

ORIGINAL ARTICLE

Variants in mannose-binding lectin and tumour necrosis factor α affect survival in cystic fibrosis

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Background: Patients with cystic fibrosis with the same mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene differ widely in survival suggesting other factors have a substantial role in mortality.

Objective: To determine if the genotype distribution of variants in three putative cystic fibrosis modifier genes (tumour necrosis factor α (*TNF α*), transforming growth factor β 1 (*TGF β 1*) or mannose-binding lectin (*MBL2*)) differed among patients with cystic fibrosis grouped according to age and survival status.

Methods: Genotypes of four variants (*TNF α -238*, *TNF α -308*, *TGF β 1-509* and *MBL2 O*) were determined in three groups of Caucasians from a single medical centre: 101 children with cystic fibrosis (aged <17 years; mean age 9.4 years), 115 adults with cystic fibrosis (aged \geq 17 years; mean age 30.8 years) and 38 non-surviving adults with cystic fibrosis (21 deceased and 17 lung transplant after 17 years of age). Genotypes of 127 healthy Caucasians in the same geographical region were used as controls. Kaplan–Meier and Cox hazard regression were used to evaluate the genotype effect on cumulative survival.

Results: Genotype frequencies among adults and children with cystic fibrosis differed for *TNF α -238* (G/G vs G/A; $p=0.022$) and *MBL2* (A/A vs O/O; $p=0.016$). When adults with cystic fibrosis were compared to non-surviving adults with cystic fibrosis, genotype frequencies of both genes differed (*TNF α -238* G/G vs G/A; $p=0.0015$ and *MBL2*: A/A vs O/O; $p=0.009$). The hazard ratio for *TNF α -238* G/G vs G/A was 0.25 (95% CI 0.06 to 1.0, $p=0.04$) and for *MBL2* O/O vs A/A or A/O was 2.5 (95% CI 1.3 to 4.9, $p=0.007$).

Conclusions: *TNF α -238* G/A and *MBL2* O/O genotypes appear to be genetic modifiers of survival of cystic fibrosis.

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Cystic fibrosis is the most common life-limiting autosomal recessive disease in Caucasians. Although the median age of survival for patients with cystic fibrosis is now almost 35 years, age at death among patients with cystic fibrosis varies substantially.¹ Measures of the severity of lung disease, such as FEV₁ and airway microbiology, are significant predictors of survival of cystic fibrosis as expected for a disease where 90% of mortality is attributed to pulmonary insufficiency.^{2–4} Epidemiologic analyses of the US CF Foundation Patient Registry have identified a number of other factors (eg, sex, birth cohort, symptoms and age at presentation, nutritional status and household income) that contribute to mortality.^{4–6} Although variation in the cystic fibrosis transmembrane conductance regulator (*CFTR*), the gene responsible for cystic fibrosis, has also been associated with survival, mortality of patients with the same *CFTR* genotype varies substantially.^{4,7} However, little is known about the contribution of other genes to cystic fibrosis survival.⁸

One study has demonstrated an association of variants in the mannose-binding lectin gene (*MBL2*) with severity of lung disease and survival in patients with cystic fibrosis.⁹ MBL binding of bacteria and viruses facilitates activation of an alternative complement pathway serving as primary defense especially in infant life. The "O" structural variants of *MBL* act in a partial dominant negative fashion to reduce the amount of functional *MBL* multimers in heterozygous (A/O) individuals, whereas primarily non-functional monomeric forms of *MBL* are found in the plasma of homozygous (O/O) individuals.¹⁰ A number of other studies have replicated the association

