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Short communication

MBL2 polymorphisms screening in a regional Italian CF Center

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Abstract

We performed *MBL2* genotyping in 47 CF patients—cared of at the regional CF Centre of Trieste—trying to establish a correlation within allelic variants of *MBL2* and modification of patients' clinical outcome. FEV1 values were significantly lowered and a significantly earlier age at onset of *Pseudomonas aeruginosa* colonisation was found in CF patients with at least one *MBL2* variant.

Keywords: Mannose-binding lectin; *MBL2*; Cystic fibrosis; Modifier genes; Respiratory infections

1. Introduction

The importance of the *MBL2* gene as a modifier locus in the phenotype of CF patients has been demonstrated by several authors [1–3]. Mannose-binding lectin (MBL) is a serum protein that plays a pivotal role in natural immune response. MBL acts as an "ante-antibody" and can enhance opsonisation, or can activate the classical pathway of the complement on certain bacteria, viruses and fungi [4,5]. Three dominant missense mutations—at codon 52, 54 and 57—were described in the first exon of the *MBL2* gene, which encodes MBL and are proved responsible for reduced levels of the protein in serum [6].

In this study, we investigated whether *MBL2* variants could modify the clinical outcome of selected CF patients admitted to the Cystic Fibrosis Regional Centre of Trieste (Friuli Venezia Giulia, Italy) with the aim of providing new insights on the prognostic factors influencing the course of lung diseases in CF patients.

2. Methods

2.1. Patients

We examined 47 patients admitted to the Regional CF Centre of Trieste (Italy), whose diagnosis was made after two positive sweat tests performed according to Gibson and Cooke [7]. Patients have been regularly followed-up since 1990.

Since all patients were able to secrete sputum, *Pseudomonas aeruginosa* (PA) and *Burkholderia cepacia* (BC) colonisations were defined as the presence of PA or BC in the sputum culture at least in three consecutive samples collected in a 6-month period.

Pulmonary function was evaluated on the basis of forced expiratory volume in 1 s (FEV1) expressed as a percentage of expected values corrected for sex and height [8]. For every patient, the best value of all records collected during each calendar year was taken into consideration. FEV1

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Table 1
Clinical characteristics of the 47 CF patients

Patients	M	F	Total
Number	25	22	47
Median age at diagnosis (years)	2.76	0.68	
Present median age (years)	19.90	16.95	
PA colonization	20	19	39
Median age of PA colonization (years)	10.23	7.04	
BC colonization	4	1	5
Mean FEV1	88%	83%	
Severe/severe *CFTR* genotype	14	14	28
Mild/unknown *CFTR* genotype	11	8	19

values were collected in patients not previously treated with a bronchodilator.

2.2. CFTR and MBL2 genotyping

CFTR genotyping was performed using the INNO-LiPA CF kit (Innogenetics, Ghent, Belgium). When INNO-LiPA CF kit was not able to detect mutations the whole *CFTR* gene was screened by DHPLC (Transgenomic, Omaha, NE, USA) and direct sequencing. *CFTR* genotype and CF phenotype severity were assessed using the data published in scientific literature [9]. *MBL2* exon I genotyping was performed using the Melting Temperature Assay (MTA) [10].

2.3. Statistics

MBL2 genotype and allele frequencies were calculated by direct gene counting, Chi square test and 2×2 contingency tables were used for the comparison of all categorical variants, while ANOVA tests were performed to calculate differences within average values. All statistical analyses were performed using the SPSS Software v. 7.0 (SPSS Inc., Chicago, IL).

3. Results

Clinical features of patients admitted to the CF Centre are shown in Table 1. The group we analysed consists of 25 females (present average age 16.95) and 22 males (present average age 19.90). There are no differences between males and females in median age of the CF diagnosis, number of individuals with PA colonization and median age of the colonization. Only one patient (male) out of 47 showed pancreatic sufficiency. Table 2 shows *MBL2* allele and genotype frequencies in the 47 CF patients and in 130 pan-ethnic healthy controls. As expected, allele and genotype frequencies agreed with the Hardy–Weinberg equilibrium. Clinical characteristics of CF patients normal MBL producers (A/A) and CF patients low MBL producers (A/0 and 0/0) are shown in Table 3. *CFTR* and *MBL2* genotypes are reported in Table 4.

