

Link between CFTR mutations and ABPA: a systematic review and meta-analysis

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Summary

There is a biological plausibility on the link between cystic fibrosis transmembrane conductance regulator (CFTR) mutations and allergic bronchopulmonary aspergillosis (ABPA). The aim of the systematic review was to investigate this link by determining the frequency of CFTR mutations in ABPA. We searched the PubMed and EmBase databases for studies reporting CFTR mutations in ABPA. We pooled the odds ratio (OR) and 95% confidence intervals (CI) from individual studies using both fixed and random effects model. Statistical heterogeneity was evaluated using the I^2 test and the Cochran-Q statistic. Publication bias was assessed using both graphical and statistical methods. Our search yielded four studies (79 ABPA, 268 controls). The odds of encountering CFTR mutation was higher in ABPA compared with the control group (OR 10.39; 95% CI, 4.35–24.79) or the asthma population (OR 5.53; 95% CI 1.62–18.82). There was no evidence of statistical heterogeneity or publication bias. There is a possible pathogenetic link between CFTR mutations and ABPA. However, because of the small numbers of patients, further studies are required to confirm this finding. Future studies should adopt a uniform methodology and should screen for the entire genetic sequence of the CFTR gene.

Key words: *Aspergillus*, cystic fibrosis, asthma, cystic fibrosis transmembrane conductance regulator, allergic bronchopulmonary aspergillosis.

Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a complex hypersensitivity reaction secondary to the presence of *Aspergillus fumigatus* in the tracheobronchial tree.^{1–3} It complicates the course of 2–32% of asthmatics and 1–15% of patients with cystic fibrosis (CF).^{4,5} The higher prevalence does not reflect the population prevalence but is representative of data of the special asthma/chest clinics.⁶ First described by Hinson *et al.* in 1952⁷, the disease presents with diverse clinical and radiological manifestations usually with uncontrolled asthma, pulmonary infiltrates and

central bronchiectasis.⁸ The condition is glucocorticoid-responsive, and early diagnosis and treatment can prevent development of bronchiectasis, a manifestation of irreversible lung damage.^{6,9} It was initially thought that exposure to large concentrations of *A. fumigatus* causes ABPA.^{7,10–13} As not all asthmatics develop ABPA despite the ubiquitous presence of *A. fumigatus*,¹⁴ it has been hypothesised that specific host genetic factors may be important for the development of the disease.^{15,16} Furthermore, familial occurrence has been reported, suggesting a genetic basis in some patients.¹⁷

Cystic fibrosis is an autosomal recessive multisystem disorder secondary to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene located on the long arm of chromosome 7. The CFTR gene encodes a chloride channel that is involved in the regulation of ion and water balance across epithelial membranes. Dysfunction in CFTR leads to an alteration in the viscosity and tenacity of mucus produced

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at the epithelial surfaces with resultant bronchopulmonary infection, pancreatic insufficiency and a high sweat chloride concentration in its classic form.¹⁸ ABPA shares many characteristics with CF-related lung disease including the presence of wheezing, pulmonary infiltrates, proximal bronchiectasis and mucus plugging. Furthermore, ABPA can also complicate the course of CF raising the possibility that mutations in the CFTR gene may be involved in the pathogenesis of ABPA.

In patients with CF, the CFTR dysfunction affects not only the epithelial cells but also the lymphocytes.¹⁹ Moreover, the cytokine production is dysregulated in CFTR-deficient peripheral lymphocytes.²⁰ Interestingly, CFTR is important to maintain the Th1/Th2 balance in CD4+ lymphocytes. CFTR deficient naive CD4+ T cells demonstrate a spontaneous Th2 bias *in vitro*, and the absence of CFTR leads to an excessive Th2 inflammatory response to *A. fumigatus* antigens *in vivo*.^{21,22} CFTR thus plays an important role in influencing CD4+ T cell differentiation and is probably necessary for the effectiveness of the immune response to *A. fumigatus*. All these findings suggest a strong biological plausibility on the link between CFTR mutations and ABPA. The aim of this systematic review is to determine the occurrence of CFTR mutations in ABPA complicating bronchial asthma using a meta-analytic technique.

