

SHORT REPORT

Alpha₁-antitrypsin phenotypes in patients with cryptogenic fibrosing alveolitis: a case-control study

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Alpha₁-antitrypsin phenotypes in patients with cryptogenic fibrosing alveolitis: a case-control study. R. Hubbard, Y. Baoku, N. Kalsheker, J. Britton, I. Johnston. ©ERS Journals Ltd 1997.

ABSTRACT: Cryptogenic fibrosing alveolitis (CFA) is an interstitial lung disease, which by definition is of unknown aetiology. Recent evidence has suggested that smoking and occupational exposure to dusts may be environmental risk factors for the disease, but there has been little research into potential host risk factors. One previous study has suggested that the prevalence of abnormal alpha₁-antitrypsin phenotypes may be increased in patients with CFA. Since alpha₁-antitrypsin is important in regulating inflammation within the lung in response to environmental exposures, such abnormalities may be of aetiological importance in this disease.

We have compared the alpha₁-antitrypsin phenotypes of 189 patients with CFA with 189 age-, sex-, and community-matched controls. This sample size was sufficient to provide more than 95% power to detect an odds ratio (OR) of 2.5. Alpha₁-antitrypsin phenotype was established by isoelectric focusing, and the prevalence of abnormal phenotypes in cases and controls was compared by conditional logistic regression. Personal smoking histories were obtained by postal questionnaire.

The prevalence of abnormal alpha₁-antitrypsin phenotypes was similar in cases and controls (12.7 versus 15.3%; OR 0.88; 95% confidence interval 0.49–1.57; p=0.66). No interaction was found between the presence of abnormal alpha₁-antitrypsin phenotypes and a history of smoking.

We conclude that cryptogenic fibrosing alveolitis is not associated with abnormal alpha₁-antitrypsin phenotypes.

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Cryptogenic fibrosing alveolitis (CFA) (also called idiopathic pulmonary fibrosis), is an interstitial lung disease which affects up to 20 per 100,000 people in the USA [1]. The registered mortality from CFA is increasing in a number of countries [2]. Although, by definition the disease is of unknown aetiology, there is increasing evidence that certain environmental exposures, including smoking and occupational dust exposure [3–6], may be important risk factors for CFA. A familial type of CFA does occur but it is rare, and for the majority of cases no host risk factor has been clearly identified. In the familial disease, the defective gene is believed to lie on chromosome 14 [7]. The gene for alpha₁-antitrypsin also lies on chromosome 14, and in one family the coexistence of alpha₁-antitrypsin deficiency and familial CFA has been reported [8].

Alpha₁-antitrypsin is a protease inhibitor, which protects the alveolar tissues from neutrophil elastase. Homozygote deficiency of alpha₁-antitrypsin is known to cause emphysema, but there have been reports of increased heterozygote phenotype deficiency in patients with rheumatoid arthritis, juvenile polyarthritis and vasculitis [9]. In one widely cited study, patients with rheumatoid arthritis complicated by fibrosing alveolitis were found to be more likely to have abnormal alpha₁-antitrypsin phenotypes than healthy controls [10]. Because of this finding, a

further 49 patients with CFA were studied and found to be two and a half times more likely to have abnormal alpha₁-antitrypsin phenotypes than healthy controls. To our knowledge, however, this reported evidence that the prevalence of abnormal alpha₁-antitrypsin phenotypes is increased in patients with CFA has never been confirmed. We have, therefore, compared the alpha₁-antitrypsin phenotypes of 189 patients with CFA to 189 age-, sex-, and community-matched controls.

Methods

For this study, we used serum samples from cases and controls identified as part of an investigation into the role of occupational dust exposures in the aetiology of CFA, details of which have been published previously [5]. Briefly, we attempted to identify all cases of CFA attending nine hospitals within the Trent region of the UK, and recruited all prevalent cases alive on Day 1 of the study, and all incident cases diagnosed over a prospective 18 month recruitment period. The diagnostic criteria for cases, which were designed to be relevant to usual clinical practice, are presented in table 1. Controls were matched

Table 1. – Diagnostic criteria for cryptogenic fibrosing alveolitis (CFA)

1. Basal inspiratory pulmonary crackles on examination.
2. Bilateral interstitial lung shadowing on chest radiography.
3. No documentation history of exposure to asbestos or other recognized fibrogens, including birds.
4. No clinical evidence of coexisting collagen vascular disease.
5. No other coexisting cause of interstitial lung disease.
6. Restrictive lung function defined as FEV₁/FVC >70% together with an FVC or TL_{CO} <80% of predicted. In the absence of restrictive lung function, patients were included if there were pathognomonic changes of CFA on a high-resolution computed tomography scan.

FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; TL_{CO}: transfer factor of the lung for carbon monoxide.

for age and sex and were drawn from the list of the same general practitioner (GP) *i.e.* family doctor, as the cases. Controls were not matched on the basis of smoking habit, because in our original study cigarette smoking was one of the aetiological factors under investigation. However, detailed data on participant smoking history were available from the previous study [5] for use in the analysis, and additional clinical data were available for cases from review of the hospital clinical records. The survival status of each incident case was established from the GP 18 months after the completion of patient recruitment. Of the 218 cases in the present series, 189 (87%) consented to have a blood sample taken. Blood samples were also obtained from one matched control per case, approaching the controls in order of closeness of age to the case. Sera were separated and stored at -70°C prior to assay. The study was approved by the Nottingham City Hospital Ethics Committee.