between *MBL2* variants and severity of cystic fibrosis lung disease but the association with cystic fibrosis survival has not been replicated.^{11–14} Functional variants in tumour necrosis factor α (*TNF α*) and transforming growth factor β 1 (*TGF β 1*) have also shown reproducible association with cystic fibrosis lung disease severity,^{15–18} but neither gene has been evaluated for survival effect. *TNF α* is a potent pro-inflammatory cytokine secreted by macrophages, lymphocytes and adipocytes in response to stimuli such as lipopolysaccharide. In the airways, *TNF α* binding induces the release of cytokines IL 6 and IL 8 and increases mucous production via modulation of the TNF receptor-associated factor–TNFR1-associated death domain protein–nuclear factor κ B (TRAF–TRADD–NF κ B) pathway.^{19,20} The profile of inflammatory mediators elevated in cystic fibrosis lung matches the transcriptional effect of NF κ B, leading to the proposal that dysregulation of the NF κ B pathway is central to the abnormal inflammatory status in patients with cystic fibrosis.²¹ Transforming growth factor β -1 (*TGF β 1*) is a multi-functional cytokine involved in many cellular functions. In the lungs, *TGF β 1* secreted by bronchial epithelial cells promotes fibroblast proliferation in response to inflammation leading to lung fibrosis.¹⁶ *TGF β 1* has been associated with lung diseases in animal models and in humans.

We hypothesised that as patients with cystic fibrosis age, genetic variants that affect cystic fibrosis survival should be easier to detect. Based on national cystic fibrosis data, annual

Abbreviations: MBL, mannose-binding lectin; SNP, single nucleotide polymorphism; TGF, transforming growth factor

mortality rates are relatively low before 17 years of age (1–2.4%) but rapidly increase thereafter (3–5.4%).¹ Thus, we performed a case–control study of patients with cystic fibrosis grouped according to age as a proxy for survival. Genetic variants that increase survival were predicted to be enriched in patients aged >17 years when compared with patients aged <17 years. Conversely, genetic variants that decrease survival were predicted to be reduced in frequency in patients aged >17 years. We then compared genotypes of three genes implicated as cystic fibrosis modifiers (*MBL2*, *TNF α* and *TGF β 1*) among patients stratified according to survival status and age (children, aged <17 years and adults, aged \geq 17 years). To exclude the effects due to differences in the treatment of cystic fibrosis, we compared the frequency of genetic variants in the patients with cystic fibrosis surviving beyond 17 years of age with patients from the same birth cohort and medical centre who had died from complications of cystic fibrosis after 17 years of age.

MATERIALS AND METHODS

Study subjects

This study was approved by the Institutional Review Board of the Johns Hopkins Medical Institutions, and written informed consent was obtained from all participants or their parents. From the Johns Hopkins cystic fibrosis clinics, 254 Caucasian patients with cystic fibrosis (101 children \leq 17 years, 115 adults \geq 17 years and 38 “non-surviving” adult patients with cystic fibrosis (21 had died of cardiopulmonary complication and 17 had lung transplantation)) were recruited. The majority of cystic fibrosis adults were recruited between 1984 and 1988 while the children with cystic fibrosis were recruited from 2001 to 2003. All patients lived in the same geographical region (Baltimore and nearby areas). In all, 127 healthy Caucasian adults from the same geographical region as the patients with cystic fibrosis recruited from 1995 to 2003 were used as controls for this study. The forced expiratory volume in 1 s (FEV₁) for each adult patient with cystic fibrosis was derived from at least two pulmonary function tests performed in 2002. Bacterial infection was documented after three separate oropharyngeal or sputum cultures were positive for the same organism.

Genotyping

Genotyping was performed by the sequence-specific oligonucleotide-PCR method as previously described.^{22–23} Briefly, DNA extracted from blood specimens by the phenol chloroform method was amplified by multiplex PCR using biotinylated primers. Hybridisation of the biotinylated PCR products to immobilised oligonucleotide probes specific to the alleles under study allowed colorimetric determination of genotype. Six single nucleotide polymorphisms (SNPs) of three genes (*MBL2* B (rs 1800450), C (rs 1800451), D (rs 5030737) alleles; *TNF α* -238 (rs 361525) and -308 (rs 1800629) alleles, *TGF β 1*-509 (rs 1800469) alleles) were typed. To evaluate the validity of the sequence-specific oligonucleotide-PCR method, 44 masked subjects had *MBL2* genotypes determined by DNA sequencing. A concordance of 100% was observed between the two methods.

Statistical analysis

Genotype frequencies were determined by counting and distributions of genotypes between patient and control groups were evaluated using χ^2 and Fisher’s exact test. The effect of *MBL2* and *TNF α* genotypes on survival was evaluated by Kaplan–Meier survival analysis. The log rank test was used to assess the statistical significance of the Kaplan–Meier plots. Hazard ratios (HRs) with 95% CIs and p values were calculated using Cox proportional hazards regression. Results of life-table analysis were evaluated for significance using the Wilcoxon

statistic. Comparisons of multiple groups were assessed using analysis of variance (ANOVA). All statistical methods were performed using SPSS V.11.5. Statistical evaluation was performed under the a priori assumption that each gene was a cystic fibrosis modifier. A p value of \leq 0.05 was deemed significant.