Material and methods

Search strategy and selection criteria

All the authors independently searched two internet databases, PubMed and EmBase for relevant studies published from 1990 to 2010 using the following free text terms: 'allergic bronchopulmonary aspergillosis' and 'ABPA', restricting the language of the publications to English. We also reviewed the reference lists of primary studies, reviews and editorials. In addition, we reviewed our personal files. The initial database created from the electronic searches was compiled and all duplicate citations were eliminated. Two reviewers (RA and AK) screened these citations, without blinding, by title and abstract review to capture the relevant studies. This database was then screened again to include only primary articles, and the full text of each citation was obtained and reviewed. Studies were eligible for inclusion if they reported CFTR mutation in ABPA complicating asthma vs. a control group. No specific inclusion criterion for ABPA was defined for purpose of this review however we recorded the criteria used by different authors.

Data abstraction

Data was recorded on a standard data extraction form, and the following items were extracted: (a) publication details – citation, title, and author[s], (b) criteria used for defining ABPA, (c) type and number of CF mutations studied in cases and controls, (d) methodology used for analysing the mutations.

Determination of pooled effect

The statistical package Comprehensive Meta-analysis (Version 2.2 for MS-Windows, NJ, USA, <http://www.meta-analysis.com>) was used to perform all the statistical analysis. We calculated the odds ratio (OR) and 95% confidence intervals (CI) from the individual studies to assess the presence of CFTR mutations in ABPA vs. the control groups from individual studies. The results from individual studies were pooled using both fixed effects model of Mantel-Haenszel and random effects model of DerSimonian-Laird.^{23,24}

Assessment of heterogeneity

The impact of heterogeneity on the pooled estimates of the individual outcomes was assessed using the I^2 test and the Cochran Q statistic. The I^2 test measures the extent of inconsistency among the results of the studies, and an I^2 value more than 50% indicates significant heterogeneity.²⁵ The Cochran test calculates the weighted sum of squared differences between individual study effects and the pooled effect across studies. The level (P value) at which heterogeneity should be diagnosed is unclear, given that the Q statistic has low power, and Fleiss *et al.* has recommended a value of at least 0.1.²⁶

Assessment of publication bias

We checked for the presence of publication bias using the Begg's funnel plot.²⁷ The funnel plot is a measure of the log OR (x-axis) against the standard error of the log OR (Y-axis). In the absence of publication bias, the estimates from smaller studies are expected to be scattered above and below the summary estimate, producing a triangular or funnel shape.

We also checked for publication bias using two statistical tests: (a) Egger test²⁸ which is a test for asymmetry of the funnel plot. This is a test for the Y intercept = 0 from a linear regression of normalised effect estimate (estimate divided by its standard error) against precision (reciprocal of the standard error of the

estimate) and (b) Begg and Mazumdar's test²⁹ which tests the interdependence of variance and effect size using rank correlation method.

An institutional review board clearance was not required for this study as this was a meta-analysis of published studies.

Results

Our initial database search retrieved a total of 1806 citations (Fig. 1). Of these 1144 studies were excluded as they did not involve ABPA. Five studies have assessed CFTR mutations in ABPA.^{30–34} Of the five studies, two had used the DNA samples of the same patients.^{32,34} Hence, one study was excluded from meta-analysis.³⁴ Thus, four studies that met our inclusion criteria were included in the final analysis.^{30–33} All were case-control studies that had evaluated the occurrence of CFTR mutations in ABPA vs. control group (Table 1). The characteristics of the study population, the diagnostic criteria used for defining ABPA, the number of CFTR mutations screened and the methodology used for identifying the CFTR mutation is shown in Table 1. The mutations encountered in ABPA were most frequently of class II ($n = 13$) followed by class I and IV (4 each).

