

# **DISCUSSION**

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Asthma is a chronic inflammatory disorder of the airways characterized by variable and reversible airflow obstruction and airway hyper-responsiveness (AHR). It is thought to be caused by a combination of genetic and environmental factors.<sup>(5)</sup> Its diagnosis is usually made based on the pattern of symptoms and/or response to therapy over time.<sup>(6)</sup>

A key feature of asthmatic airways is remodeling which involves thickening of the airway wall, altered deposition of extracellular matrix (ECM) proteins<sup>(177,178)</sup> and increased airway smooth muscle (ASM) mass. These structural changes may result from an aberrant repair process in the lung, which includes increased proliferation of the ASM cells.<sup>(179,180)</sup> The ECM maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function.<sup>(181)</sup> The ECM deposited by asthma derived ASM cells is altered such that increased amounts of collagen I and laminin<sup>(182-184)</sup>, as well as fibronectin (FN) are produced which mediate a range of cellular interactions including migration, growth and differentiation.

The fibulins are a family of proteins that are associated with basement membranes and elastic extracellular matrix fibers. They are minimally defined as having a series of epidermal growth factor (EGF)-like modules, followed by a carboxy-terminal fibulin-type module. Fibulins are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular ECM structures.

The first member of the fibulin family — now referred to as fibulin-1 — was identified in affinity chromatography experiments.<sup>(47)</sup> The main component that was isolated from human placenta had a molecular mass of 90–100 kDa (from electrophoresis measurements), was shown to bind calcium and was considered to be a connecting element between  $\beta$ 1 integrins and some cytoskeletal structures. It shows a distinct arrangement of typical ECM modules that are grouped together as domains I, II and III.<sup>(49,58)</sup> (FIG. I).

The amino terminal domain I consists of three anaphylatoxin-like (AT) modules. The central domain II is composed of nine epidermal growth factor (EGF)-like modules. The carboxy-terminal domain III resembles a new protein module (FC; fibulin-type carboxyl terminus) that is shared by fibulins and fibrillins at the carboxyl terminus.<sup>(54)</sup>

Four isoforms of FBLN-1 have been identified to date in humans, designated FBLN-1A, 1B, 1C, and 1D (Figure III).<sup>(68)</sup>

Fibulin-1 is incorporated into FN-containing matrix fibers. It plays a role in cell adhesion and migration along protein fibers within the ECM and is important for certain developmental processes. Fibulin-1 contributes to the supramolecular organization of ECM architecture, in particular to that of the basement membrane. It is implicated in cellular transformation and tumor invasion, and can behave both as an oncosuppressor and oncogene depending on tissue environment. It also plays a role in hemostasis and thrombosis owing to its ability to bind fibrinogen and incorporate into clots and plays a significant role in modulating the neurotrophic activities of APP, particularly soluble APP.<sup>(48)</sup>

Fibulin-1 (FBLN-1), a secreted glycoprotein, assists in stabilizing the ECM. It associates with FN and a variety of other ECM proteins including laminin and fibrinogen.<sup>(105)</sup> Mice deficient in FN and FBLN-1 die perinatally due to abnormal lung development.<sup>(128)</sup> FBLN-1 expression has been reported in human lung tissue using microarray technology.<sup>(189,190)</sup>

The present study aimed at investigation of possible association between the fibulin-1 levels in asthmatic patients and its relation to asthma severity.

The study was carried out on forty five asthmatic patients with different degrees of asthma severity and thirty age and sex- matched normal control subjects.

According to our results, we found that fibulin-1 increased in serum of asthmatic patients without relation to asthma severity and highly increased in BAL of severe degree asthmatic patients than in mild and moderate degrees. So, fibulin-1 in BAL can be used as a marker in diagnosis of severe degrees of bronchial asthma.

Transforming growth factor beta 1 (TGF- $\beta$ 1) is a pro-fibrotic cytokine which is increased in several forms of acute and chronic adult lung diseases such as asthma<sup>(185)</sup>, COPD<sup>(196,197)</sup>, and pulmonary fibrosis.<sup>(198,199)</sup> It is considered to play a crucial role in the pathogenesis of tissue fibrosis, stimulating the production of various collagens and ECM proteins.<sup>(200,201,202)</sup> Disruption of the TGF- $\beta$ 1 gene in mice results in an extensive infiltrate of lymphocytes and plasma cells in various organs including the lungs, leading to tissue necrosis and death of the animals. This suggests that TGF- $\beta$ 1 plays a critical role in situ in lung homeostasis.<sup>(203,204)</sup> Although TGF- $\beta$ 1 has been detected in BAL of normal human individuals<sup>(205)</sup>, the location of this cytokine has not yet been demonstrated in normal human lung, nor has the nature of the cells containing TGF- $\beta$ 1 been established. The highest concentration for TGF- $\beta$ 1 was seen in ciliary epithelial cells. Smooth muscle cells, both bronchial and vascular, also contained TGF- $\beta$ 1, although to a lesser extent than epithelial cells. In endothelial cells, pleural cells, fibroblasts, and alveolar epithelial cells TGF- $\beta$ 1 was not detectable.

