HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2017 March 01.

Published in final edited form as:

J Allergy Clin Immunol. 2016 March ; 137(3): 767–73.e6. doi:10.1016/j.jaci.2015.05.053.

Airway Factor XIII associates with type-2 inflammation and airway obstruction in asthma

Stephane Esnault, PhD[1], **Elizabeth A. Kelly, PhD**[1], **Ronald L. Sorkness, PhD**[2], **Michael D. Evans, MS**[3], **William W. Busse, MD**[1], and **Nizar N. Jarjour, MD.**[1,*]

[1]Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

[2]The School of Pharmacy, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

[3]Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

Abstract

Background—Coagulation factor XIII (FXIII) plays an important role in wound healing by stabilizing fibrin clots and crosslinking extracellular matrix proteins. FXIII is expressed in cells of the monocyte/macrophage and dendritic cell lineages in response to type-2 cytokines.

Objective—We sought to determine the association between FXIII and asthma pathobiology.

Methods—We analyzed the expression of FXIII mRNA and protein level in bronchoalveolar lavages obtained before and after segmental allergen challenge from mild asthma subjects, and in induced sputum samples collected from subjects with mild-moderate and severe asthma.

Results—FXIII mRNA and protein were highly upregulated in bronchoalveolar cells and fluid after allergen challenge and mRNA level correlated with protein amount. In sputum of asthmatic subjects, FXIII was positively correlated with type-2 immune response and markers of the dendritic cells (CD209 and CD207). FXIII expression was also associated with increased airflow limitation (FEV_1/FVC and RV/TLC) and greater reversibility to β-agonist.

Conclusions—FXIII was upregulated in the airway of asthma subjects after allergen exposure. Its expression in the sputum of asthma patients correlated with the type-2 immune response and airflow limitation. Excessive activity of FXIII could contribute to the pathophysiology of airway obstruction in asthma.

Keywords

Factor XIII; asthma; severe asthma; allergy; pulmonary function; airway obstruction; air trapping; inflammation; eosinophils; IL-13

[*]Corresponding author: Nizar N Jarjour, Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, CSC K4/928, Madison, WI 53792-9988, nnj@medicine.wisc.edu, Tel: +1 608-263-3035; FAX: +1 608-263-3104.

Conflicts of interest: none

This article has online Supporting Information

Introduction

Asthma is characterized by both airway inflammation and remodeling. Airway remodeling is reflected by thickening of the reticular basement membrane, mucus gland hypertrophy, deposition of extracellular matrix, increased smooth muscle mass and angiogenesis [1–4]. Collectively, these changes contribute to persistent airflow limitation and asthma severity.

Airway inflammation is associated with plasma extravasation, and the exposure of plasma to tissue factor triggers a cascade of coagulation factors that leads to thrombin activation and fibrin clotting [reviewed in [5]]. We and others have reported increased thrombin activity in airways of patients with asthma [6, 7]. Wagers *et al.* have observed fibrin deposition in the airway of a patient who died in status asthmaticus [8]. Mechanistically, the study by Wagers *et al.* suggested that fibrin reduces surfactant function which then ultimately leads to airway closure and hyperresponsiveness. In severe asthma, the pro-fibrinogenic pathway is increased compared with less severe disease [9, 10].