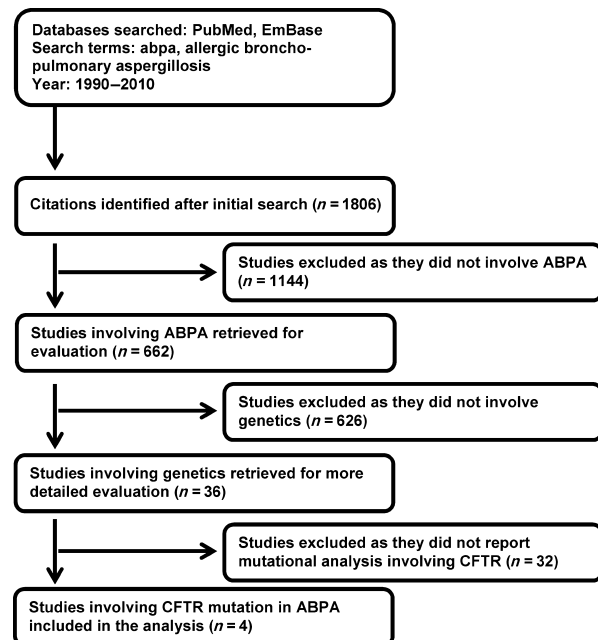


Figure 1 Flow diagram showing the trial selection process for this systematic review.

CFTR mutations in ABPA vs. the control population

Four studies have evaluated the presence of CFTR mutations in ABPA vs. the control population (79 ABPA, 268 controls).^{30–33} The pooled results from the four studies suggest a higher odds (OR 10.39; 95% CI, 4.35–24.79) in the occurrence of one CFTR mutation in ABPA vs. the control group (Fig. 2). Only one study found two CFTR mutations in a single patient, and in those with two CFTR mutations, segregation analysis was not performed. Hence, one cannot be certain whether the occurrence of two CFTR mutations is *in cis* or *in trans*.

CFTR mutations in ABPA vs. asthmatics

Two studies (52 ABPA, 94 asthmatics) have investigated the occurrence of CFTR mutations in ABPA vs. the asthma population.^{32,33} The odds (OR 5.53; 95% CI, 1.62–18.82) of encountering CFTR mutations were higher in ABPA compared with the asthma population (Fig. 3).

Heterogeneity and publication bias

There was no statistical heterogeneity with an I^2 value being 25.3% and Cochran Q statistic of 4.017 (P value = 0.26) although there was clinical heterogeneity in the different number of mutations screened in the individual studies, and the varying methodology used for diagnosis of CFTR mutations. There was no evidence of publication bias either on visual examination of the funnel plot (Fig. 4) or on the statistical tests (Begg–Mazumdar: Kendall's tau = 0.1667, $P = 0.73$; Egger: intercept = 5.7463, $P = 0.46$).

Discussion

The pathogenesis of ABPA remains largely speculative; however, the familial occurrence of ABPA does suggest a possible genetic contribution to the disease.^{35,36} The manifestations of chronic lung disease in ABPA is similar to CF, and the development of ABPA in CF suggests a possible role of CFTR mutations in ABPA. The finding of a higher occurrence of CFTR mutations in ABPA compared with the general population (91% higher chance) and asthmatics (85% higher chance) in this study further supports this hypothesis. Increased knowledge of potential ABPA-causing CFTR mutations (or genotypes) is crucial to identify risk groups for ABPA. This could lead to a more intensive and regular screening for ABPA from early age and, possibly, help to conduct studies targeting therapeutic ABPA prevention.

Table 1 Details of the study participants and the protocols used in various studies included in the systematic review.