RESULTS

Table 1 shows the demographics of the patient and control groups used in this study.

Adults with cystic fibrosis and non-surviving adults with cystic fibrosis are from the same birth cohort while the adult control group has older individuals. All individuals were typed for six SNPs selected from three genes (*MBL2*, *TNF α* and *TGF β 1*) that have been associated with the severity of cystic fibrosis lung disease. To test for association with survival, we compared the genotype frequencies of each SNP between cystic fibrosis adults and children. Variants in two genes, *MBL2* and *TNF α* , demonstrated significant differences in genotype distribution between these two groups (table 2). For the *MBL2* gene, the difference in genotype distribution is due to a deficiency of O/O genotypes in adults with cystic fibrosis compared to children with cystic fibrosis. The distribution of A/A and A/O genotypes does not deviate significantly between these two groups. For *TNF α* , the difference between the two groups is due to a higher frequency of the *TNF α* -238 G/A genotype in adults with cystic fibrosis compared to children with cystic fibrosis. These results suggest that *MBL2* O/O is associated with reduced survival beyond 17 years of age while *TNF α* -238 G/A appears to be associated with an increased chance of surviving beyond 17 years of age (table 2).

To test these associations, we compared the genotype frequencies of the SNPs in adults with cystic fibrosis to adults with cystic fibrosis from the same birth cohort and cystic fibrosis clinic who had died from the disease after 17 years of age. The O/O genotype of *MBL2* was higher in non-survivors compared to the adults with cystic fibrosis as expected for a negative modifier of survival (table 3). In addition, the *TNF α* -238 G/A genotype was absent in non-surviving adults with cystic fibrosis compared to the adults with cystic fibrosis, consistent with positive modifier effect on survival (table 3).

To evaluate the contribution of *CFTR* genotype, homozygotes for the common cystic fibrosis mutation *AF508* were compared with “other” *CFTR* genotypes and there were no differences. However, the non-surviving adults with cystic fibrosis had a higher fraction of *AF508* homozygotes (n = 24; 63%) compared with surviving adults with cystic fibrosis (n = 60; 52%), and the difference approached statistical significance (tables 2 and 3).

The distributions of the *MBL2* and *TNF α* genotypes in each group did not deviate from the distributions predicted by the Hardy–Weinberg equation (not shown). Furthermore, when adults with cystic fibrosis and non-surviving adults with cystic

Table 1 Demographics of the patient and control groups

	Children with CF	Adults with CF	Non-surviving adults with CF	Healthy controls
n	101	115	38	127
Year of birth (SD)	1993 (4.1)	1972 (9.4)	1970 (9.5)	1956 (13.9)
Age range (years)	0–16	17–66	17–50*	23–77
Mean age (years)	9.4	30.8	28.7*	46.3
Sex (male:female)	51:50	57:58	20:18	NA

CF, cystic fibrosis.

*Age at death or lung transplant.

Table 2 Comparison of genotype distributions of variants in three candidate genes in children and adults with cystic fibrosis

	Children with CF	Adults with CF	Fisher's exact*	χ^2 †
	n (%)	n (%)		
MBL2‡				
A/A	63 (62.4)	77 (66.9)	A/A vs	0.057
A/O	29 (28.7)	36 (31.3)	O/O: 0.016	
O/O	9 (8.9)	2 (1.7)		
TGFβ1-509				
C/C	56 (55.4)	49 (42.6)	C/C vs	0.100
C/T	38 (37.6)	60 (52.2)	T/T: 0.230	
T/T	7 (6.9)	6 (5.2)		
TNFα-238				
G/G	92 (91.1)	94 (81.7)	G/G vs	0.074
G/A	9 (8.9)	21 (18.3)	G/A: 0.022	
A/A	0	0		
TNFα-308				
G/G	67 (66.3)	84 (73)	G/G vs	0.349
G/A	32 (31.7)	27 (23.5)	A/A: 0.290	
A/A	2 (2)	4 (3.5)		
CFTR				
$\Delta F508/\Delta F508$	54 (53.5)	60 (52.2)	$\Delta F508/\Delta F508$	0.956
Other genotypes	47 (46.5)	55 (47.8)	vs other: 0.107	

CF, cystic fibrosis.