MBL2 genotype affects both the onset of PA colonisation as well as patients' FEV1 values. In fact, the median age of PA colonisation was significantly lower in *MBL2* A/0 and

Table 2
MBL2 allele and genotype frequencies in CF patients and healthy controls

MBL2	CF patients (47)	Healthy controls (130)
Genotype		
A/A	24/47 (51.1%)	78/130 (60%)
A/0	21/47 (44.7%)	45/130 (35%)
0/0	2/47 (4.2%)	7/130 (5%)
Allele		
A	69/94 (73.4%)	201/260 (77.3%)
0	25/94 (26.6%)	59/260 (22.7%)

Table 3
General and clinical characteristics of CF patients divided into normal MBL producers (A/A) and lower MBL producers (A/0 and 0/0)

CF patients	*MBL2* genotypes		
	A/0 and 0/0	A/A	*p*
N	23	24	
Gender (M/F)	13/10	12/12	ns
Median age at diagnosis (years)	1.48	1.66	ns
Actual median age (years)	17.3	19.9	ns
Mean follow-up duration (years)	15.1	17.9	ns
PA colonization	20	19	ns
Median age of PA colonization (years)	6.29	11.24	0.037*
BC colonization	3	2	ns
Severe/severe *CFTR* genotype	14	14	ns
Mild/unknown *CFTR* genotype	9	10	ns
Mean FEV1 values	71.3%	94%	0.04*
Mean FEV1 in non-PA colonized	85%	92%	ns
Mean FEV1 in PA colonized	70%	90.2%	0.04*
Mean FEV1 in severe/severe CF phenotypes	74.6%	90.2%	0.01*
Mean FEV1 in mild/unknown CF phenotypes	66%	82.7%	ns

* $p = 20$ (p = B2 0.5).

Table 4
CFTR and *MBL2* genotypes

CFTR genotypes	*MBL2* genotypes		
	AA	A0	00
Severe/Severe CFTR genotype			
deltaF508/deltaF508 (20)	10	8	2
deltaF508/N1303K (1)	0	1	0
deltaF508/621+1G→T (3)	2	1	0
1717-1G→A/1717-1G→A (1)	1	0	0
deltaF508/1677delTA (1)	1	0	0
G542X/G542X (1)	0	1	0
deltaF508/1717-1G→A (1)	0	1	0
Total 28	14	12	2
Mild; unknown/unknown CFTR genotype			
R1162X/2789+5G→A (6)	3	3	0
2183 AA→G/4016insT (4)	2	2	0
R1162X/R1162X (3)	1	2	0
DI507/2183 AA→G (4)	2	1	0
S466X/R1070Q; 5T (2)	2	1	0
Total 19	10	9	0

0/0 CF patients (6.29 years) when compared to A/A patients (11.24; $p=0.037$). Moreover, the average FEV1 percentage is significantly lower in low MBL producers (71.3%) than in normal MBL producers (94%; $p=0.04$).

The influence of the *MBL2* genotype on FEV1 is strictly related to PA colonisation, *CFTR* mutations/CF phenotype. In fact, the mean FEV1 values were significantly different between low and normal MBL producers only in PA colonized patients ($p=0.04$) and in patients showing a severe genotype ($p=0.01$). The average FEV1 values of CF patients not colonized by PA and CF patients presenting a mild or unknown genotype did not vary significantly between low and normal MBL producers.

Although a 16.7% difference between low and normal MBL producers was evidenced among mean FEV1 values in patients with mild/unknown CF genotypes, the statistical significance was not reached due to high standard deviations.

4. Discussion

The correlation between *MBL2* genotype and pulmonary phenotype severity in CF patients has been broadly described but it is still controversial. Recently, Davies et al. [11] found that CF patients with 2 structural *MBL2* mutations, but no heterozygotes, had more severe pulmonar clinical symptoms. These were not related to increased rates of infection with PA and there was no apparent increased susceptibility to BC. However, Carlsson et al. [12]-who investigated serum MBL deficiency, *MASP-2* gene mutation and reduced MBL pathway function in CF subjects-reported that no correlation to reduced lung function could be demonstrated except in a small group of patients colonized with *S. aureus* (which, like BC but unlike PA, binds MBL) and whose MBL-deficient genotypes were associated with decreased lung function.

Our data, even if obtained on a small group of Italian CF patients, show that low MBL producers are characterised by an earlier onset of PA colonisation of the lungs. Moreover, in our population, the *MBL2* genotype affects FEV1 values in patients colonized, but it seems not to have any effect in non-colonized ones (Table 3).

We can also hypothesize a *MBL2* involvement in the inflammation process of lungs. As already suggested by Garred et al. [3], a deficient removal of apoptotic and necrotic bodies in loco by MBL may trigger the inflammation of tissues leading then to a worsening of CF patients' clinical conditions.

In conclusion, *MBL2* behaved as a modifier gene in the cohort of CF patients admitted to our regional CF centre, while *MBL2* genotyping immediately after a CF diagnosis could be of interest for the follow-up, expecially in low MBL producer subjects.

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