Author (year)	No. of cases	No. of controls	Diagnosis of ABPA/(country of origin of participants)	No. of CFTR mutations screened	Methods used to identify mutation	Results
Miller <i>et al.</i> (1996) [30]	11 ABPA	53 chronic bronchitis	Asthma, pulmonary infiltrates, CB, immediate Af skin test positivity, total IgE >1000 ng ml ⁻¹ , positive Af precipitins, elevated Af IgG/IgE, blood eosinophilia, sweat chloride <40 mmol l ⁻¹ /(United States)	Both groups six mutations F508del, G542X, G551D, R553X, W1282X and N1303K; nine more mutations in ABPA: R117H, R347P, R347H, R334W, A455E, G551S, 2789 + 5 G>A, D1152H, and 3849 + 10 kb C>T	Reverse ASO analysis and DGGE with DNA sequencing	1 patient carried 2 CF (F508del; R347H) and 5 carried 1 CF (4 F508del; 1 R117H). Mutations seen in 6/11 ABPA vs. 1/53 controls
Aron <i>et al.</i> (1999) [31]	16 ABPA	39 subjects without allergy	Asthma, pulmonary infiltrates, CB, immediate Af skin test positivity, total IgE >1000 IU ml ⁻¹ , positive Af precipitins, elevated Af IgG/IgE, blood eosinophilia/(France)	31 CFTR mutations	Oligonucleotide ligation assay (Perkin Elmer Applied Biosystem, Foster City, Calif)	CFTR mutation in 4 of 14 patients with ABPA (1 R1162X, 1 N1303K, and 2 F508del mutations); 2/39 controls
Marchand <i>et al.</i> (2001) [32]	21 ABPA	43 allergic asthma; 142 healthy controls	Asthma, pulmonary infiltrates, CB, immediate Af skin test positivity, total IgE >450 IU ml ⁻¹ , positive Af precipitins, elevated Af IgG/IgE, blood eosinophilia >500 µl ⁻¹ . Sweat chloride <60 mmol l ⁻¹ /(Belgium)	R117H, 621 -1 G>T, R334W, F508del, I507del 10, 1717 -1 G>A, G542X, R553X, G551D, R1162X, 3849 + 10 kb C>T, W1282X, N1303K	Heteroduplex and acrylamide gel electrophoresis, ARMS, nested PCR followed by electrophoresis and DNA sequencing	One CFTR mutation in 6/21 patients (F508del [<i>n</i> = 2], G542X [<i>n</i> = 1], R1162X [<i>n</i> = 1], 1717 -1 G>A [<i>n</i> = 1], and R117H [<i>n</i> = 1]) vs. 2/43 asthmatics (1 CFTR mutation; (F508del, 1717 -1 G>A and 6/142 controls
Eaton <i>et al.</i> (2002) [33]	31 ABPA	Healthy controls (<i>n</i> = 34) Asthma (<i>n</i> = 51)	Asthma, positive SPT to Af, total IgE >1000 ng ml ⁻¹ , elevated Af-IgE, positive precipitins to Af, blood eosinophilia >350 µl ⁻¹ , pulmonary infiltrates on CXR or CB on CT/(New Zealand)	16 CF mutations -- F508del, I507del, R117H, W1282X, 621 + 1 G>T, R334W, R347P, A455E, 1717 -1 G>A, G542X, 5549N, G551D, R553X, R560T, N1303K and 3849 + 10 kb C>T	ASO hybridization and DGGE with DNA sequencing	4/31 (F508del [<i>n</i> = 3], R117H [<i>n</i> = 1]) vs. 2/51 asthma (F508del [<i>n</i> = 1], R117H [<i>n</i> = 1]) vs. 1/34 healthy controls

ABPA, allergic bronchopulmonary aspergillosis; ARMS, amplification refractory mutation system; ASO, allele-specific oligonucleotide; CB, central bronchiectasis; CFTR, cystic fibrosis transmembrane conductance regulator; DGGE, denaturing gradient gel electrophoresis; OR, odds ratio

CFTR mutation class (class I -- 1717 -1 G>A, R1162X, G542X; class II -- F508del, N1303K; class IV -- R347H, R117H).

