Analysis of FAM13A Gene In Chronic Obstructive Pulmonary Disease In Kashmir Valley of India: A Preliminary Study

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Abstract- *Chronic obstructive pulmonary disease (COPD) is a genetically convoluted disease leading to airflow obstruction in the lungs. As far as its genetics is concerned, there have been several association studies across the globe; however, very few studies have been carried out in Kashmir valley of India. One of the new entrants under candidate gene category for COPD is family with sequence similarity 13, member A (FAM13A). The aim of the current study was to investigate the association between rs2869967 variant in FAM13A gene and COPD in Kashmiri population. The preliminary findings revealed presence of a novel A>G single nucleotide polymorphism (SNP) and lack of rs2869967 variant in most of the COPD samples as compared to controls, clearly indicating possible role of this novel variant in COPD. However, further studies in large datasets from Kashmiri population are needed to validate the above findings in order to understand the functional role of FAM13A in relation to COPD susceptibility and development.*

Keywords- COPD; FAM13A; Kashmir; Profile; India; Genetics; SNP

Abbreviations: Chronic obstructive pulmonary disease COPD; family with sequence similarity 13, member A FAM13A; Human Gene Mutation Database HGMD; In Patient Department IPD; National Center for Biotechnology Information NCBI; Online Mendelian Inheritance in Man OMIM; Out Patient Department OPD; Sher-I-Kashmir Institute of Medical Sciences SKIMS; single nucleotide polymorphism SNP

I. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a chronic complex disease characterized by more or less irreversible airflow obstruction. Generally speaking, it consists of many diseases including chronic bronchitis, emphysema and chronic obstructive airways disease, thereby leading to progressive airflow limitation and compromised lung function [1]. Its prevalence across the globe varies and is believed to be higher amongst aged people [2]. The etiology of COPD has a profound genetic element which has prompted researchers to conduct genetic studies for identifying risk factors and in a way understand disease pathogenesis. Therefore, many candidate genes that could be linked to the development of disease have been examined. However, inconsistent results in different study populations have suggested the underlying mechanism for this disease to be an amalgam of genetic and environmental factors [3]. Recent studies reveal association between rs2869967 variant of family with sequence similarity 13 member A gene [(FAM13A) OMIM 613299, Entrez Gene 10144] and COPD in different populations [4-7]. FAM13A is located on chromosome 4 at position q22 and its role has been implicated in signal transduction pathways [8]. This encouraged us to explore the association between FAM13A and COPD in Kashmiri population of India by screening FAM13A gene in COPD patients and controls.

II. MATERIALS AND METHODS

2.1 Study participants

A total of 42 unrelated COPD cases from Kashmir region of India were recruited for the present study from the In Patient Department (IPD) and Out Patient Department (OPD) of the Department of Chest Medicine, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Medical College Hospital (MCH), Bemina, Kashmir, India; following the proper ethical procedures of the hospital. Written consent was obtained from all subjects prior to enrolment. All procedures performed with human participants were in accordance with the ethical standards of the institutional research committee and 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2 Blood sample collection and DNA extraction

2 to 5 ml of whole peripheral venous blood sample was collected from the subjects in EDTA coated Vacutainer tubes (ADS Hitech Polymers, India) and stored at -80°C before processing for DNA extraction. Blood samples were also collected from healthy individuals from the same geographic area to serve as controls. Genomic DNA was extracted from the blood leukocytes by using DNeasy® Blood & Tissue Kit (Qiagen, Germany), according to the manufacturer's enclosed protocol and its integrity and specificity was checked on 0.8% agarose gel by comparing with 1 kb DNA ladder (Fermentas, USA). The concentration of extracted DNA samples was determined by using NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, USA) and stored at -20°C till further processing.

2.3 PCR amplification

The primer sequences for rs2869967 variant in FAM13A were picked from [5]. PCR was carried out in thermal cycler (Benchmark TC9639, USA), at respective annealing temperatures (Tm) of primer sets obtained after optimization, in a reaction volume of 50 µl containing 50-100 ng of genomic DNA, 0.2 mM dNTPs (Thermo Scientific, USA), 1x Phusion HF Buffer (New England Biolabs, Inc. USA), 1U of Phusion DNA Polymerase (New England Biolabs, Inc. USA) and 0.4 µM of each primer (Integrated DNA Technologies, India). Amplification was performed by initial denaturation at 98°C for 5 min, followed by 30 cycles of denaturation at 98°C for 30 s, annealing at 58°C for 45 s, extension at 72°C for 1 min and a final extension at 72°C for 5 min, after the last cycle. The specificity of amplified products was checked by analyzing on 1.5% agarose gel and comparing with 100 bp DNA ladder (Thermo Fisher Scientific, USA). Purification of PCR products was done by using the GenElute™ Gel Extraction Kit (Sigma-Aldrich, USA), according to the manufacturer's instructions and samples were then stored at -20°C till sequencing.