Factor XIII (FXIII) covalently cross-links fibrin at the end of the coagulation cascade. FXIII is a transglutaminase present extracellularly as plasma FXIII (pFXIII), and intracellularly as cellular FXIII (cFXIII). pFXIII is activated by thrombin and possesses a multitude of substrates that participate in the stability of the fibrin clot during the wound healing process [11, 12]. These substrates include fibronectin, thrombospondin-1, α2-antiplasmin, thrombin-activatable fibrinolysis inhibitor, actin, von Willebrand factor, and plasminogen activator inhibitor-2. Because of its function to stabilize fibrin clots, FXIII plays a major role in acute thrombotic events such as myocardial infarction, ischemic stroke, deep vein thrombosis, and pulmonary embolism [13]. Furthermore, fibrin plugs with or without eosinophilic inflammation are found in the life-threatening plastic bronchitis, which causes implicate several pulmonary diseases including asthma[14, 15]. However, the presence and role of FXIII in the airway of asthmatic subjects have yet to be analyzed. *In vitro*, allergen-activated peripheral blood mononuclear cells (PBMC) from asthmatic patients express more FXIII mRNA than untreated cells [16]. Also, FXIII protein level was augmented in bronchoalveolar lavage fluid (BAL) from children with bronchoalveolar inflammation compared with lavage fluid from normal children [17]. Recently, expression quantitative trait locus mappings identified cis-acting expression-associated variants in FXIII in relationship with asthma pathogenesis in children [18]. In addition recently, accumulation of FXIII+ dendritic cells was reported in the lung tissue from individuals who died from asthma[19].

FXIII expression is upregulated in IL-4- and IL-13-activated macrophages [20, 21] suggesting FXIII is a marker of alternatively activated macrophages. Production of FXIII in alternatively activated macrophages was further confirmed in nasal polyps from patients with chronic rhinosinusitis [22] where FXIII is thought to contribute to fibrin deposition. Using whole human genome expression microarrays, we have previously shown that FXIII was one of the transcripts upregulated in BAL cells following an *in vivo* segmental allergen challenge (SBP-Ag) in asthma [23].

Based on these observations we hypothesized that FXIII production by airway cells may be associated with type-2 immune characteristics and a loss of pulmonary function in asthma. To test this hypothesis, we analyzed FXIII in both BAL fluids after a SBP-Ag, and induced sputum samples from a group of asthmatic subjects enrolled in the Severe Asthma Research Program (SARP) at the University of Wisconsin.

Methods

Subjects, BAL cell preparations and study designs

The study protocol was approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board (IRB). Informed written consent was obtained from subjects prior to participation.

All 7 subjects undergoing the SBP-Ag and BAL were atopic, with at least one positive skin prick test. These subjects had a history of mild asthma with airway reversibility to albuterol. None of the subjects were using inhaled or oral corticosteroids. Detailed methods for bronchoscopy, SBP-Ag, and BAL cell preparation have previously been described [24]. Blood eosinophils (EOS) were purified by negative selection as previously described [25]. More details are provided in the online supplement.

Induced sputum was obtained by standard methods [26, 27] from 56 subjects enrolled in the Severe Asthma Research Program (SARP) at the University of Wisconsin. Additional details are provided in the online supplement. The subjects had severe (n=22) or non-severe asthma (n=34) as defined by the American Thoracic Society criteria. Sputum samples were processed in 2 sets. Set 1 (n=23) includes sputum samples obtained over a period of 12 months (2010 to 2011). Set 1 was used for association analyses between transcripts. 33 sputum samples (set 2) obtained between 2007 and 2010 were added to samples from set 1 for association analyses between FXIII with asthma characteristics. The subject characteristics were obtained as described in the online supplement.

RNA, real-time qPCR and ELISA

Total RNA was extracted from unfractionated BAL cells or purified BAL EOS using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Total RNA from sputum samples was extracted according to the Trizol Reagent manufacturer's recommendations. Typically, 400–800 ng of total RNA were recovered from the sputum samples. The reverse transcription reaction was performed using the Superscript III system (Invitrogen/Life Technologies, Grand Island, NY, USA). Expression of mRNA was determined by qPCR using SYBR Green Master Mix (SABiosciences, Frederick, MD, USA). Data are expressed as fold change using the comparative cycle threshold (ΔΔCt) method as described previously [28]. The values presented and used for correlations are fold change = ($2^{\Delta\Delta Ct}$) compared to the lowest expression among the asthmatic subjects, which was fixed at 1. Sputum samples with housekeeping gene (GUSB) Ct values >25 were excluded of the study. More details are provided in the online supplement.