As alveolar macrophages can synthesise TGF- $\beta$ 1 *in vitro*<sup>(206,207)</sup>, it has been suggested that this cell population is the main source of TGF- $\beta$ 1 in airways. A number of other cell types are potential producers of TGF- $\beta$ 1, however, including lymphocytes, monocytes, macrophages, neutrophil and eosinophil polymorphonuclear cells, epithelial cells, and mesenchymal cells.<sup>(208-211)</sup> The regulation of ECM production by TGF is often different between primary mesenchymal lung cells from people with fibrotic lung diseases in comparison to those without.

TGF- $\beta$ 1 is a pleiotropic cytokine with various immunological properties which are potentially relevant to lung physiology. Some of these are immunosuppressive, imbalanced by other effects on immune cell differentiation. TGF- $\beta$ 1 inhibits the macrophage cytotoxicity and monocyte synthesis of cytokines, but it respects the macrophage capacity of phagocytosis. It downregulates the proliferation of lymphocytes and limits their responses to cytokines.<sup>(212-218)</sup> One can therefore speculate that TGF- $\beta$ 1 in distal airways might allow macrophages to clear pathogens without inducing unwarranted immune reactions and excessive lymphocyte activation. In addition, as it favours a Th1 cytokine secretion profile<sup>(219)</sup>, TGF- $\beta$ 1 could prevent pulmonary hypersensitivity reactions.<sup>(220)</sup> By stimulating maturation of T lymphocytes into memory cells<sup>(221)</sup>, it could regulate the phenotype and

function of pulmonary resting T cells.<sup>(222)</sup> Also, by triggering B cell differentiation into IgA-producing cells<sup>(223)</sup>, TGF- $\beta$ 1 could contribute to the IgA-mediated local response.<sup>(224)</sup> Other properties of TGF- $\beta$ 1, concern tissue homeostasis and repair and are also potentially relevant in the lung. TGF- $\beta$ 1 strongly inhibits epithelial cell proliferation in vitro. It stimulates squamous differentiation of bronchial epithelial cells<sup>(225)</sup>, and also triggers the expression of extracellular matrix components in various kinds of cells including pulmonary fibroblasts.<sup>(208,209)</sup> Thus, TGF- $\beta$ 1 probably contributes to the maintenance of the lung architecture and the regulation of local repair processes.

Levels of the pro-fibrotic cytokine TGF- $\beta$ 1 are elevated in the BAL fluid in asthma<sup>(195)</sup>, and are increased in bronchial tissue.<sup>(185)</sup>

TGF- $\beta$ 1 increased the expression of FBLN-1 in ASM cells. The increased FBLN-1 resulted in exaggerated proliferation and wound repair in asthma derived ASM cells.

TGF- $\beta$ 1 not only increased FBLN-1 in asthma derived ASM cells, but also enhanced its deposition in the asthmatic ECM. The architecture of the asthmatic airway often undergoes prominent and permanent structural changes, including alterations of the molecular composition of the ECM. In particular, ECM protein deposition is increased in the lamina reticularis<sup>(226)</sup>, resulting in basement membrane thickening<sup>(227)</sup>. Given that TGF- $\beta$ 1 levels may alter the ECM assembly process, it is likely that the raised TGF- $\beta$ 1 levels in the asthmatic airway may contribute to an increase in the ECM thereby augmenting the effects of airway remodeling.

AHR is a hallmark feature of asthma that has been linked to the pathological events associated with remodeling of the airway wall. TGF $\beta$ -1 has also been linked to the mechanism underpinning AHR in asthma. In particular, TGF- $\beta$ 1 administration to the airways in mice induces peribronchial fibrosis that results in the development of AHR.<sup>(228)</sup> When antisense oligomer targeting FBLN-1 (AOs) was used in mice treatment, TGF- $\beta$ 1 induced AHR was inhibited demonstrating a critical role for FBLN-1 in the mechanism of TGF- $\beta$ 1 induced AHR. The regulated expression of FBLN-1 downstream of inflammation and its regulation of the remodeling/AHR axis identify the therapeutic potential of targeting this glycoprotein.

