Serum folliculin is related to lower pulmonary function in patients with asthma

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To the Editor,

Folliculin, a protein expressed in various types of cells including airway epithelial cells, encoded by the *FLCN* gene, is associated with the 5' AMP-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1) signaling pathways and, it is thought to alter cell-to-cell adhesion and contribute to the pathogenesis of cystic lung disease in Birt-Hogg-Dubé syndrome (1-5). In addition, the gene*FLCN* regulates the E-cadherin-LKB1-AMPK axis, which controls lung epithelial cell survival and alveoli size (2). In a recent study, serum folliculin levels were found to be higher in patients with asthma than in healthy control groups and high folliculin levels were associated with increased airway hyperresponsiveness in patients with asthma. In vitro data demonstrated the eosinophil-induced release of folliculin from epithelial cells. These clinical and in vitro observations suggest that folliculin may play some role in the interaction between the eosinophils and airway epithelium (6).

To investigate the relationship between clinical characteristics and the level of folliculin in asthmatics, the data of a total of 404 patients with asthma and 94 of controls were enrolled and retrospectively reviewed. To correct for the heavily skewed distributions of the serum folliculin levels, values were log-transformed. Study methods, design and definitions used can be found in the online supplement (Study S1).

The proportions of males and smokers were significantly higher among the patients with asthma than in the controls, and the mean serum folliculin level in asthmatics was significantly higher than that in controls (4.80 pg/mL versus 4.13 ng/mL; $P < 0.001$) (Table S1). As the control group was comprised only of males and a significant difference in smoking history was noted, adjusting for sex and smoking history was performed and significantly higher serum folliculin levels were still observed ($P < 0.001$). We compared the serum folliculin levels between asthmatics and controls subdivided by sex and smoking status. In these subgroups, the serum folliculin levels were still significantly higher in asthmatics than in the control group (Table S2). ROC curve analysis revealed a significant difference in serum folliculin levels between asthmatics and controls (area under the curve = 0.846, confidence interval [CI] 0.80–0.89, $P < 0.001$); the optimal cut-off value of serum folliculin level that distinguished asthma patients from controls was 4.31 pg/mL after log-transformation, correlating with 83.91% sensitivity and 77.66% specificity (Figure 1). When we perform ROC curve analysis with only the males, the optimal cut-off value of serum folliculin level was 4.33 pg/mL (Figure S1).

We compared folliculin levels among the four groups divided by pre- pre-bronchodilator (BD) predicted FEV1 (%) and found a significant difference in serum folliculin level ($P < 0.001$, Figure S2). Simple and multiple linear regression analysis was performed to determine the correlation between serum folliculin level and lung function in patients with asthma. In simple linear regression analysis, serum folliculin level were significantly correlated with pre-BD FEV1% predicted (β-coefficient = -4.848, $P = 0.013$), however significance was only marginal after adjusting for age and sex (β-coefficient = -3.199,$P = 0.096$) in multiple linear regression analysis. This is because, firstly, there was collinearity of folliculin level and age in our data, and secondly, the rate of smokers (85.22%) among males was higher than among females (14.91%), so it seems that lung function in females is higher.

Patients with asthma were divided into two groups using the mean value of the logarithmic serum folliculin levels (4.80 pg/mL). Patients in the high-folliculin group were older at the onset of symptoms, heavier smokers and had a significantly lower lung function. The number of acute exacerbations occurring per year was more frequent in the high folliculin group than in the low folliculin group, but no statistical significance was noted (Table 1). When patients with asthma were divided into the upper quartile of folliculin levels and the lower three quartiles combined, those from the high folliculin group in the upper 25 percentile were found to be older and had lower atopy and lung function than the lower folliculin group with the lower 75 percentile combined. (Table S3). Likewise, we also divided the patients into 4 quantile groups according to serum folliculin levels and identified differences in each group in lung function and age (Table S4).

A previous in vitro study showed that human airway epithelial cells (HAECs) exposed to leukotriene E4 and peripheral blood eosinophils released folliculin and interleukin (IL)-8, which resulted in the destruction of the integrity of the epithelial cells. The knockdown of folliculin expression resulted in a decrease in IL-8 release and suppression of epithelial cell activation, which restored the epithelial integrity in HAECs. In their study, folliculin was suggested to be associated with a higher serum transforming growth factor-β1 level, which was associated with worsening of airway inflammation and remodeling (6,7). Consistent with theses result, a higher serum folliculin level in patients with asthma than in healthy controls was also observed in our study, in addition, we showed that an increase in serum folliculin level was associated with a decrease in basal lung function. As folliculin is released from bronchial epithelial cells in response to compressive stress that mimics a bronchospasm (8, 9), we postulate that chronic airway inflammation produces mechanical stress on the airway epithelium, thereby inducing oxidative damage and release of folliculin with changes in the epithelial cell structure. Therefore, we assume that folliculin is associated with the airway inflammation and remodeling pathway in patients with asthma. In our study, serum folliculin level showed no association with serum laboratory variables, suggesting that the increase in folliculin level following mechanical stress is independent of other serum inflammatory markers.

In conclusion, our study demonstrates for the first time that serum folliculin concentration is higher in patients with asthma, and it is associated with worse lung function independent to other serum inflammatory markers. Thus, folliculin may represent a novel biomarker related to lower pulmonary function in patients with asthma and further studies are warranted to evaluate the mechanism and test our hypothesis.

