

TGF- β_1 genotype and accelerated decline in lung function of patients with cystic fibrosis

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Abstract

Background—Polymorphisms in transforming growth factor (TGF)- β_1 associated with variations in cytokine levels are linked to fibrosis in a number of tissues. However, the contribution of this cytokine to organ fibrosis in patients with cystic fibrosis is presently unclear. This study was undertaken to examine the association between TGF- β_1 gene polymorphisms and the development of pulmonary dysfunction in patients with cystic fibrosis.

Methods—Polymorphisms in the TGF- β_1 gene defining amino acids of codons 10 and 25 were determined by ARMS-PCR using DNA stored on 171 Caucasian patients who were homozygous for the $\Delta F508$ mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Clinical information on the patients was obtained from medical records.

Results—Patients with cystic fibrosis of a TGF- β_1 high producer genotype for codon 10 had more rapid deterioration in lung function than those with a TGF- β_1 low producer genotype. The relative risk of accelerated decline in forced expiratory volume in one second (FEV₁) to 50% predicted and forced vital capacity (FVC) to 70% predicted of patients with a high producer genotype was 1.74 (95% CI 1.11 to 2.73) compared with 1.95 (95% CI 1.24 to 3.06) for those with a low producer genotype.

Discussion—TGF- β_1 genotypes may have a role in mediating pulmonary dysfunction in patients with cystic fibrosis. Further work is required to determine whether inhibition of TGF- β_1 activity in these patients may slow disease progression.

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Keywords: cystic fibrosis; transforming growth factor (TGF)- β_1 ; polymorphisms; lung function tests

Cystic fibrosis is the most common fatal inherited condition in the western world. Despite advances in the medical management of cystic fibrosis over the last three decades, there are still a number of unexplained differences in its clinical course, especially in the rate of development of respiratory failure of patients who appear to have the same CFTR genotype, degree of bacterial colonisation, and compliance with medication.^{1,2} In this study we have tested

the hypothesis that this difference may be due partly to inherited variation in the ability of patients to express the profibrotic cytokine, transforming growth factor (TGF)- β_1 .

TGF- β_1 is produced by many cells throughout the body and promotes the proliferation of fibroblasts and the deposition of collagen.^{3,4} Production of TGF- β_1 varies between individuals and partly depends on polymorphisms in the TGF- β_1 gene at positions +869 and +915 encoding codons 10 and 25.⁵⁻⁸ The bi-allelic polymorphism, either a T or C in the DNA sequence at position +869 (relative to the major transcription start site), changes the amino acid at codon 10 from leucine to proline. Similarly, the bi-allelic polymorphism, either a G or C at position +915, results in the amino acid at codon 25 being either arginine or proline. For both polymorphisms the allele encoding proline is associated with lower TGF- β_1 synthesis in vitro and in vivo.

In human lung tissue TGF- β_1 produced by bronchial epithelial cells stimulates fibroblasts to proliferate in vitro.⁹ Previous animal and human studies have shown that high TGF- β_1 producers develop significantly more lung fibrosis in response to a number of inflammatory triggers such as radiation,¹⁰ chemotherapy,¹¹ and lung transplantation.⁵ Little is known about the role of TGF- β_1 in cystic fibrosis. Examination of lung tissue in two patients with cystic fibrosis showed strong immunohistological staining of inflammatory tissue using an antibody against this cytokine.¹² In this study we have investigated the correlation between high TGF- β_1 production and pulmonary dysfunction in cystic fibrosis by determination of polymorphisms in the TGF- β_1 gene.

Methods

PATIENTS AND CLINICAL RECORDS

The Department of Clinical Genetics at the Royal Manchester Children's Hospital, Manchester has stored DNA from all patients with cystic fibrosis in the North West region of the United Kingdom who have been referred for genetic mutation testing over the last two decades. Data from 171 patients who attended one of three major cystic fibrosis units in Manchester (Royal Manchester Children's Hospital, Booth Hall Children's Hospital or Wythenshawe Hospital) were examined. Clinical information was obtained from hospital notes and physiotherapy records in which formal lung function tests were regularly recorded. At the time of blood collection consent had been obtained to use their DNA for studies which

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Table 1 Frequencies of TGF- β_1 polymorphisms

TGF- β_1 polymorphisms		Controls (n = 107)	CF patients (n = 171)
+869 (codon 10)	+915 (codon 25)		
Allele frequencies			
T	Any	139 (65%)	224 (66%)
C	Any	75 (35%)	118 (34%)
Any	G	193 (90%)	319 (93%)
Any	C	21 (10%)	23 (7%)
Genotype frequencies			
T/T*	-	44 (41%)	67 (39%)
T/C	-	51 (48%)	90 (53%)
C/C	-	12 (11%)	14 (8%)
-	G/G*	87 (81%)	149 (87%)
-	G/C	19 (18%)	21 (12%)
-	C/C	1 (1%)	1 (1%)
T/T	G/G	44 (41%)	66 (39%)
T/C	G/G	38 (36%)	72 (42%)
C/C	G/G	5 (5%)	11 (6%)
T/T	G/C	0 (0%)	1 (0%)
T/C	G/C	13 (12%)	17 (10%)
C/C	G/C	6 (6%)	3 (2%)
T/T	C/C	0 (0%)	0 (0%)
T/C	C/C	0 (0%)	1 (0%)
C/C	C/C	1 (1%)	0 (0%)

