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Original Article

FAM13A is a modifier gene of cystic fibrosis lung phenotype regulating rhoa activity, actin cytoskeleton dynamics and epithelial-mesenchymal transition

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Abstract

Background: Cystic fibrosis (CF) lung disease severity is highly variable and dependent on several factors including genetic modifiers. Family with sequence similarity 13 member A (*FAM13A*) has been previously associated with lung function in the general population as well as in several chronic lung diseases, such as chronic obstructive pulmonary disease (COPD), we examined whether *FAM13A* is a modifier gene of CF lung phenotype. We also studied how *FAM13A* may contribute to the physiopathological mechanisms associated with CF.

Methods: We investigated the association of *FAM13A* with lung function in CF French patients (n = 1222) by SNP-wise analysis and Versatile Gene Based Association Study. We also analyzed the consequences of FAM13A knockdown in A549 cells and primary bronchial epithelial cells from CF patients.

Results: We found that *FAM13A* is associated with lung function in CF patients. Utilizing lung epithelial A549 cells and primary human bronchial epithelial cells from CF patients we observed that IL-1β and TGFβ reduced FAM13A expression. Knockdown of FAM13A was associated with increased RhoA activity, induction of F-actin stress fibers and regulation of epithelial-mesenchymal transition markers such as E-cadherin, α-smooth muscle actin and vimentin.

Conclusion: Our data show that FAM13A is a modifier gene of CF lung phenotype regulating RhoA activity, actin cytoskeleton dynamics and epithelial-mesenchymal transition.

Keywords: Cystic fibrosis; Modifier genes; FAM13A

Abbreviations: CF, Cystic fibrosis; FAM13A, Family with sequence similarity 13 member A; COPD, Chronic obstructive pulmonary diseases; CFTR, CF transmembrane conductance regulator; GAP, GTPase activating protein; VEGAS, Versatile Gene Based Association Study; EMT, epithelial to mesenchymal transition; KNoRMA, Kulich normalized mortality adjusted CF-specific lung phenotype; hAECBs, human airway epithelial cells from bronchi; MAF, minor allele frequency; FEV_1, forced expiratory volume in 1 s; siFAM13, siRNA for FAM13A; siCTRL, siRNA negative control.

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1. Introduction

Cystic fibrosis (CF) is a monogenic disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. However, lung disease severity is highly variable and dependent of several factors including genetic modifiers [1,2]. The modifier genes are indeed thought to contribute to approximately 50% of the lung phenotype in CF patients harboring the common *CFTR* F508del mutation [3]. Family with sequence similarity 13 member A (*FAM13A*) has been previously shown to be associated with the lung function in the general population [4] as well as in patients with asthma, COPD, idiopathic pulmonary fibrosis and lung cancer [5–10]. The protein sequence of FAM13A contains a rho GTPase activating protein (GAP) domain. GAPs inactivate GTPases by the conversion of GTP to GDP. GTPases are known to play a role in the actin cytoskeleton and remodeling as they regulate assembly of focal adhesions and F-actin stress fibers [11]. Therefore, variants affecting the on/off switch of this GTPase feedback loop via the FAM13A-GAP domain may modify disease progression possibly by dysregulating Rho signaling and disrupting the cytoskeleton. The most common rho GTPases include RhoA, RhoB, RhoC, Rac1 and Cdc42. Perturbed rho GTPase signaling is associated with several lung diseases, including asthma, pulmonary hypertension, chronic obstructive pulmonary disease (COPD), and lung cancer [12]. In chronic lung diseases, RhoA is the most commonly associated GTPase for changes in the barrier function and actin cytoskeleton. Interestingly, RhoA has been reported to be upregulated in CF cells [13].

To date, no study has explored the role of *FAM13A* as a potential candidate for modifying the CF lung phenotype. In this study, we investigated whether *FAM13A* variants associated with CF lung phenotype. Considering the numerous publications involving *FAM13A* in lung disease progression, susceptibility and severity, and the potential role of the rhoGAP domain, we also examined the function of FAM13A in the lung and how it could participate in the CF lung physiopathology.

2. Materials and methods

2.1. Patients and lung phenotype

The French CF Gene Modifier Consortium has been recently described [14]. Characteristics of 1222 French patients included in this study are shown in Table 1. Written informed consent was obtained from adults. For patients < 18 years old consent from parents or guardians was given for participation in the study. The study was approved by the French ethical committee (CPP n°2004/15) and the information collection was approved by CNIL (n°04.404).

We transformed forced expiratory volume in 1 s (FEV_1) to the Kulich normalized mortality adjusted CF-specific lung phenotype (KNoRMA) [15], a standardized consortium lung phenotype which allows for direct comparison of the lung function of CF patients irrespective of age and gender.

Table 1
Characteristics of CF patients enrolled in *FAM13A* genotyping.

Cohort	
n	1222
Mean age (±SD)	21.0 (9.2)
Range	6.0–57.6
Male n (%)	627 (51.3)
European [a] n (%)	1211 (99.1)
F508del/F508del n (%)	716 (58.6)
Pancreatic exocrine insufficient n (%)	1222 (100.0)

[a] On the basis of Eigenstrat principal components analysis and closeness to CEU.

2.2. Reagents

Lipopolysaccharide (LPS, *Pseudomonas aeruginosa* 10), anti-FAM13A (HPA038109) and anti-β-actin antibodies were from Sigma-Aldrich (Saint-Quentin Fallavier, France) and TNF-alpha (TNF-α) and IL-1-beta (IL-1β) were from Immunotools (Friesoythe, Germany). Transforming Growth Factor-beta (TGF-β) was from PeproTech (Rocky Hill, NJ, USA). Anti-E-cadherin, anti-vimentin, anti-rabbit and anti-mouse-Horseradish peroxidase (HRP) antibodies were from Cell Signaling Technology (Danvers, MA, USA). Anti-RhoA antibody and rhotekin-RBD beads are from cytoskeleton (Denver, USA). Rhosin (Rho inhibitor) was from Millipore (Billirica, MA, USA). FAM13A expression (p.FAM13A) and control plasmids (p.CTRL) were from Origene (RC216561, Rockville, MD, USA). Silencer® Select siRNA for FAM13A (siFAM13) and negative control (siCTRL) were from Ambion (Austin, TX, USA). Lipofectamine 3000 was from invitrogen (Carlsbad, CA, USA).

2.3. Cell cultures

We used two types of respiratory epithelial cells: A549 (Alveolar origin; ATCC®-CCL185, Rockville, MD, USA), and commercial primary human airway epithelial cells from bronchi (hAECBs, Epithelix, Geneva, Switzerland) (Table 2). A549 cells were cultured as previously described [16] then seeded in plates (TPP, Techno Plastic Products, Trasadingen, Switzerland) as described in the figure legends. A549 cells were stimulated for 6 h with LPS, TNF-α 10 ng/mL, or IL-1β 10 ng/mL. For TGF-β experiments, A549 cells were serum starved for 6 h and then treated with 5 ng/mL of TGF-β for 24 h (mRNA quantification) or 48 h (protein expression) as indicated in the figure legends. Primary hAECBs were isolated from the bronchi of healthy individuals or CF patients and were cultured as recommended by the manufacturer.

2.4. RT-qPCR

Total RNA was extracted using a nucleospin extract II kit (Macherey Nagel, Duren, Germany). Reverse transcription (RT) was performed using the ABI high-capacity cDNA kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed using an ABI StepOnePlus™. Each reaction contained 10 μL

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