*Since χ^2 may be inaccurate when any cell frequency is <5, Fisher's exact was used to compare genotype frequencies in 2x2 tables. P values are shown for comparisons when any cell frequency was <5. All other comparisons had non-significant p values.†p Values for χ^2 test using 2x3 table; 2 df except for TNF-238 when a 2x2 table was used.‡The three independent amino acid substitutions termed B (G54D), C (G57E) and D (R52C) in exon 1 of MBL2 are collectively designated as O alleles whereas the wild type is termed A.⁹**Table 3** Comparison of genotype distribution variants in three candidate genes in adults with cystic fibrosis and non-surviving adults with cystic fibrosis

	Adults with CF	Non-surviving adults with CF	Fisher's exact*	χ^2 †
	n (%)	n (%)		
MBL2‡				
A/A	77 (66.9)	21 (55.3)	A/A vs	0.012
A/O	36 (31.3)	12 (31.6)	O/O: 0.009	
O/O	2 (1.7)	5 (13.1)		
TGFβ1-509				
C/C	49 (42.6)	12 (31.6)	C/C vs	0.320
C/T	60 (52.2)	22 (57.9)	T/T: 0.186	
T/T	6 (5.2)	4 (10.5)		
TNFα-238				
G/G	94 (81.7)	38 (100)	G/G vs	0.010
G/A	21 (18.3)	0	G/A: 0.0015	
A/A	0	0		
TNFα-308				
G/G	84 (73)	27 (71.0)	G/G vs	0.883
G/A	27 (23.5)	9 (23.7)	A/A: 0.303	
A/A	4 (3.5)	2 (5.3)		
CFTR				
$\Delta F508/\Delta F508$	60 (52.2)	24 (63.2)	$\Delta F508/\Delta F508$ vs	0.321
Other genotypes	55 (47.8)	14 (36.8)	other: 0.076	

CF, cystic fibrosis.

*Because χ^2 may be inaccurate when any cell frequency is <5, Fisher's exact was used to compare genotype frequencies in 2x2 tables. p Values are shown for comparisons when any cell frequency was <5. All other comparisons had non-significant p values.†p Values for χ^2 test using 2x3 table; 2 df except for TNF-238 when a 2x2 table was used.‡The three independent amino acid substitutions termed B (G54D), C (G57E) and D (R52C) in exon 1 of MBL2 are collectively designated as O alleles whereas the wild type is termed A.⁹

fibrosis were reconstituted into a single birth cohort, the distribution of *MBL2* and *TNF α* genotypes did not differ from children with cystic fibrosis, or from healthy controls from the same geographical region (table 4). These results indicate that the genotype differences between adults with cystic fibrosis and non-surviving adults with cystic fibrosis are not due to biased ascertainment of either group.

The *CFTR* genotype distribution in the combined adult with cystic fibrosis group did not differ significantly from the children with cystic fibrosis (table 4), or the 17 836 genotyped patients enrolled in the US-CF Foundation Registry (data not shown). Finally, no significant difference was observed in the distribution of *MBL2* or *TNF α* genotypes in the adults with cystic fibrosis and non-surviving adults with cystic fibrosis homozygous for $\Delta F508$ compared with patients with other *CFTR* genotypes (table 5).

Kaplan-Meier survival analyses of the adults with cystic fibrosis and non-surviving adults with cystic fibrosis based on genotype illustrated that individuals with cystic fibrosis with *MBL2* O/O genotype have a survival disadvantage (p = 0.004, log rank), while those with *TNF α -238* G/A genotype have a marked survival advantage (p = 0.03, log rank, fig 1A,B). *CFTR* genotype does not influence survival until patients surpass 33 years of age, after which, homozygosity for $\Delta F508$ is associated with reduced survival compared to other *CFTR* genotypes (p = 0.03, log rank, fig 1C). Life-table analysis showed that median survival time of patients with *MBL2* A/A or A/O genotype differed significantly from patients with the O/O (41 and 27 years, respectively; p = 0.02; Wilcoxon statistic). Similarly, median survival of patients with *TNF α* G/G genotypes

