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Short communication

Association of interleukin-10 gene haplotypes with *Pseudomonas aeruginosa* airway colonization in cystic fibrosis

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Abstract

We genotyped three polymorphisms of the promoter region of the interleukin-10 (IL-10) gene in 220 CF patients from the CF Center of Bari, and tested for an association between genetic variants of the cytokine and chronic airway colonization with *Pseudomonas aeruginosa*. We found that carriers of the high-IL-10-producing-GCC haplotype had significantly higher risk of chronic pulmonary infection with the pathogen.

Keywords: Interleukin-10; Cystic fibrosis; *Pseudomonas aeruginosa*; Gene polymorphism

1. Introduction

Chronic infection with *Pseudomonas aeruginosa* (*P. aeruginosa*) represents a major cause of lung disease in cystic fibrosis (CF) [1]. The observation that the severity of pulmonary disease usually does not correlate with the CFTR genotype, has raised the hypothesis that other environmental and genetic factors could affect the CF lung phenotype [2]. Several studies suggest that IL-10 plays a pivotal role in modulating an excessive activity in the inflammatory cascade, down-regulating the production of some cytokines [3,4]. It has been demonstrated that bronchoalveolar lavage fluid (BALF) from CF patients contains high concentrations of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, but negligible amounts of IL-10, as compared to healthy volunteers [5]. Bonfield et al. [6] have also demonstrated that bronchial epithelial cells from CF patients produce lower amounts of IL-10 compared to control subjects. The same authors documented that IL-10 deficiency results in increased local and systemic morbidity in mice with chronic endobronchial infection from *P. aeruginosa* [7]. Recent studies support also the concept that polymorphisms in the promoter region of IL-10 gene could affect the production of this cytokine in several inflammatory disorders [8–10].

The purpose of the present study was to test the hypothesis that a genetically determined variation in the production of IL-10 might influence the development of chronic infection with *P. aeruginosa* in patients with CF.

2. Methods

2.1. Patients

Clinical charts were examined of 220 Caucasian patients who attended a regional referral CF Center in Southern Italy from 1996 to 2006. The diagnosis of CF was established on the basis of the results of 2 sweat chloride tests (>60 mmol/L), and the identification of mutations in the CFTR gene [11,12]. The CFTR genotypes were grouped in severe/severe (carriers of classes I, II and III mutations), mild (carriers of classes IV and V mutations) and unknown/unknown. The study protocol was approved by the local Ethics Committee. Clinical, biological, and functional data were obtained retrospectively from patients' hospital records. Results of pulmonary function tests (measurements of forced expiratory volume in 1 s, FEV1,

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Table 1
Clinical features of the study population

Patients characteristic	
Male/female ratio	1/1
Median age at diagnosis in years (range)	2.4 (1–14)
Present median age in years (range)	16 (1–46)
P. aeruginosa colonization	124/175 (71%)
Median age of colonization in years (range)	9.0 (4.5–19)
Median age of first infection in years (range)	5.8 (3.2–8)
Median FEV1 as % of predicted (range)	76 (20–126)
Severe/severe CFTR genotype [a]	179/220 (81%)
Mild; unknown/unknown CFTR genotype [a]	41/220 (19%)
Median % FEV1 (range) in severe/severe CF phenotypes	77 (20–126)
Median % FEV1 (range) in mild/unknown CF phenotypes	67 (32–112)
PS/PI ratio	0.1/1

[a] Severe CFTR genotype = mutations of classes I, II, III; Mild CFTR genotype = mutations of classes IV and V; PS/PI = Pancreatic sufficiency/insufficiency.

expressed as % of predicted values) were obtained from 157 (71%) patients. Sputum swabs were collected in 175 (79%) subjects during a routine visit (patients were regularly examined at 4-month intervals) as well as twice during hospitalization. Chronic *P. aeruginosa* infection was defined as the presence of this pathogen in the sputum cultures in at least three consecutive samples collected in a 6-month period [13]. Informed consent on DNA analysis was obtained from the participants, or in case of minors, from parents.

2.2. Molecular genetic methods

Genomic DNA was extracted from blood samples, and the single nucleotide polymorphisms (SNPs) at position -1082 (A/G), -819 (C/T) and -592 (C/A) in the promoter region of the IL-10 were genotyped by use of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), as described elsewhere [10]. The alleles combination of the SNPs gives rise to three main haplotypes in Caucasians: GCC, ACC, ATA [9,10].

2.3. Processing of sputum samples, culture of P. aeruginosa and phenotypic analysis

Sputum samples from all patients were mixed with equal volumes of 1% dithiothreitol (Merck, Darmstadt, Germany) before incubation at 37 °C for 30 min. All specimens were examined microscopically and cultured. Isolates obtained from the samples were identified by the Phoenix (Becton Dickinson, Sparks, MA, USA) automated system.