FXIII protein was measured in the BAL fluid using the Zymutest Factor XIII-A kit from Hyphen Biomed, France. Free FXIII or FXIII complexed with FXIIIB were both measurable

by the ELISA kit. Samples were processed as recommended by the manufacturer. The assay sensitivity was ~1ng/ml.

Statistical analysis

To compare expression of genes in total BAL cells and purified BAL EOS by RT-qPCR or ELISA, data were analyzed using the Wilcoxon signed-rank test. Demographic factors were compared between non-severe and severe asthma using the Wilcoxon rank sum test and the chi square test for association. In the sputum, FXIII expression levels were compared among groups using the Wilcoxon rank sum test and to continuous measurements using the Spearman rank correlation coefficient. A trend test for an association between increasing ICS dose and FXIII expression was obtained by regressing log FXIII on ICS dose where dose levels none through high were re-coded as 0 through 3. Because FXIII expression tended to increase with age, all analyses were also performed using age-adjusted FXIII expression levels, which were calculated by regressing log FXIII on age and obtaining the standardized residuals. A two-sided p-value less than 0.05 was regarded as statistically significant.

Results

FXIII is increased in BAL after a SBP-Ag

FXIII mRNA level was assessed by RT-qPCR of unfractionated BAL cells before and 48 h after SBP-Ag and BAL EOS purified 48 h after SBP-Ag (Figure 1). FXIII mRNA level increased 300-fold in BAL cells after SBP-Ag compared to BAL cells obtained from the same subjects at baseline (before allergen challenge). The main cell population (74 % of the total) recruited into the airway 48 h after SBP-Ag was EOS (Supplementary Table 2). FXIII mRNA was low in purified BAL EOS compared to unfractionated BAL cells (Figure 1) demonstrating that EOS are not the main source of FXIII mRNA. FXIII protein amount in BAL fluid was close to the sensitivity of the ELISA before SBP-Ag and increased to ~30 ng/ml after allergen challenge (Figure 1). In BAL fluid obtained 48 h after SPB-Ag, FXIII protein amount was correlated to mRNA levels (Supplementary Figure 1). The ratio of albumin to total protein was similar in BAL fluid and plasma (Supplementary Figure 1). In contrast, the ratio of FXIII to albumin in BAL fluid diverged from the ratio in plasma, suggesting FXIII-expressing cells rather than blood provided FXIII protein in the BAL fluid.

FXIII mRNA level correlates with expression of markers of the type-2 immune response and expression of markers of the dendritic cell family

In a first set of the sputum samples (set 1), 23 asthmatic subjects were analyzed for FXIII expression vis-à-vis expression of several transcripts coding for type-2, type-1 or type-17 immune response, as well as cellular markers. The characteristics of these subjects are described in the Supplementary Table 3. Table 1 shows that FXIII mRNA level correlates with expression of a type-2 cytokine (IL-13) and chemokine (CCL17/TARC), and with the type-2 cytokine (IL-4 and IL-13)-induced membrane protein, CD23β/FcεRII.

FXIII is expressed by platelets and the monocyte/macrophage/dendritic cell family [29, 30]. Therefore, expression of markers of these cell types was analyzed for association with

FXIII. Platelet factor 4 (PF4) a marker of platelets, did not correlate with FXIII expression (Table 1). CD64, the high affinity immunoglobulin gamma Fc receptor 1 and CD163, which is induced by anti-inflammatory mediators (glucocorticoids or IL-10) and suppressed by pro-inflammatory cytokines (IFN-γ and TNF) [31, 32], are monocyte/macrophage specific markers. CD64 and CD163 did not relate to FXIII expression (Table 1). These findings led to an assessment of whether a restricted and specific monocyte/macrophage phenotype, such as alternatively activated (M2) macrophages, was the source of FXIII. Thus, in addition to CD163, CCL18 expression was measured and used as a marker of M2 macrophages; CCL18 did not show association with FXIII (Table 1). However, both CD209 (DC-SIGN, CLEC4L) a marker of immature dendritic cells (DC) and CD207 (CLEC4K) expressed by Langerhans cells highly correlated with FXIII expression levels (Table 1), suggesting that the DC family could be an important source of FXIII in sputum.