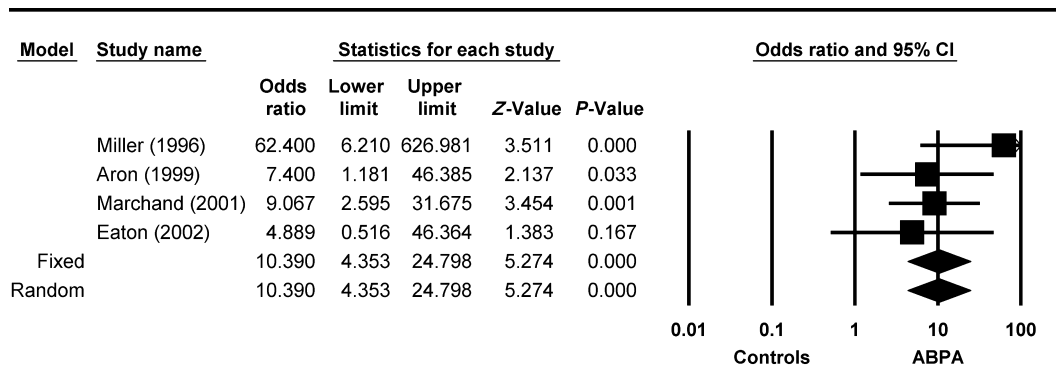


Figure 2 Forest plot showing the occurrence of CFTR mutations in ABPA vs. the control population. There is a higher chance of encountering CFTR mutation in ABPA compared with the control population.

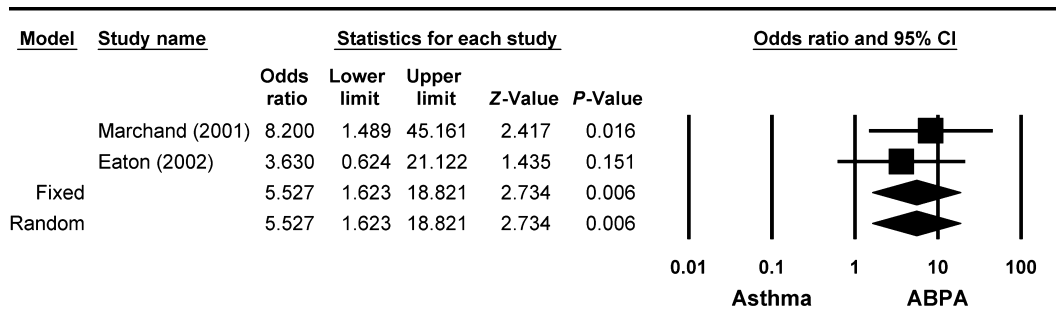


Figure 3 Forest plot showing the occurrence of CFTR mutations in ABPA vs. asthma. The odds of finding CFTR mutations in the ABPA group is significantly higher compared with asthmatics.

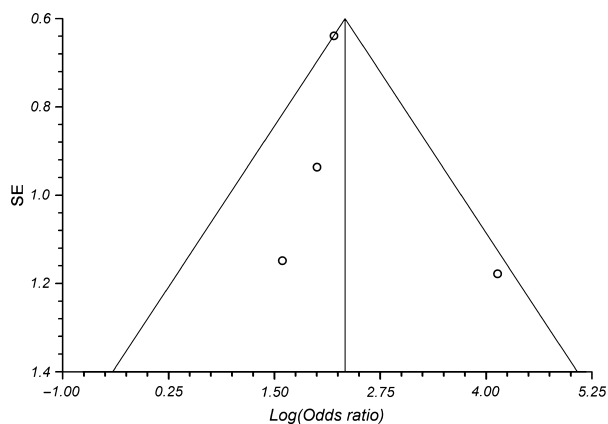


Figure 4 Funnel plot comparing log odds ratio vs. the standard error of log OR. Each open circle represents an individual study in the meta-analysis. The line in the center indicates the summary log OR and the other two lines indicate the 95% CI. There is no evidence of publication bias.