It is still unknown whether TGF- $\beta$ 1 induced the deposition of FBLN-1 into the ECM directly through the activation of the cell surface receptors such as integrins, or if TGF- $\beta$ 1 increases other ECM proteins, which may bind to soluble FBLN-1 and anchor it in the ECM. In support of the possibility of integrin involvement, it is known that TGF- $\beta$ 1 increased FN deposition occurs via an integrin receptor on human ASM cells.<sup>(200)</sup> However, it is also known that FBLN-1 is not incorporated into the ECM by cells which fail to assemble FN<sup>(144)</sup>, indicating that these two proteins have a critical interaction in other cell systems.

While FBLN1 is normally found in the blood, there is not much known about how the body regulates FBLN1 levels or where the molecule comes from. TGF- $\beta$ 1 causes increased release of soluble FBLN1 from airway epithelial cells, which may contribute to the increased levels found in the serum and BAL of people with asthma.

## ***Discussion***

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Lau JY, et al. <sup>(229)</sup> found that FBLN-1 is increased in the serum and BAL of people with asthma, and shown that FBLN-1 regulates airway smooth muscle (ASM) cell proliferation, therefore highlighting the potential role of FBLN-1 in airway wall remodeling.

They also found that there was no correlation between the FBLN-1 level detected in the serum or BAL from the asthmatic volunteers and their FEV1/FVC ratio.

In contrast, Chen L, et al. <sup>(230)</sup> found that TGF- $\beta$ 1 decreased FBLN-1 mRNA, and that FBLN-1 protein production was controlled. Therefore it was hypothesized that the increased ECM FBLN-1 following TGF- $\beta$ 1 stimulation was due to sequestration of soluble FBLN-1 and not de-novo synthesis.

# **SUMMARY**

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Bronchial asthma is the common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and bronchospasm.

Asthma is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV1), and peak expiratory flow rate (PEFR).

A key feature of asthmatic airways is remodeling which involves thickening of the airway wall, altered deposition of extracellular matrix (ECM) proteins and increased airway smooth muscle (ASM) mass. These structural changes may result from an aberrant repair process in the lung, which includes increased proliferation of the ASM cells.

The fibulins are a family of proteins that are associated with basement membranes and elastic extracellular matrix fibers.

The fibulins are minimally defined as having a series of epidermal growth factor (EGF)-like modules, followed by a carboxy-terminal fibulin-type module.

Fibulins are hypothesized to function as intramolecular bridges that stabilize the organization of supramolecular ECM structures, such as elastic fibers and basement membranes.

Fibulin-1 was the first family member to be identified and it shows a distinct arrangement of typical ECM modules that are grouped together as domains I, II and III.

The synthesis of fibulin-1 starts at early stages of embryonic development and it is a constituent of most basement membranes in the avian embryo.

Fibulin-1 is incorporated into FN-containing matrix fibers. It plays a role in cell adhesion and migration along protein fibers within the ECM and is important for certain developmental processes.

Four isoforms of FBLN-1 have been identified to date in humans, designated FBLN-1A, 1B, 1C, and 1D.

Fibulin-1 is widely expressed in elastic fibers and the ECM of Fibulin-1 can be detected in human serum at relatively high concentrations (10–50 µg/ml).

Several studies have reported that fibulin-1 is overexpressed in various human neoplasias and it is implicated in processes such as invasion, motility, and in vivo tumor growth. Fibulin-1 inhibits in vitro adhesion and motility of various carcinoma cell lines.

Fibulin-1 (FBLN-1) assists in stabilizing the ECM which maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function.

The present study aimed at investigation of possible association between the fibulin-1 levels in asthmatic patients and its relation to asthma severity.

## *Summary*

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The study was carried out on forty five asthmatic patients and thirty age and sex-matched normal control subjects.

It was found that fibulin-1 increased in serum of asthmatic patients but without relation to asthma severity and it also increased in BAL of severe degree bronchial asthma than in mild and moderate degrees.

# CONCLUSIONS

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Fibulin-1, a secreted glycoprotein, plays a role in cell adhesion and migration along protein fibers within the ECM and is important for certain developmental processes.

It assists in stabilizing the ECM which maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function.

Fibulin-1 was found in two forms, soluble and tissue form.

It was found that fibulin-1 increased in serum of asthmatic patients but without relation to asthma severity and it also increased in BAL of severe degree bronchial asthma than in mild and moderate degrees.