FXIII expression in sputum samples from non-severe versus severe asthmatic individuals

For further analysis of FXIII mRNA levels in relation to asthma severity and characteristics of asthma including pulmonary functions, 33 more asthmatic subjects (set 2) were added to set 1. The characteristics of the 56 asthmatic patients (34 non-severe and 22 severe) are described in Table 2. Patients with severe asthma were older (p=0.0002) and had reduced pulmonary functions compared to the non-severe asthma group (FEV_1 % predicted, p=0.008). Markers of atopy (IgE and skin prick test) and sputum inflammation (EOS and neutrophils) were similar between severe and non-severe asthma. Also, of 56, 23 subjects were using no or low level of corticoids while 33 subjects were taking medium or high levels of corticoids, with 22 of 33 belonging to the severe population.

The median of FXIII expression level was ~3.5 fold greater in the severe compared to the non-severe asthma population (45 [13, 80] vs. 13 [7, 27], p=0.02; Supplementary Figure 2A); though part of this difference may be explained by the severe group being older on average, as age-adjusted FXIII did not differ significantly between groups (p=0.23). Given that a primary difference between severe and non-severe asthma was the dose of inhaled corticosteroids, we analyzed whether FXIII levels were dependent on the daily corticosteroid doses. The subjects were divided in 4 groups composed of individuals taking either no (n=15), low (n=8), medium (n=11) or high (n=22) levels of corticoids (Supplementary Figure 2B). No significant association between FXIII and corticosteroid dose was seen (p=0.23).

FXIII expression and measures of pulmonary functions

Because fibrin deposition is found in the lung tissue of severe asthmatic subjects and FXIII substrates include fibrin and fibronectin, the latter of which is a major factor in airway remodeling [33], we speculated that FXIII expression level would associate with decreased lung function. Table 3 shows inverse correlation between FXIII with the ratio FEV_1/FVC % predicted (r= −0.42, p=0.001, n=56), and to a lesser extent with FEV_1 or FEV_1/FVC % predicted after β-agonist (FEV_1/FVCMX PP). FXIII also correlated with reversibility of FEV_1 to β-agonist (r= 0.45, p=0.005), and to RV/TLC % predicted (r=0.37, p=0.008) (Table 3). The association between FXIII and FEV_1/FVC % predicted or reversibility of FEV_1 to β-

agonist were graphed and shown in Figure 2 where values from each independent subjects were plotted.

The predicted pulmonary functions take into consideration ethnicity, height, sex, and age [34, 35]. However, due to the difference of age in the severe versus non-severe population (Table 2), FXIII was also analyzed for correlation to lung physiology characteristics after an adjustment to age. Age-adjusted FXIII still correlated with FEV_1/FVC % predicted, % reversibility of FEV_1 to β-agonist and RV/TLC % predicted (Table 3). Collectively, these data suggest that higher airway FXIII expression may lead to increased airflow limitation and air-trapping.

Sputum FXIII expression and relationship with a type-2 environment

The majority of the asthmatic subjects included in this study were allergic (Table 2) as indicated by allergen sensitization (47 positive skin prick test of the 55 tested individuals). Also, 46 of the 56 asthmatic subjects had total blood IgE levels above 30 IU/ml, and 20 of the 51 sputum samples had ≥ 1.5% EOS.

In accordance to Table 1 (set 1), FXIII was positively associated to IL-13 expression in sputum samples (Table 4). Also, FXIII correlated with total blood IgE, and sputum or blood EOS (Table 4). By contrast, macrophages inversely correlated with FXIII (Table 4). The association between FXIII and IL-13 mRNA level or the percentage of EOS in sputum samples were graphed and shown in Figure 3 where values from each independent subject were plotted.