2.4 Sequencing

For exploration of sequence alterations in FAM13A, the amplified products were sequenced commercially through the services of SciGenom Labs, Pvt., Ltd., Kerala, India (http://www.scigenom.com). Nucleotide sequences of the amplicons were viewed and analyzed for alterations by comparing with NCBI reference sequence using ClustalX Version 2 [9, 10], Chromas Pro Version 1.49 beta 2 (http://www.technelysium.com.au) and Finch TV (http://www.geospiza.com). Databases such as Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk), NCBI′s SNP (http://www.ncbi.nlm.nih.gov/snp) and PubMed databases (http://www.ncbi.nlm.nih.gov/pubmed) were consulted for the detection of previously reported and novel sequence variations in FAM13A.

III. RESULTS AND DISCUSSION

The present study comprised of 42 COPD patients as well as unrelated controls from the Indian data set, covering the patients from its northern region Kashmir. All the subjects were of the Kashmiri ethnic group. Agarose gel electrophoresis revealed successful extraction of DNA from blood samples of both cases as well as controls (Fig. 1). The extracted DNA was used as a template for amplification of FAM13A gene and PCR products on analysis showed specific bands on the agarose gel as per expected size of 126bp (Fig. 2).

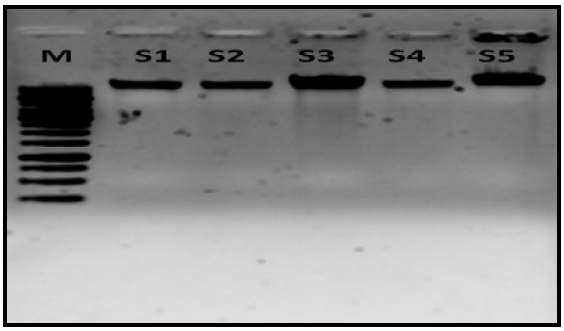

Figure 1. Representative 0.8% agarose gel showing DNA extracted from study subjects. 2 µl (100-200 ng/µl) of each sample was loaded in each well. [Lane M shows separation pattern of 1 kb ladder; and lanes S1 to S5 show genomic DNA].

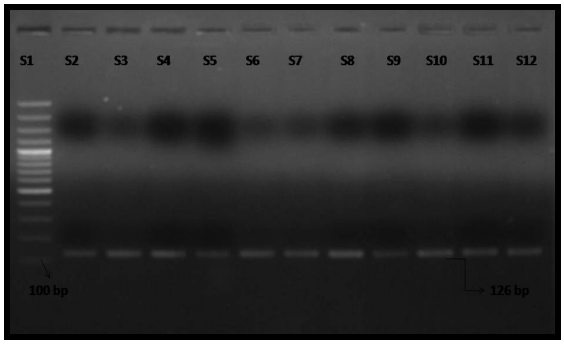

Figure 2. Representative 1.5% agarose gel showing specific PCR amplification product of FAM13A (126bp) in study subjects. 2 µl (100-200 ng/µl) of each sample was loaded in each well. [Lane S1 shows separation pattern of 100 bp ladder; and lanes S2 to S12 show desired product].

After sequence alignment of 15 samples, some COPD samples showed A to G change in the sequence. Further, analysis of sequencing chromatograms also indicated

an overlapping peak suggesting the possible variation (Fig. 3). Several control DNA samples were also sequenced simultaneously to check for presence of any polymorphic variations. The A>G transition was found to be present in majority of the COPD patients as compared to controls; however, exact number is not available presently due to insufficient data. The database search revealed that this variation appeared to be novel and was present in the heterozygous state in the intronic region of gene (Table 1). The characteristics of FAM13A SNPs are given in Table 1. Additionally, sequence analysis showed lack of reported variation rs2869967 in COPD samples as well as controls.