differed significantly from patients with the G/A (50 and > 50 years, respectively; p = 0.04; Wilcoxon statistic). Patients with cystic fibrosis carrying the *MBL2* O/O genotype compared with patients with cystic fibrosis carrying A/A or A/O have a hazard ratio (HR) of 2.5 (95% CI 1.3 to 4.9, p = 0.007). Other comparisons of *MBL2* genotypes were not significant. The *TNF-238* G/A genotype is associated with a reduced HR of 0.25 (95% CI 0.06 to 1.0, p = 0.04) when compared with patients with cystic fibrosis bearing the G/G genotype. The absence of patients with the *TNF-238* A/A genotype precluded calculation of HRs for this genotype. The HR for *CFTR* genotype does not reach statistical significance (not shown).

Mean percentage predicted FEV₁ (the measurement of pulmonary function most predictive of the severity of cystic fibrosis lung disease) and the frequency of *Pseudomonas aeruginosa* and *Burkholderia* infection (bacterial pathogens that contribute to reduced longevity in patients with cystic fibrosis) did not differ between surviving adult patients with cystic fibrosis carrying the A/A and A/O MBL genotypes (mean % predicted FEV₁ 61% vs 58%; *Pseudomonas* 94% vs 89%; *Burkholderia* 2.6% vs 2.8%, respectively). Too few surviving patients with the *MBL2* O/O genotype were available for meaningful analysis of FEV₁. All seven adult patients with cystic fibrosis (two surviving, five deceased) with the *MBL2* O/O genotype had *P. aeruginosa* infection and none had *Burkholderia* infection. The difference in mean percentage predicted FEV₁ between surviving adult patients with cystic fibrosis carrying the *TNF-238* G/A and those with the G/G genotype was not statistically significant (G/A 65.3%; n = 21; G/G 59.9%; n = 89; ANOVA p = 0.12). The frequency of *P. aeruginosa* and *B. cepacia*

Table 4 Comparison of genotype distribution of *TNF α* , *MBL2* and *CFTR* genotypes between combined adults with cystic fibrosis and two control groups

	Combined adults with CF*	Children with CF	Healthy controls
	n (%)	n (%)	n (%)
<i>MBL2</i>			
A/A	98 (64)	63 (63)	70 (55)
A/O	48 (31)	29 (29)	46 (36)
O/O	7 (5)	9 (9)	11 (9)
<i>TNFα-238</i>			
G/G	132 (86)	92 (91)	115 (90)
G/A	21 (14)	9 (9)	11 (9)
A/A	0	0	1 (1)
<i>CFTR</i>			
$\Delta F508/\Delta F508$	84 (55)	54 (53.5)	
Other genotypes	69 (45)	47 (46.5)	

CF, cystic fibrosis.

*Adults with cystic fibrosis and non-surviving adults with cystic fibrosis.

infection did not differ significantly between surviving adults with cystic fibrosis with G/A and G/G genotypes (*P aeruginosa* 95.2% vs 93.6%; *B cepacia* 0% vs 4.2%, respectively).

DISCUSSION

Numerous studies have investigated the association between candidate genes and different aspects of the cystic fibrosis phenotype.^{8–24} Replication of associations in multiple independent patient populations is a key step in confirming a pathologic role for a protein variant in a disease process. *CFTR* genotype has been associated with survival⁷ and Kaplan–Meier analysis of patients in this study suggest that the survival effect of *CFTR* may be age dependent (ie, after 33 years of age). We show that variation in *MBL2* is associated with reduced survival, replicating the finding of Garred *et al.*⁹ However, in this study, only the less common O/O *MBL2* genotype appeared to influence survival. We have also discovered an association between a relatively common variant in *TNF α* and mortality due to cystic fibrosis. Although two other studies have shown association between variants of *TNF α* and lung disease severity,^{15–16} this is the first study to suggest that variation in *TNF α* modifies cystic fibrosis survival. Finally, *TNF α* appears to be the first modifier gene with a genotype associated with an improved outcome in cystic fibrosis.