The first step in the development of ABPA is sensitization to *Aspergillus*, and ABPA can be conceptualised as an exaggerated form of *Aspergillus* sensitization.⁸ In

fact, there is a spectrum from mild to moderate asthma to severe asthma with fungal sensitization and ABPA.³⁷ The immune response to *Aspergillus* antigens in ABPA complicating asthma (and CF) is a Th2 CD4+ cell response.³⁸ However, not all patients with asthma and CF develop ABPA despite being exposed to *A. fumigatus*. For instance the isolation of *Aspergillus* from the respiratory secretions of CF patients is common with growth rates as high as 57% but the prevalence of ABPA in CF ranges from 5–15%.^{4,39} The allergic inflammatory response in ABPA appears to be quantitatively greater than that in *Aspergillus* sensitive asthma and CF, and this step is probably influenced by the genetic characteristics of an individual.⁴⁰ Indeed, CFTR deficient lymphocytes produce a higher level of Th2 cytokines compared with wild T cells in response to *A. fumigatus*.²¹

The phenotypic expression of CF is diverse with a wide spectrum of disease manifestations ranging from the classic presentation with pancreatic insufficiency, lung disease and elevated sweat chloride levels to atypical manifestations that include congenital bilat-

eral absence of vas deferens (CBAVD), diffuse bronchiectasis and infertility with sufficient pancreatic function and normal chloride levels.⁴¹ In fact, CBAVD is believed to be a genital manifestation of CF.⁴² Do the results of this study suggest that ABPA could be one such atypical manifestation of CF? The diagnosis of CF is based on a consistent phenotype with evidence of CFTR channel dysfunction (abnormal sweat chloride values or nasal potential difference), or identification of two disease causing CFTR mutations *in trans*.⁴³ However, in the current study not all patients with ABPA were shown to possess even one mutation and only a small number of patients were found to have two CFTR mutations. Can a single CFTR mutation i.e. the heterozygous state, lead to ABPA? The frequency of CFTR mutations were definitely higher in ABPA compared with the control population and this lends credence to the hypothesis that ABPA is a CFTR-related disorder. It may also be possible that many patients with ABPA carried mutations that were either not looked for or could not be discovered by the techniques performed in the individual studies. Marchand *et al.* in their study found six of 21 patients with ABPA heterozygous for one CFTR mutation when they screened for 13 CFTR mutations,³² whereas they could identify CFTR mutations in 12 of 18 patients of the same cohort when they screened them for more than 1300 mutations currently considered as potential CF causing.³⁴

Another important question is the relationship between complex alleles (occurrence of two CFTR mutations on the same allele) or heterozygous CFTR mutation and ABPA. The putative relevance of these mutations in ABPA has not been functionally characterised and thus the pathogenic potential remains an enigma. The demonstration of higher CFTR mutation frequency in asthma and chronic rhinosinusitis supports the hypothesis that heterozygous CFTR state is also pathogenic,^{44–48} and could explain the occurrence of single CFTR mutation in ABPA. However some studies have shown no greater evidence of chronic lung disease in CF carriers, and heterozygous CFTR state has even been shown to be protective against asthma.^{49,50} In ABPA, it may be hypothesised that mutation in one allele leads to subtle (undetectable) changes in the mucus properties. This can cause persistence of *A. fumigatus* triggering the immune cascade similar to the immunopathogenesis of ABPA in CF because of the abnormal mucus resulting from underlying homozygous CFTR mutations.⁵¹ In fact, a recent study demonstrated a continuum of increasing airway CFTR dysfunction (assessed by nasal potential difference