2.4. Statistical analysis

Statistical analyses were performed using SPSS® for Windows software (SPSS Inc., version 11.0, Chicago, IL). Pearson Chi-square or Fisher Exact test were used for categorical data. Differences between groups were tested with the nonparametric Kruskal–Wallis test. Linear regression analyses were calculated for determination of the influence of confounding variables (gender, birth date, age at diagnosis of CF, CFTR genotype, and pancreatic status) on the outcome. Haplotypes were inferred from promoter IL-10 SNPs by Bayesian methods as implemented in the Phase software [14]. Odds ratios (OR) with 95% confidence intervals (CI) for infection susceptibility of specific IL-10 haplotypes were determined using association analysis with FBAT, adjusting for confounders [15].

3. Results

Clinical features of patients enrolled to the study are summarized in Table 1. We found a high rate (71%) of chronic *P. aeruginosa* infection in our CF population. Demographic and clinical characteristics of chronic *P. aeruginosa* colonized and non-colonized CF patients are shown in Table 2. There were significant differences between these two groups of CF patient in median age and median FEV1 values. CFTR mutations/CF phenotype did not affect both the *P. aeruginosa* colonization status as well as patients FEV1 values.

The frequencies of alleles, genotypes and haplotypes of IL-10 gene are shown in Table 3. The genotype–allele distribution for the IL-10 SNPs followed the Hardy–Weinberg equilibrium. The -819 and the -592 SNPs were in complete linkage disequilibrium in our population. Table 3 shows that, although not significantly, the frequency of the -1082AA genotype tended to be higher than the GG genotype in *P. aeruginosa* non-colonized patients compared with colonized subjects (63% vs 10% and 46% vs 17%, $p=0.09$). This different genotype frequency was reflected in the haplotype distribution in the two study groups: the ACC haplotype was observed twice as frequent as the GCC haplotype among non-colonized patients compared to colonized subjects (46% vs 24% and 34% vs 35%, $p=0.01$). Single -1082 SNP analysis also revealed that out of 33 patients homozygous for GG, 27 (81%) were colonized and 6 (18%) were not colonized (Table 4), while of 101 patients carrying the AA genotype at the same SNP, 67 (66%) were colonized and 34 (34%) were non-colonized

Table 2
General and clinical characteristics of CF patients divided into *P. aeruginosa* colonized and non-colonized patients

Patients characteristic	*P. aeruginosa* colonized ($n=124$)	Non-colonized ($n=51$)
Male/female ratio	1/1	0.8/1
Median age at diagnosis in years (range)	1.4 (1–9)	1.6 (1–14)
Present median age in years (range)	22 (4–46)	8 (3–27) [b]
Median FEV1 as % of predicted (range)	68 (20–126)	92 (62–119) [c]
Severe/severe CFTR genotype [a]	107/124 (86%)	47/51 (92%)
Mild; unknown/unknown CFTR genotype [a]	17/124 (14%)	4/51 (8%)
Median % FEV1 (range) in severe/severe CF phenotypes	74 (20–126)	94 (62–119)
Median % FEV1 (range) in mild/unknown CF phenotypes	60 (55–67)	81 (73–89)
PS/PI ratio	0.1/1	0.2/1

[a] Severe CFTR genotype = mutations of classes I, II, III; Mild CFTR genotype = mutations of classes IV and V; PS/PI = Pancreatic sufficiency/insufficiency.
[b] χ^2 $p=0.001$.
[c] χ^2 $p=0.006$.

Table 3
Alleles, genotypes and haplotypes frequencies in the study population divided into *P. aeruginosa* colonized and non-colonized patients

IL-10 alleles and genotypes	[a]Frequency in all patients (n=220)	[a]Frequency in colonized (n=124)	[a]Frequency in non-colonized (n=51)
-1082 (A/G)			
A	0.65 (288/440)	0.65 (160/248)	0.76 (77/102)
G	0.35 (152/440)	0.35 (88/248)	0.24 (25/102)
A/A	0.46 (101/220)	0.46 (57/124)	0.63 (32/51)
A/G	0.39 (86/220)	0.37 (46/124)	0.27 (14/51)
G/G	0.15 (33/220)	0.17 (21/124)	0.10 (5/51)
-819 (C/T)			
C	0.71 (313/440)	0.68 (169/248)	0.69 (70/102)
T	0.29 (127/440)	0.32 (79/248)	0.31 (32/102)
C/C	0.52 (115/220)	0.51 (63/124)	0.47 (24/51)
C/T	0.38 (83/220)	0.34 (42/124)	0.43 (22/51)
T/T	0.10 (22/220)	0.15 (19/124)	0.10 (5/51)
-592 (C/A)			
C	0.71 (313/440)	0.68 (169/248)	0.69 (70/102)
A	0.29 (127/440)	0.32 (79/248)	0.31 (32/102)
C/C	0.52 (115/220)	0.51 (63/124)	0.47 (24/51)
C/A	0.38 (83/220)	0.34 (42/124)	0.43 (22/51)
A/A	0.10 (22/220)	0.15 (19/124)	0.10 (5/51)
IL-10 haplotypes			
GCC	0.34 (151/440)	0.35 (88/248)	0.24 (24/102)[b]
ACC	0.38 (165/440)	0.34 (83/248)	0.46 (47/102)
ATA	0.28 (124/440)	0.31 (77/248)	0.30 (31/102)