A number of studies have suggested that *MBL2* alleles are associated with the severity of lung disease in cystic fibrosis. In three studies, patients with cystic fibrosis who carried *MBL2*

genotypes A/O or O/O alleles had more severe lung disease as judged by FEV₁% predicted than their A/A counterparts (p values 0.03, 0.04 and 0.002, respectively).^{9–11–14} One study found that only O/O patients had significantly worse lung function (p<0.05).²⁵ This study involved 260 children and 298 adults with cystic fibrosis although the effect of the *MBL2* O/O genotype was confined to adults. We also did not find a difference in lung function when comparing adults with cystic fibrosis with A/A and A/O genotypes and too few O/O individuals were available for meaningful comparisons. A study utilising the concentration of MBL in plasma found that mean FEV₁% predicted was significantly lower in MBL-deficient patients aged >15 years.¹⁴ Together, these studies suggest that the deleterious consequences of MBL deficiency become apparent in patients with cystic fibrosis as they age, particularly in those with severe MBL deficiency associated with the O/O genotype. Thus, survival would be expected to reduce in patients with the *MBL2* O/O genotype after adolescence. This concept is consistent with our observation that *MBL2* O/O is under represented in adults with cystic fibrosis compared with children with cystic fibrosis and over represented in patients that died after 17 years of age compared with adults with cystic fibrosis.

A recent multi-centre study involving 808 $\Delta F508$ homozygote patients with cystic fibrosis did not reveal association between lung disease severity and *MBL2* genotype, and *MBL2* genotype frequencies did not differ significantly in 56 non-surviving patients with cystic fibrosis.¹⁸ A possible clue to lack of *MBL2* association may be due to differences in study design; the large-scale case–control study of 808 patients required recruitment from many cystic fibrosis centres whereas the studies that found association between *MBL2* and lung function primarily involved patients from a single cystic fibrosis centre. Collection of study subjects from a single centre affords a degree of control over variation in non-genetic factors (ie, access to care, treatment and phenotype definition) that is difficult to control in multi-centre studies. Thus, single-centre studies can have higher power than multi-centre studies to detect genetic contribution to outcomes that have a substantial non-genetic component.²⁴ Second, the non-survivor group studied by Drumm and colleagues had a greater proportion of transplanted patients (47/56; 84%) compared with the single-centre studies that found an association with survival (17/38; 45% in this study and 12/26; 46%). Criteria for lung transplantation have a low positive predictive value for mortality (~30%) making transplantation an imperfect proxy in survival studies.²⁶ The high proportion of transplantation patients in the Drumm study may have made their non-survivor group less representative of mortality risk factors than the current study and the study of Garred and colleagues.⁹ Finally, the Drumm study primarily involved adult patients with cystic fibrosis in whom the effect of the *MBL2* O/O genotype on lung function and

Table 5 Distribution of *MBL2* and *TNF α* genotypes in adults with cystic fibrosis stratified by *CFTR* genotype

	n	<i>MBL2</i>	$\Delta F508/\Delta F508$	Other
Adults with CF	115	O/O	2	0
		A/A or A/O	58	55
Non-surviving adults with CF	38	O/O	5	0
		A/A or A/O	19	14
	n	<i>TNFα-238</i>	$\Delta F508/\Delta F508$	Other
Adults with CF	115	G/A	12	9
		G/G	48	46
Non-surviving adults with CF	38	G/A	0	0
		G/G	24	14

CF, cystic fibrosis.