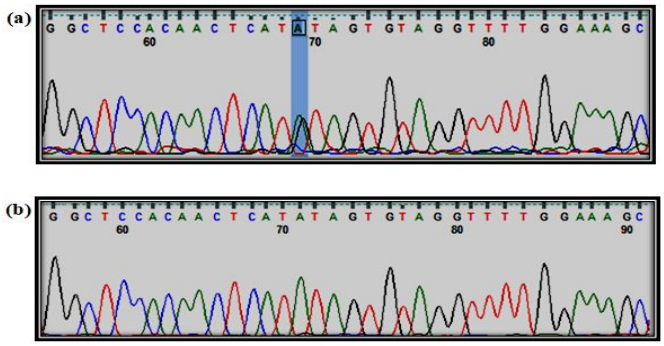

Figure 3. Representative sequence chromatograms showing the presence of novel A>G SNP (shown in blue) in FAM13A in COPD cases (a) in contrast to corresponding normal sequence from controls (b).

Table 1 Characteristics of FAM13A SNPs

S. No.	SNP Type	Gene location	SNP ID	Alleles
1.	Reported	Intron	rs2869967	C/T
2.	Novel	Intron		A/G

SNP single nucleotide polymorphism

To a large extent, progress has been attained in recognizing the candidate genes and gene defects that underlie inherited predisposition to COPD. Therefore, screening of candidate genes can further clarify the relationship between COPD genetics and its development. Yet, in spite of some progress made in the genetics of this chronic disease, to a large extent the exact role of several genes in its pathogenesis remains concealed. Keeping in view the association between rs2869967 variant of FAM13A with COPD, we examined this variant in ethnic cohort of Kashmiri patients as our population being small, isolated, and containing conserved gene pool makes it a right candidate for gene analysis study. Sequence analysis revealed a novel A>G transition in most of the COPD samples as compared to controls, which clearly shows its role in the development of COPD or its predisposition in Kashmiri population. Moreover, the results did not reveal presence of reported variation rs2869967 in any case or control. At the same time, the findings of our study are inconclusive due to very small sample size in relation to disease prevalence in the region; therefore, A>G transition might be a new SNP with or without functional implications in relation to COPD development or risk in Kashmiri population.

IV. CONCLUSION

To the best of our knowledge, this is the first study from India which assessed the role of FAM13A in Kashmiri COPD patients. Our findings are not concrete but just preliminary, indicating possible role of novel A>G SNP in COPD in Kashmir valley of India. However, nothing can be inferred presently which makes it mandatory to conduct further comprehensive studies to validate our data and thereby elucidate the exact role of novel A>G variant in COPD in Kashmiri population.

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The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

REFERENCES

[1] Vestbo, J., et al., *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary.* Am J Respir Crit Care Med, 2013. **187**(4): p. 347-65.

[2] Prince, M.J., et al., *The burden of disease in older people and implications for health policy and practice.* Lancet, 2015. **385**(9967): p. 549-62.

[3] Postma, D.S., A. Bush, and M. van den Berge, *Risk factors and early origins of chronic obstructive pulmonary disease.* Lancet, 2015. **385**(9971): p. 899-909.

[4] Cho, M.H., et al., *Variants in FAM13A are associated with chronic obstructive pulmonary disease.* Nat Genet, 2010. **42**(3): p. 200-2.

[5] Guo, Y., et al., *Genetic analysis of IREB2, FAM13A and XRCC5 variants in Chinese Han patients with chronic obstructive pulmonary disease.* Biochem Biophys Res Commun, 2011. **415**(2): p. 284-7.

[6] Kim, W.J. and S.D. Lee, *Candidate genes for COPD: current evidence and research.* Int J Chron Obstruct Pulmon Dis, 2015. **10**: p. 2249-55.

[7] Kim, W.J., et al., *Association of lung function genes with chronic obstructive pulmonary disease.* Lung, 2014. **192**(4): p. 473-80.
[8] Cohen, M., et al., *Cloning and characterization of FAM13A1--a gene near a milk protein QTL on BTA6: evidence for population-wide linkage disequilibrium in Israeli Holsteins.* Genomics, 2004. **84**(2): p. 374-83.
[9] Larkin, M.A., et al., *Clustal W and Clustal X version 2.0.* Bioinformatics, 2007. **23**(21): p. 2947-8.
[10] Thompson, J.D., et al., *The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools.* Nucleic Acids Res, 1997. **25**(24): p. 4876-82.