measurements) associated with the bearing of zero, one, or two CFTR mutations.⁵²

The severity of lung disease in CF varies, and one important mechanism for the variability of the pulmonary phenotype in CF is the presence of genetic modifiers. These are genetic variations not directly related to the CFTR gene but influence the severity or clinical manifestations of the disease. The genes encoding mannose-binding lectin (MBL) 2, an innate immune response protein and transforming growth factor-beta (TGF β), a potent suppressor of T cell activation have been implicated as pulmonary modifiers in CF.^{53–55} It has been seen that approximately 20% of patients with classic CF carry genetic variants in one or both of these genes.⁵⁶ Recently, Wright *et al.* carried out a genome-wide association study in about 3500 individuals with CF, and demonstrated a significant association between lung disease severity and a single nucleotide polymorphism on chromosome 11 and a locus at chromosome 20.⁵⁷ It is possible that a combination of single CFTR mutation in presence of genetic modifiers could lead to ABPA phenotype. Although polymorphisms in MBL and TGF β have been described in ABPA,^{58–60} no study has evaluated the association of genetic modifiers and CFTR mutations in ABPA, and future studies should also evaluate this factor.

One more reason for the lack of demonstration of CFTR mutations in all patients with ABPA could be the association between disease severity of ABPA and CFTR mutations. In fact, a higher proportion of CFTR mutations were seen in severe ABPA.^{30,33} In the study by Eaton *et al.* four cases in which mutations were seen were glucocorticoid-dependent reflecting a severe disease,³³ raising a possibility of an association between severity of ABPA and CFTR mutations. Recently, it has been shown that high attenuation mucus is related to the severity of the disease.^{61–63} Thus studies evaluating the presence of CFTR mutations in ABPA should classify ABPA according to severity of the disease based on high attenuation mucus and analyse genetic associations accordingly. On the other hand, the 'severity' of CFTR mutations is also correlated with *A. fumigatus* colonization. In one study, patients with 'minimal' CFTR function (mutation class I–III) had a higher hazard than patients with 'residual' function (mutation class IV–V) for *A. fumigatus* colonization. Also, these individuals with minimal CFTR function acquired infection at a younger age than those with residual function.⁶⁴

Finally, it may well be possible that ABPA unlike CF is a disorder with polygenic inheritance wherein mutations in different pathways interplay with environmen-

tal factors culminating in the phenotypic manifestation. Abnormalities in innate and/or the adaptive immune response other than CFTR gene can predispose to ABPA.^{16,40} Numerous studies have found association with various genetic mutations/polymorphisms in mediators of the innate and adaptive immunity including HLA (DR2, DR5 restriction), collectins (surfactant protein A, MBL, toll like receptor-9), cytokines (IL-10 and TGF- β) or their receptors (IL-4R α).^{58–60,65–73}

The current study is not without limitations. The major limitation is the small sample size of the study. Hence, large systematic studies are needed. This could possibly be addressed by formation of ABPA registry akin to the CF registries that analyse the extensive data in many countries or regions. The other major limitation is the limited panel of mutations that have been tested in the studies included in the meta-analysis. More than 1500 CFTR mutations have been described, and phenotypic characteristics of many CFTR mutations are also likely to be available soon (<http://www.cff.org>). Also, the severity of ABPA, which could have a bearing on outcome, was not reported in all the studies. Although, there is no statistical heterogeneity or publication bias, the small number of studies and the wide range of odds ratio limits the generalisability of the results.

Conclusions

In conclusion, there seems to be an association between CFTR mutations and ABPA as demonstrated by a higher frequency of CFTR mutations in ABPA in this systematic review. Due to the small sample size of the studies, further data is required to confirm this finding. Future studies should adopt a uniform methodology and should screen for the entire genetic sequence of the CFTR gene including gene modifiers to detect the presence of these mutations.

Financial disclosure

None

Conflict of interests

None

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