After age adjustment, FXIII remained significantly associated with IgE and IL-13 with a trend for sputum and blood EOS (Table 4). These data further demonstrate a significant association between FXIII expression levels in airway cells and a type-2 environment in asthma.

Discussion

The role of the coagulation cascade proteins, particularly FXIII, is largely unknown in asthma. We found that FXIII was upregulated in the airway of mild asthmatic subjects after allergen challenge. Furthermore, FXIII expression in the sputum of mild-to-severe asthma subjects correlated with type-2 immune response and airway obstruction. Our results indicate FXIII may be a component of the molecular mechanisms that enhance airway obstruction in asthma. FXIII stabilizes fibrin clots, binds and activates fibroblasts, and increases collagen stabilization [36–40], all of which are mechanisms implicated in wound healing, tissue repair and remodeling. In addition, our study supports the hypothesis that FXIII expression is associated with the type-2 immune response, particularly with a type-2 cytokine (IL-13), chemokine (CCL17) and a type-2-cytokine-responsive gene (CD23β). These results are in accordance with reports showing upregulation of FXIII in IL-4- or IL-13-activated monocytes/macrophages [20, 21].

In addition to IL-4- or IL-13-activated monocytes/macrophages, differentiation of DC by IL-4 plus GM-CSF also induces cellular FXIII [41]. FXIII protein has been found in the

intracellular compartment of dermal DC, which express CD209 (DC-SIGN) and CD11b (ITGAM) [30 and reviewed in 42] and in leishmaniasis skin lesions, where they functioned as antigen-presenting cells similar to CD207+ Langerhans cells 43. We found that the expression of FXIII correlates with transcript levels of CD209 and CD207, suggesting that the DC may be an important source of FXIII in the airway of asthmatic subjects. Three main DC subsets have been reported in human lung 44, 45 but expression of CD209 or FXIII by these DC subsets has not been reported. The lack of association between FXIII expression and the presence of macrophages or markers of macrophages (CD163 or CCL18), suggests that M2 or type-2-cytokine-activated macrophages might not be the main source of FXIII in the airways of asthma patients. These data are in contrast of the reported findings in nasal polyps in patients with rhinosinusitis where FXIII expression was highly correlated with markers of M2 macrophages 22. However, our data agree with the work of Jayo *et al*, which found increased FXIII expression during differentiation of monocytes into DC using GM-CSF and the type-2 cytokine, IL-4 41. Nevertheless, our study suggests that, *in vivo*, and in asthma, type-2 cytokines might further favor differentiation and/or recruitment of FXIII+ DC rather than FXIII+ M2-macrophages. A recent work by Cagnoni *et al* 19 also supports that DC are the source of FXIII in the large airways of fatal asthma cases. However, we cannot rule out that other airway cells such as epithelial cells, which are known as fibrinogen producer cells, may also produce FXIII. The exact source of FXIII and the presence of FXIII in airway DC from asthmatic subjects will require further investigations.

In addition to its function as an extracellular protein, FXIII is also functional intracellularly. Intracellular FXIII (cFXIII) is activated by low Ca^{2+} concentrations in the absence of thrombin 46. cFXIII has many known intracellular substrates including, actin, myosin, vinculin, filamin, the type-1 angiotensin II receptor-associated protein and tubulin 11. Because of its known targets and its association with microfilaments 47, cellular FXIII function in cytoskeletal remodeling appears evident. Particularly, cFXIII has been implicated in the Fcγ and complement-mediated phagocytic activities of macrophages 48, and regulation of the migration of monocyte-derived DC 41. This latter function is important because DC migrate from tissue to lymph nodes, which is a crucial step in the development of the adaptive immune response. Although Cagnoni *et al* propose that FXIII+ DC remain resident in the lung tissue where they reactivate primed lymphoid cells19. Therefore, a high type-2 environment leading to higher FXIII expression by DC may induce or enhance the immune response to a specific allergen.