Keywords: asthma; folliculin; biomarker

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to declare.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

TABLE

Table 1 Clinical characteristics of asthma patients with low and high log-transformed folliculin levels

	Low folliculin group	**High folliculin group**	*P* **value**
	(n = 210)	**(n = 194)**	
Log [folliculin level] (pg/mL)*	4.4 ± 0.3	5.2 ± 0.4	**< 0.001**
Age (mean ± SD, years)	48.8 ± 14.2 (n = 206)	53.1 ± 14.7 (n = 188)	**0.003**
Sex (n, % male)	42.72 (n = 88)	46.81 (n = 88)	0.475
BMI (kg/m^2)	23.7 ± 3.3 (n = 202)	23.8 ± 3.0 (n = 187)	0.688
Age of diagnosis (year)	43.8 ± 15.7 (n = 196)	46.1 ± 17.5 (n = 177)	0.091
Age of symptom onset (year)	38.6 ± 16.3 (n = 202)	42.7 ± 17.6 (n = 185)	**0.018**
Smoking (pack-years)	7.2 ± 13.3 (n = 197)	10.6 ± 18.0 (n = 182)	**0.043**
Smoker [a)] (%)	45.7 (n = 90)	49.5 (n = 90)	0.528
Smoking status (%)			0.615
Never-smoker	54.3 (n = 107)	50.6 (n = 92)	
Ex-smoker	31.5 (n = 62)	36.3 (n = 66)	
Current smoker	14.2 (n = 28)	13.2 (n = 24)	
Skin prick test (% positive, n/total)	42.4 (n = 64)	34.5 (n = 39)	0.242
Acute exacerbation, past 1 year (yes, %)	17.5 (n = 36)	13.8 (n = 26)	0.393
Acute exacerbation (/1 year)	3.3 ± 5.0 (n = 53)	4.4 ± 8.3 (n = 70)	0.349
Pre-BD FEV1(%)	73.5 ± 19.8 (n = 201)	69.1 ± 22.8 (n = 183)	**0.045**
Pre-BD FVC (%)	88.5 ± 18.6 (n = 201)	84.3 ± 18.4 (n = 183)	**0.026**
Pre-BD FEV1/FVC	0.69 ± 0.13 (n = 202)	0.67 ± 0.16 (n = 184)	0.098
Post-BD FEV1 predicted (%)	76.3 ± 21.4 (n = 98)	70.0 ± 22.0 (n = 117)	**0.034**
Post-BD FVC predicted (%)	89.4 ± 18.2 (n = 98)	83.6 ± 17.4 (n = 117)	**0.018**
Post-BD FEV1/FVC predicted	0.69 ± 0.13 (n = 98)	0.67 ± 0.19 (n = 117)	0.507
PC20	4.5 ± 5.0 (n = 89)	5.6 ± 6.3 (n = 59)	0.260
Blood eosinophils (%)	5.6 ± 5.6 (n = 177)	4.7 ± 4.8 (n = 148)	0.131
Blood eosinophil count (cells)	396.6 ± 445.0 (n = 177)	336.2 ± 334.9 (n = 148)	0.164
Neutrophils (%), sputum	44.7 ± 37.7 (n = 24)	39.0 ± 34.5 (n = 29)	0.557
Eosinophils (%), sputum	18.9 ± 27.0 (n = 24)	14.4 ± 24.3 (n = 29)	0.495
CRP (mg/dL)	0.6 ± 1.4 (n = 111)	0.6 ± 1.6 (n = 104)	0.902
Serum total IgE (IU/mL)	362.5 ± 490.6 (n = 60)	589.5 ± 1034.1 (n = 53)	0.161

BMI, body mass index; pre-BD FEV1, pre-bronchodilator forced expiratory volume in 1 s; pre-BD FVC, pre-bronchodilator forced vital capacity; post-BD FEV1, post-bronchodilator forced expiratory volume in 1 s; post-BD FVC, post-bronchodilator forced vital capacity; PC20, methacholine provocative concentration causing a 20% drop in FEV1; CRP, C-reactive protein.

Low folliculin group: patients with log transformed folliculin level < 4.80.

High folliculin group: patients with log transformed folliculin level [?] 4.80.

[a)] The combination of current smokers and ex-smokers. Each percentage is calculated after excluding patients with unknown smoking status.

Acute exacerbation, previous 1 year: patients who had an acute exacerbation at least once during the previous 1 year at the time of enrollment.

Acute exacerbation (/1 year): the average number of acute exacerbations that occurred during the first 3 years after enrollment.

P values are based on the$t$ -test and Wilcoxon test for continuous variables and the chi-square test for categorical variables. P values less than 0.05 are in bold.

*Statistical significance defined as $P < 0.05$.

FIGURE LEGENDS

Figure 1 . Receiver operating characteristic (ROC) curve of serum folliculin level; area under the curve = 0.846, confidence interval [CI] 0.80–0.89,$P < 0.001$. The cut-off value of serum folliculin level that discriminated patients with asthma from healthy controls was 74.45 pg/mL (4.31 pg/mL after log-transformation) with 83.91% sensitivity and 77.66% specificity.

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