical significance of serum autoantibodies in idiopathic interstitial pneumonia. J Korean Med Sci. 2013;28(5):731–7.

20. Ghang B, Lee J, Chan Kwon O, Ahn SM, Oh JS, Hong S, Kim YG, Yoo B, Jeong WS, Kim J, Lee CK. Clinical significance of autoantibody positivity in idiopathic pulmonary fibrosis. Respiratory medicine. 2019;155:43–8.

21. Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. Nature medicine. 2014;20(11):1242–53.

22. Alpi AF, Chaugule V, Walden H. Mechanism and disease association of E2-conjugating enzymes: lessons from UBE2T and UBE2L3. Biochem J. 2016;473(20):3401–19.

23. Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D’Andrea AD, Dutta A. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Molecular cell. 2006;23(4):589–96.

24. Wen M, Kwon Y, Wang Y, Mao JH, Wei G. Elevated expression of UBE2T exhibits oncogenic properties in human prostate cancer. Oncotarget. 2015;6(28):25226–39.

25. Hu W, Xiao L, Cao C, Hua S, Wu D. UBE2T promotes nasopharyngeal carcinoma cell proliferation, invasion, and metastasis by activating the AKT/GSK3beta/beta-catenin pathway. Oncotarget. 2016;7(12):15161–72.
26. Yu H, Xiang P, Pan Q, Huang Y, Xie N, Zhu W. Ubiquitin-Conjugating Enzyme E2T is an Independent Prognostic Factor and Promotes Gastric Cancer Progression. Tumour biology: the journal of the International Society for Oncodevelopmental Biology Medicine. 2016;37(9):11723–32.

27. Ueki T, Park JH, Nishidate T, Kijima K, Hirata K, Nakamura Y, Katagiri T. Ubiquitination and downregulation of BRCA1 by ubiquitin-conjugating enzyme E2T overexpression in human breast cancer cells. Cancer research. 2009;69(22):8752–60.

28. Liu J, Liu X. UBE2T silencing inhibited non-small cell lung cancer cell proliferation and invasion by suppressing the wnt/β-catenin signaling pathway. Int J Clin Exp Pathol. 2017;10(9):9482–8.

29. Luo C, Yao Y, Yu Z, Zhou H, Guo L, Zhang J, Cao H, Zhang G, Li Y, Jiao Z. UBE2T knockdown inhibits gastric cancer progression. Oncotarget. 2017;8(20):32639–54.

30. Schuliga M, Pechkovsky DV, Read J, Waters DW, Blokland KEC, Reid AT, Hogaboam CM, Khalil N, Burgess JK, Prêle CM, Mutsaers SE, Jaffar J, Westall G, Grainge C, Knight DA. Mitochondrial dysfunction contributes to the senescent phenotype of IPF lung fibroblasts. J Cell Mol Med. 2018;22(12):5847–61.

31. Oldham JM, Adegunsoye A, Valenzi E, Lee C, Witt L, Chen L, Husain AN, Montner S, Chung JH, Cottin V, Fischer A, Noth I, Vij R, Strek ME. Characterisation of patients with interstitial pneumonia with autoimmune features. Eur Respir J. 2016;47(6):1767–75.

32. Behr J, Prasse A, Wirtz H, Koschel D, Pittrow D, Held M, Klotsche J, Andreas S, Claussen M, Grohé C, Wilkens H, Hagmeyer L, Skowasch D, Meyer JF, Kirschner J, et al. Survival and course of lung function in the presence or absence of antifibrotic treatment in patients with idiopathic pulmonary fibrosis: long-term results of the INSIGHTS-IPF registry. Eur Respir J. 2020;56(2):1902279.

33. Azuma A, Taniguchi H, Inoue Y, Kondoh Y, Ogura T, Homma S, Fujimoto T, Sakamoto W, Sugiyama Y, Nukiwa T. Nintedanib in Japanese patients with idiopathic pulmonary fibrosis: A subgroup analysis of the INPULSIS(R) randomized trials. Respirology. 2017;22(4):750–7.

34. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, Cottin V, Flaherty KR, Hansell DM, Inoue Y, Kim DS, Kolb M, Nicholson AG, Noble PW, Selman M, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014;370(22):2071–82.

35. Enomoto N, Homma S, Inase N, Kondoh Y, Saraya T, Takizawa H, Inoue Y, Ishii H, Taguchi Y, Izumi S, Yamano Y, Tanino Y, Nishioka Y, Toyoshima M, Yokomura K, et al. Prospective nationwide multicentre cohort study of the clinical significance of autoimmune features in idiopathic interstitial pneumonias. Thorax. 2021.

36. Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. Curr Opin Pulm Med. 2012;18(5):441–6.

37. Ishikawa N, Hattori N, Yokoyama A, Kohno N. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. Respiratory investigation. 2012;50(1):3–13.

38. Nobuo K, Seishi K, Yukikazu A, Hirofumi F, Michio Y, Mitoshi A. New Serum Indicator of Interstitial Pneumonitis Activity: Sialylated Carbohydrate Antigen KL-6. Chest. 1989;96(1):68–73.
Figure 1

Comparison of serum anti-UBE2T antibody levels in various lung diseases. Anti-UBE2T antibody levels were significantly higher in the PF-ILD group than the healthy control group. HP, chronic hypersensitive pneumonitis; PF-ILD, progressive-fibrosing idiopathic interstitial pneumonia.
Figure 2

Immunohistochemistry of UBE2T in lung specimens. A, B: Healthy subject. UBE2T is observed sparsely in the bronchiole epithelium and macrophages. C, D: Usual interstitial pneumonia. UBE2T is expressed in the lining epithelium of the honeycomb structure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- renamed98ad5.docx