*TGF- β_1 high producer genotypes^{5,6}; all others are TGF- β_1 low producer genotypes.

would enhance scientific knowledge of their condition. Polymorphism frequencies were compared with those of 107 healthy laboratory workers and renal transplant donors analysed previously in our laboratory.⁶

All 171 patients were Caucasian and homozygous for the $\Delta F508$ CFTR gene mutation; 45% were male with ages ranging from 6 to 54 years (mean (SD) 23 (9) years). Forty three (25%) had died at a mean (SD) of 22 (8) years

(range 6–50) and 85 (50%) had developed respiratory failure with forced expiratory volume in one second (FEV₁) of <50% predicted at 20 (7) years (range 5–35). 15% of patients had cirrhosis with portal hypertension (heterogeneous appearance of liver, dilated portal vein, and splenomegaly on abdominal ultrasound scan) and the same percentage had insulin dependent diabetes. Only 4% of patients had both cirrhosis and diabetes.

TGF- β_1 GENE POLYMORPHISM STUDIES

Archived DNA used in this study had been extracted from 5 ml whole blood using standard methods. The technique used to genotype individual samples for the two TGF- β_1 alleles under investigation was an amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method using primers already described.⁷

STATISTICAL ANALYSIS

Survival analysis was carried out by Kaplan-Meier and Cox regression analysis using the SPSS statistical package. Medians and 95% confidence intervals (CI) are given.

Results

TGF- β_1 POLYMORPHISM FREQUENCIES

The relative frequencies of the two polymorphisms of TGF- β_1 gene are shown in table 1 and are similar to those found in healthy adults.⁶ There was no skewing of the distribution of these polymorphisms in patients who had died or in those with FEV₁ <50% predicted, with cirrhosis, or with diabetes.

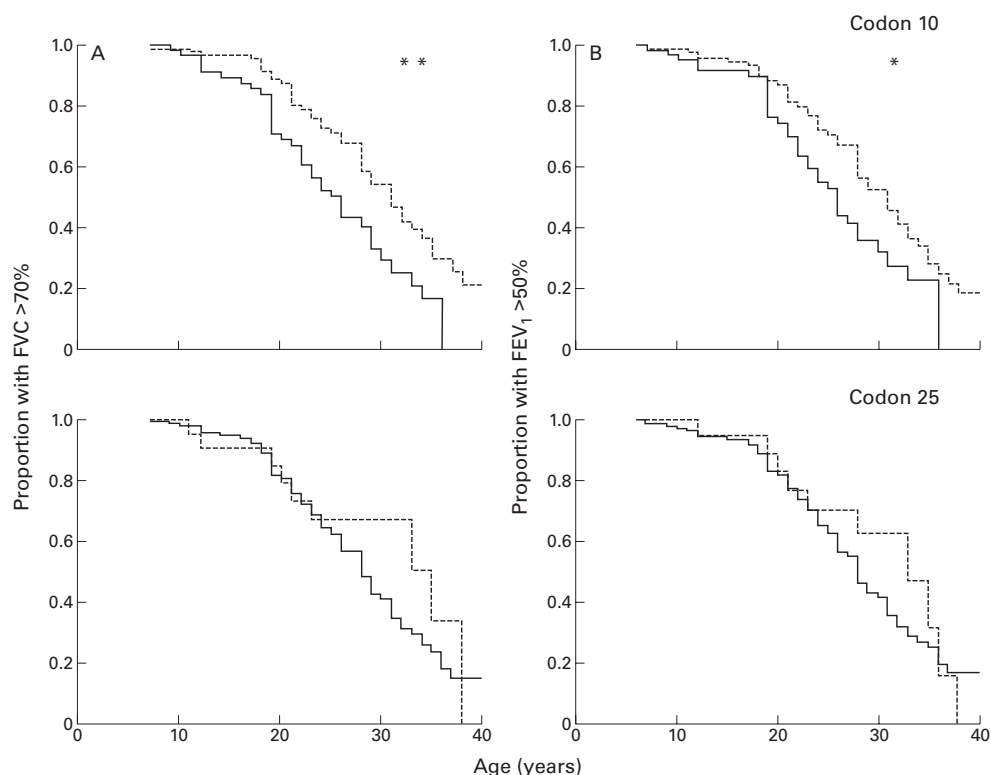


Figure 1 Proportion of patients with (A) FVC >70% predicted and (B) FEV₁ >50% predicted with increasing age for polymorphisms in TGF- β_1 codon 10 and codon 25. Cases were censored from the analysis after the age of final follow up. Continuous line = TGF- β_1 high producer genotype; broken line = TGF- β_1 low producer genotype. * p <0.01, ** p <0.005, Kaplan-Meier analysis.