FXIII levels in the airway cells of asthmatic individuals undergoing an exacerbation were not evaluated. However, recruitment of FXIII+ cells and/or upregulation of FXIII expression in sputum correlated with airway obstruction (FEV_1/FVC and RV/TLC) and reversibility indicating that FXIII expression level in sputum may change over time with asthma symptoms and exacerbation. Importantly, the patterns of airflow limitation, premature airway closure/air-trapping, and incomplete reversibility with bronchodilator are the characteristics of severe asthma 34, and indicate substantial dysfunction in peripheral airways. FXIII may prove to be a biomarker of the corticosteroid-refractory processes that underlie severe asthma.

The measurement of FXIII in various fluids from human subjects could be a challenge due to the numerous FXIII substrates and its presence in complex protein aggregates. Brims *et al* [9] expressed concerns regarding the measurement of FXIII in sputum from asthmatic subjects and suggested that FXIII was either trapped by fibrin in the tissue or degraded by neutrophil elastase. This dilemma demonstrates the suitability of measuring transcripts rather than protein in sputum samples, but it also indicates the need to develop antibodies that recognize substrate-bound FXIII. Despite this challenge, we were able to use ELISA to quantify FXIII protein in BAL fluid after SBP-Ag, while the levels in sputum samples were too close to the level of detection to make conclusive measurements. The inability to measure FXIII in the sputum may indicate that it is bound to other proteins, or that sufficient accumulation of FXIII in sputum samples might require asthma symptoms or exacerbations.

In conclusion, we analyzed FXIII expression in asthmatic subjects and demonstrated that airway FXIII production is upregulated by an *in vivo* allergen challenge in mild asthmatic patients. In addition, in mild-to-severe asthma, FXIII expression in sputum samples correlated with DC markers, type-2 environment and airflow limitation. These findings suggest a potential contribution of FXIII to asthma pathology and disease severity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This work was supported by the Severe Asthma Research Program Grant R01 HL069116, U10 HL109168, Program Project Grant P01 HL088594, and Clinical and Translational Research Center Grant UL1 RR025011 and UL1 TR000427 from the National Institutes of Health.

The authors thank Erin Billmeyer, Michele Wolff and Holly Eversoll for patient recruitment, screening, and assessments; Gina Crisafi, Katie Popp and Helen Werner for preparing sputum samples; Gina Crisafi for protocol coordination; Larissa DeLain for laboratory technical support. We thank Mats Johansson for his inputs on the manuscript. We acknowledge contributions from all investigators, staff, and participants in the SARP study.

Abbreviations

BAL	bronchoalveolar lavage
DC	dendritic cells
EOS	eosinophils
FXIII	coagulation factor XIII
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
ICS	Inhaled corticosteroids
RV	residual volume
SARP	Severe Asthma Research Program

SBP-Ag	segmental bronchoprovocation with an allergen
TLC	total lung capacity

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Key Messages

- Airway FXIII is allergen-induced in asthma
- Airway FXIII associates with airflow limitation and characteristics of severe asthma
- FXIII, a cross-linker of fibrin and fibronectin could contribute to pathophysiology in asthma