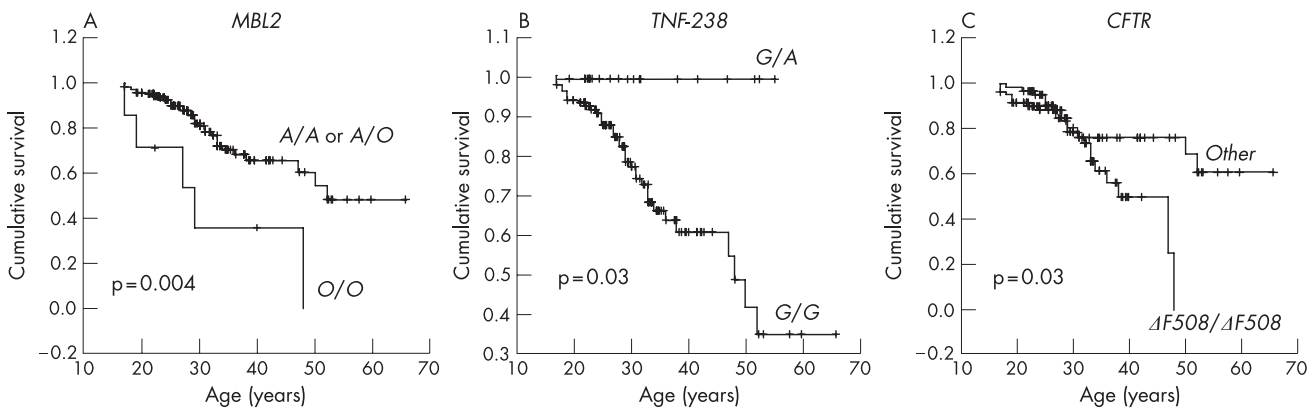


Figure 1 Kaplan-Meier plots of cumulative survival based on genotype. A. The *MBL2* *O/O* genotype is associated with decreased survival compared with patients with the *A/A* or *A/O* genotype ($p=0.004$, log rank test). B. The *TNF α* *G/A* genotype is associated with increased survival compared with the *G/G* genotype ($p=0.03$; log rank test). C. The *CFTR* $\Delta F508/\Delta F508$ genotype is associated with reduced cumulative survival compared with patients with "other" *CFTR* genotypes ($p=0.03$; log rank test).

survival might have been difficult to discern without comparing to a younger cystic fibrosis cohort (as noted above).

Although this study found the *TNF α* -238 *G/A* allele associated with survival, we were unable to correlate this allele with mean FEV₁. Likewise, the -238 alleles did not show association with pulmonary function measurements in a study of 113 patients with cystic fibrosis.¹⁶ These results suggest that the -238 *A* allele might improve survival by affecting other life-limiting manifestations in patients with cystic fibrosis. Monocytes bearing the -238 *A* variant have been shown to produce lower amounts of *TNF α* in response to stimulation than those homozygous for -238 *G*.²⁷ The presence of a transcriptional repressor site that incorporates nucleotides between -254 and -230 suggests that the reduced expression might be attributed directly to variation at -238.²⁸ Alternatively, sequence variants such as *TNF α* -376 *A* that are in linkage disequilibrium with -238 *A* may be responsible for altered transcription. The -376 *A* variant has been shown to regulate *TNF α* transcription by altering binding of the transcription factor OCT-1.²⁹ Thus, patients with cystic fibrosis bearing the -238 *A* allele might have global reductions in *TNF α* levels leading to a life-long decrease in inflammatory status compared with patients who are homozygous for the *G* allele.

Association between *TGF β 1* alleles and lung function in patients with cystic fibrosis was first reported in 171 *ΔF508* homozygotes. Individuals carrying the *T/T* codon 10 genotype (+869) reached an FEV₁% predicted below 50% significantly earlier in age than patients with other genotypes at this SNP.¹⁷ Average age at death did not differ for *TGF β 1* genotypes at codon 10 or codon 25 (+915). A second study involving 808 *ΔF508* homozygote patients with cystic fibrosis reported that the *C/C* genotype of codon 10 and the *T/T* genotype at -509 were associated with severe lung disease. Survival analysis was not performed. Although we were unable to show a significant association between *TGF β 1*-509 genotypes and survival, the frequency of the -509 *T* allele in adults with cystic fibrosis (0.26) compared with non-surviving adults with cystic fibrosis (0.40) approached significance ($p=0.07$). Thus, the current study may be under powered to detect the effect of *TGF β 1* variation upon cystic fibrosis survival.

In summary, we have replicated and refined the observation that variation in *MBL2* contributes to cystic fibrosis survival.⁹ In addition, we show that a putative promoter polymorphism in *TNF α* influences survival of patients with cystic fibrosis. Both of these genes encode proteins involved in the inflammatory

response. These findings are consistent with the potential role of inflammatory mediators in cystic fibrosis pathophysiology that has been emphasised in numerous studies, and also highlights the potential role of anti-inflammatory agents in the treatment of cystic fibrosis.²¹⁻³⁰

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