Alpha₁-antitrypsin phenotyping of samples was performed, blind to disease status, by isoelectric focusing. For the analysis, the normal MM phenotype was defined as the baseline, and other phenotypes as abnormal, and the odds ratios (ORs) of CFA in relation to the presence of abnormal alpha₁-antitrypsin phenotypes by conditional logistic regression were calculated.

This analysis was performed for cases as a whole, and also for incident cases separately to look for possible survival bias. A subset analysis was then performed to look for associations with individual abnormal phenotypes. The above analyses were repeated adjusting for the effects of smoking history, using first the binary variable "ever or never smoker" and then the quantitative variable "pack-years of smoking". Evidence of interaction between the presence of abnormal alpha₁-antitrypsin phenotypes and the variable "ever or never smoker" was also sought. Finally, we examined whether the presence of abnormal alpha₁-antitrypsin phenotypes had any effect on survival for patients with CFA using Cox's proportional hazards analysis, for incident cases only.

Assuming the prevalence of abnormal alpha₁-antitrypsin phenotypes in the general population to be 15%, it was calculated that using the 189 matched case-control sets of sera available, would provide in excess of 95% power to detect an OR of 2.5, *i.e.* of similar magnitude to that reported by GEDDES *et al.* [10].

Table 2. – Alpha₁-antitrypsin phenotypes for all cases of cryptogenic fibrosing alveolitis and matched controls, and separately for incident cases and matched controls

	Alpha ₁ -antitrypsin phenotype				
	MM	MS	MZ	SS	SZ
All cases (n=189)	165	20	4	0	0
Controls (n=189)	160	14	11	3	1
Incident cases (n=57)	52	4	1	0	0
Controls (n=57)	47	4	3	2	1

Results

The mean (SD) age of the cases was 67 (11) yrs, 131 (69%) were male, and 57 (30%) were incident cases. At presentation, the mean percentage predicted (SD) forced vital capacity (FVC) was 79 (21)% and transfer factor of the lung for carbon monoxide (TL_{CO}) was 51 (17)%. A history of being a current or exsmoker was more common amongst cases than controls, although not to the point of statistical significance (77 *versus* 67%; OR 1.48; 95% confidence interval (95% CI) 0.87–2.51; p=0.15). The mean number of pack-years accumulated by cases who had smoked was higher than that for controls, although this difference was nonsignificant (37 *versus* 35 pack-years; p=0.6). The median duration of follow-up for the incident cases was 2.1 yrs, during which time 30 (53%) cases died.

The results of phenotyping (table 2) demonstrated no evidence of association between CFA and the presence of abnormal alpha₁-antitrypsin phenotypes either before (OR 0.88; 95% CI 0.49–1.57; p=0.66), or after adjustment for the effect of cigarette smoking (OR 0.80; 95% CI 0.43–1.49; p=0.49). Similarly, for incident cases only, no relationship was found between alpha₁-antitrypsin phenotype and CFA either before (OR 0.43; 95% CI 0.11–1.66; p=0.22), or after adjustment for the effects of smoking (OR 0.29; 95% CI 0.06–1.38; p=0.12). Use of pack-years rather than the binary variable "ever or never smoker" in these analyses produced similar results. No evidence of any interaction between smoking habit and alpha₁-antitrypsin was found.

Subset analysis revealed no evidence of association between CFA and any particular alpha₁-antitrypsin phenotype. The presence of abnormal alpha₁-antitrypsin phenotypes had no effect on the survival of incident cases (hazard ratio 0.49; 95% CI 0.07–3.60; p=0.5), even after adjustment for age at presentation, sex, FVC at presentation, and smoking habit (hazard ratio 1.04; 95% CI 0.13–8.26; p=0.97).

Discussion

Our results provide no evidence of an association between the presence of abnormal alpha₁-antitrypsin phenotypes and cryptogenic fibrosing alveolitis, findings which clearly contrast with those of GEDDES *et al.* [10]. This negative study is not due to a lack of statistical power since we had sufficient cases, almost four times as many as GEDDES *et al.* [10], and controls to detect an OR of 2.5,

which is of similar size to the effect reported previously [10]. The prevalence of abnormal α_1 -antitrypsin phenotypes in our community-matched controls was almost identical to that seen for healthy controls in the study by GEDDES *et al.* [10], and is similar to reports from other normal populations [11]. The difference between the two studies, therefore, lies in the prevalence of abnormal α_1 -antitrypsin phenotypes in patients with CFA. In particular, GEDDES *et al.* [10] found a marked increase in the prevalence of the MZ phenotype amongst patients with CFA, but in the present study this phenotype was actually less common amongst cases than controls. It is important to note that the findings of GEDDES *et al.* [10] were not part of the primary hypothesis tested in their study, and were reported to be unexpected. That others have not reported similar findings since the original study nearly 20 yrs ago also suggests that this finding is an isolated observation, and likely to be due to chance.

Other researchers have concentrated on the normal variants of the M phenotype of α_1 -antitrypsin. One study investigated the distribution of these variants in patients with rheumatoid arthritis and scleroderma [12], and found no association with either disease, but in subset analyses an association was found between M phenotype variants and interstitial lung disease for patients with rheumatoid arthritis, but not scleroderma. Although the small number of patients in this study and the inconsistency of the data between the two diseases limit the conclusions which may be drawn, the possibility remains that variants of the normal M phenotype may be associated with the development of interstitial lung disease, and therefore of importance in CFA. In contrast, BATE *et al.* [13] have examined restriction fragment length polymorphisms of the α_1 -antitrypsin gene and found no association between these and rheumatoid arthritis or its pulmonary manifestations.

In summary, we conclude on the basis of the present study that cryptogenic fibrosing alveolitis is not associated with abnormal α_1 -antitrypsin phenotypes.

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