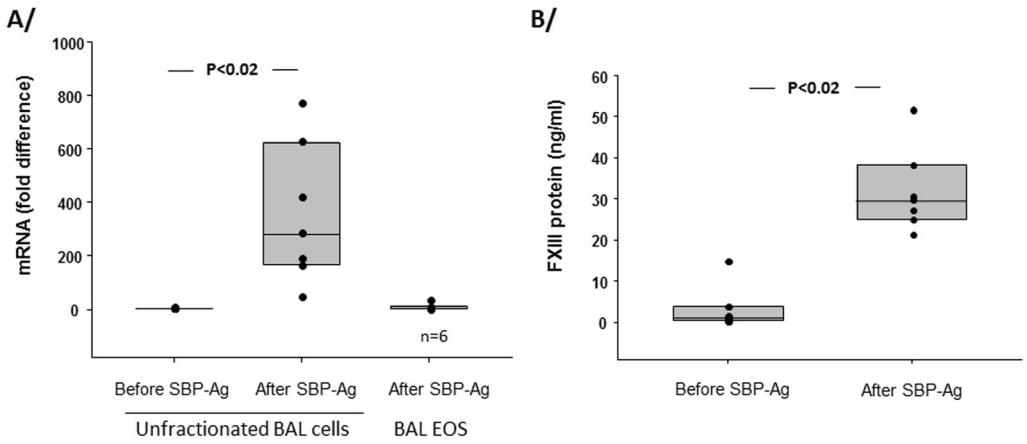

Figure 1. FXIII transcript and protein are increased in BAL after segmental bronchoprovocation with an allergen (SBP-Ag)

A/ FXIII mRNA was analyzed by RT-qPCR in unfractionated BAL cells before and 48h after SBP-Ag and purified EOS after SBP-Ag. Symbols indicate data from individual subjects, box plots are composite data from 7 subjects among whom 6 had highly purified EOS (>95%). B/ FXIII protein in BAL fluids before and after SBP-Ag was analyzed by ELISA. Box plots depict the median and the interquartile range between the 25th and 75th percentiles for 7 subjects.

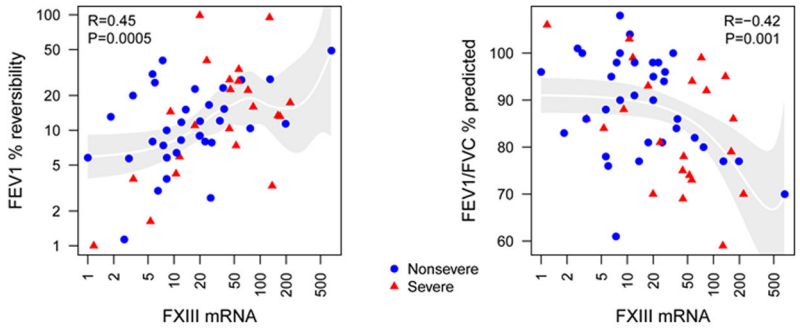

Figure 2. FXIII mRNA expression correlates with % reversibility of FEV1 % predicted to β-agonist, and with FEV_1/FVC % predicted

Some of the correlations described in Table 3 were graphed with non-severe (n=34) shown in blue circles and severe subjects (n=22) shown in red triangles.

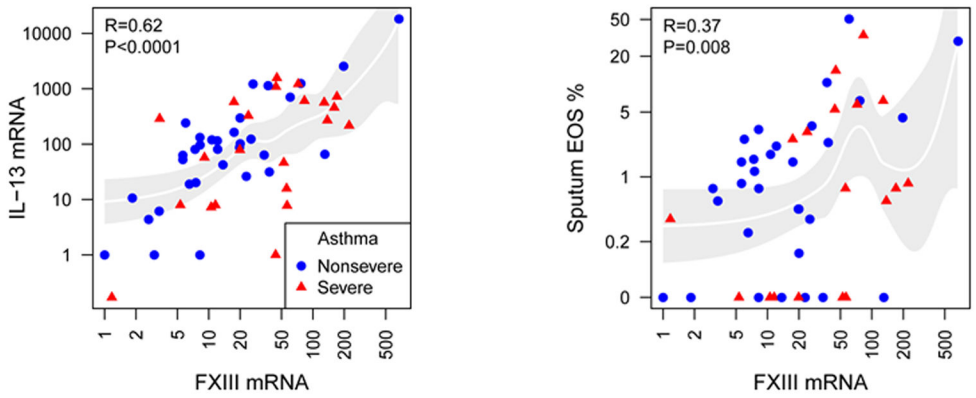

Figure 3. FXIII mRNA expression correlates with IL-13 mRNA levels, and the percentage of EOS in sputum

Some of the correlations described in Table 4 were graphed with non-severe (n=34) shown in blue circles and severe subjects (n=22) shown in red triangles.