Table 2 Hazard ratios and 95% confidence intervals (CI) for more rapid deterioration in FEV₁ to <50% predicted and FVC to <70% predicted with high producer TGF- β_1 genotype

	FEV ₁			FVC		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Codon 10						
"High"	1.74	1.11 to 2.73	<0.02	1.95	1.24 to 3.06	<0.005
"Low"	1.00			1.00		
Codon 25						
"High"	1.05	0.68 to 2.74	NS	1.40	0.70 to 2.80	NS
"Low"	1.00			1.00		
Codons 10 + 25						
"High"	1.71	1.10 to 2.69	<0.02	1.91	1.21 to 2.99	<0.005
"Low"	1.00			1.00		

NS = not significant.

MORTALITY

The average age at death or lung transplantation of patients who had the TGF- β_1 high producer genotype for codons 10 and/or 25 was not significantly different from patients with a low producer genotype using Kaplan-Meier statistical analysis.

RESPIRATORY INSUFFICIENCY

Patients with the TGF- β_1 high producer genotype for codon 10, but not codon 25, developed a forced vital capacity (FVC) of <70% predicted on average five years younger (mean 26 (range 23–29) years) than patients with a TGF- β_1 low producer genotype (mean 31 (range 28–34) years; fig 1). The relative risk of patients with a high producer genotype having a reduced FVC of <70% predicted at a younger age was double that of patients with a low producer genotype (hazard ratio 1.95 (95% CI 1.24 to 3.06), $p < 0.005$; table 2). Patients with a high producer TGF- β_1 genotype also developed severe obstructive disease (FEV₁ <50%) and respiratory failure at a significantly lower age than patients with a low producer genotype (hazard ratio 1.74 (95% CI 1.11 to 2.73), $p < 0.02$; fig 1). Of those with an FVC of <70% predicted, 86% also had an FEV₁ of <50% predicted ($p < 0.000001$; χ^2 test).

There were no significant differences between the proportion of patients with *Pseudomonas aeruginosa* or *Burkholderia cepacia* isolated from sputum culture and non-colonised patients ($n = 21$ (12%)) with a TGF- β_1 high producer genotype. The TGF- β_1 high producer genotype was also not associated with a shorter interval between lung colonisation and development of pulmonary insufficiency (FEV₁ <50% predicted).

Discussion

In this study we have shown that, in patients with cystic fibrosis, the TGF- β_1 codon 10 high producer genotype is associated with a more rapid deterioration in lung function. This is in agreement with studies of patients following lung transplantation^{5,13} and in animal models of lung injury following radiation¹⁰ and chemotherapy¹¹ where inherent production of high levels of TGF- β_1 are associated with more severe lung fibrosis and more rapid deterioration in lung function. High levels of TGF- β_1 have also been associated with increased fibro-

sis in the liver of patients with autoimmune hepatitis¹⁴ and animals models of both liver and kidney fibrosis.^{15–17}

Lung disease in patients with cystic fibrosis is characterised by both airway obstruction and interstitial fibrosis. The standard way of measuring the progress of lung disease is by spirometric testing at each outpatient attendance. We found that the risk of FEV₁ falling below 50% predicted and FVC falling below 70% predicted at a significantly younger age in patients with a TGF- β_1 codon 10 high producer genotype was almost double that of patients with a low producer genotype; 86% of our patients had both an FEV₁ of <50% predicted and an FVC of <70% predicted. One would expect a stronger correlation between polymorphisms in the profibrotic TGF- β_1 cytokine gene and lung fibrosis (restrictive lung disease) than with airway obstruction. However, because of the close correlation between obstructive and restrictive disease in patients with cystic fibrosis, it may be difficult to dissect out the relative association between the TGF- β_1 genotype and these two elements of lung disease, even in a prospective study using full lung function tests to assess the degree of restrictive lung disease.

No correlation was found between the TGF- β_1 codon 25 genotype and measures of survival or lung function. As the population is skewed markedly towards a high producer genotype (87%), the number of patients in the low producer genotype group was small ($n = 22$) which may have reduced the power of the study to detect differences between the groups. However, in a previous study with a similar sample size in which the association between TGF- β_1 polymorphisms and histological evidence of pulmonary fibrosis in patients undergoing lung transplantation was examined, we found a significant increase in the proportion of patients with high producer genotype for both codons 25 and 10. The difference was more significant for codon 10 ($p < 0.004$) than for codon 25 ($p < 0.02$).⁵ The fact that histological examination provided a much more accurate means of assessing fibrosis than lung function tests may provide part of the explanation for these differences.

In summary, TGF- β_1 high producer genotypes predict a group of cystic fibrosis patients who will develop more rapid pulmonary disease. Further work is required to determine

whether blocking the effects of this cytokine may retard the progression of lung disease.

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