Table 1

Sputum FXIII mRNA level correlates with expression of markers of both the type-2 immune response and the dendritic cell family, n=23 (set 1)

	r	P value
IL-13 mRNA	**0.48**	**0.02**
CCL17 mRNA	**0.68**	**0.0005**
IFN-γ mRNA	0.23	0.30
IL-17 mRNA	0.21	0.35
CD23β mRNA	**0.79**	**<0.0001**
PF4 mRNA	− 0.04	0.84
CD64 mRNA	0.15	0.49
CD163 mRNA	− 0.08	0.72
CCL18 mRNA	0.15	0.51
CD209 mRNA	**0.68**	**0.0005**
CD207 mRNA	**0.55**	**0.008**

Table 2

Subject characteristics, n=56

	Non-severe n=34	Severe n= 22	*p*
Age	**24 [21, 36]**	**46 [39, 53]**	**0.0002**
Gender (female/male)	15/19	11/11	0.67
Caucasian race	79% (3AA, 2H, 1AsA, 1NtA)	82% (4AA)	0.82
FEV_1 PP	**86 [75, 99]**	**71 [61, 94]**	**0.008**
% reversibility FEV_1 PP	11 [7, 20]	14 [6, 26]	0.48
FEV_1/FVC PP	90 [81, 98]	85 [74, 94]	0.15
Level of corticoid intake (none/low/medium/high)	15/8/10/1	0/0/1/21	**<0.0001**
IgE (IU/ml)	100 [38, 288]	117 [36, 405]	0.97
Skin prick test +	91%	76%	0.13
Sputum eosinophils %	0.8 [0.1, 2.4], n=31	0.7 [0.0, 5.3], n=18	0.92
Sputum neutrophils %	44 [29, 60], n=31	58 [43, 76], n=18	0.11

Median [25^{th}, 75^{th}]

AA, African American; H, Hispanic; AsA. Asian American; NtA, Native American; FEV_1, force expiratory volume in 1 second; FVC, force vital capacity; % reversibility, reversibility after β-agonist; PP, % predicted.

P values (Wilcoxon rank sum test or chi square test for association) <0.05 indicates statistical significant differences between the non-severe and the severe groups.

Table 3

Sputum FXIII mRNA level correlates with pulmonary functions in asthma, n=56

		FXIII no adjustment		FXIII age-adjusted	
	n	r	p-value	r	p-value
% reversibility FEV_1 PP	**56**	**0.45**	**0.0005**	**0.39**	**0.003**
FEV_1/FVC PP	**56**	**− 0.42**	**0.001**	**− 0.35**	**0.008**
RV/TLC PP	**51**	**0.37**	**0.008**	**0.36**	**0.01**
FEV_1/FVCMX PP	**56**	**− 0.30**	**0.02**	− 0.21	0.11
FEV_1 PP	**56**	**− 0.30**	**0.03**	− 0.20	0.15

% reversibility; reversibility after β-agonist; FEV_1, force expiratory volume in 1 second; FVC, force vital capacity; MX, maximum after β-agonist; PP, percentage predicted; RV, residual volume; TLC, total lung capacity.

Table 4

FXIII mRNA level associates with characteristics of a type-2 environment, n=56

		FXIII no adjustment		FXIII age-adjusted	
	n	r	p-value	r	p-value
Total blood IgE	**56**	**0.28**	**0.04**	**0.35**	**0.009**
Sputum IL-13 mRNA	**55**	**0.62**	**<0.0001**	**0.56**	**<0.0001**
Sputum EOS %	49	**0.37**	**0.008**	0.24	0.10
Sputum PMN %	49	0.24	0.10	0.22	0.12
Sputum MAC %	49	**− 0.44**	**0.001**	**− 0.40**	**0.005**
Blood EOS %	56	**0.29**	**0.03**	0.24	0.08

EOS, eosinophils; MAC, macrophages; PMN, neutrophils