[a] Relative allele and haplotype frequency on the total number of chromosomes; relative genotype frequency on the total number of patients.
[b] χ^2 p value GCC vs ACC=0.01, comparing colonized and non-colonized patients.

Table 4
Association between *P. aeruginosa* airway colonization, IL-10 genotypes and IL-10 haplotypes

IL-10 genotypes	Frequency of colonization	OR (95% CI)
-1082 (A/G)		
A/A	0.66 (67/101)	0.69 (0.21–2.23)
A/G	0.78 (67/86)	1.25 (0.34–4.53)
G/G	0.81 (27/33)	Ref
-819 (C/T)		
C/C	0.74 (85/115)	0.70 (0.17–2.83)
C/T	0.67 (56/83)	1.00 (0.13–7.39)
T/T	0.80 (18/22)	Ref
-592 (C/A)		
C/C	0.74 (85/115)	0.70 (0.17–2.83)
C/A	0.67 (56/83)	1.00 (0.13–7.39)
A/A	0.80 (18/22)	Ref
IL-10 haplotypes		
GCC	0.79 (120/151)[a]	2.34 (1.12–4.90)[b]
ATA	0.73 (91/124)	1.75 (0.86–3.56)
ACC	0.65 (108/165)	Ref

OR = odds ratio; CI = confidence interval; Ref = reference genotype/haplotype used for comparison.
[a] χ^2 p value GCC vs ACC=0.04.
[b] p=0.02, analyzed by logistic regression analysis, controlling for age as covariate.

Table 5
Frequency of CFTR genotypes distribution among IL-10 genotypes and haplotypes

IL-10 genotypes	Frequency of[a] severe/severe CFTR genotype (n=179)	Frequency of [b]mild; unknown/unknown CFTR genotype (n=41)
-1082 (A/G)		
A/A	0.48 (87)	0.28 (11)[c]
A/G	0.34 (60)	0.60 (25)
G/G	0.18 (32)	0.12 (5)
-819 (C/T)		
C/C	0.51 (91)	0.66 (27)[c]
C/T	0.40 (72)	0.30 (12)
T/T	0.09 (16)	0.04 (2)
-592 (C/A)		
C/C	0.51 (91)	0.66 (27)[c]
C/A	0.40 (72)	0.30 (12)
A/A	0.09 (16)	0.04 (2)
IL-10 haplotypes		
GCC	0.34 (122)	0.44 (36)[d]
ATA	0.37 (103)	0.17 (14)
ACC	0.29 (133)	0.39 (32)

[a] Severe CFTR genotype = mutations of classes I, II, III.
[b] Mild CFTR genotype = mutations of classes IV and V.
[c] χ^2 p=0.001 for the three genotype combinations of each polymorphism in the two groups.
[d] χ^2 p value GCC vs ATA vs ACC=0.07.

(p=0.09); (Table 4). The haplotype analysis confirmed that GCC was associated with an increased risk of chronic infection with *P. aeruginosa* as compared to ACC haplotype (Table 4). No association was found between haplotypes and median FEV1 values in the total CF population [GCC 73% (range 20–126%) vs ACC 75% (range 20–125%) vs ATA 84% (range 26–126%); p=0.365]. Finally, the distribution of the CFTR genotypes in the IL-10 genotypes and haplotypes is reported in Table 5. There were significant differences in the distribution of the three genotypes for each SNP in both groups of patients, stratified for CFTR mutation genotype. The haplotype analysis revealed that the distribution of the GCC, ATA and ACC haplotypes was similar among carriers of the severe CFTR genotypes (34% vs 37% vs 29%), while, although not significantly, it was different among carriers of the mild/unknown one (44% vs 17% vs 39%, p=0.07).

4. Discussion

Chronic pulmonary infection and the ensuing destructive inflammatory process is still the major cause of morbidity and mortality in CF patients despite great progresses in antimicrobial therapy. In keeping with other studies [16,17], we found that patients with *P. aeruginosa* chronic airway colonization had significantly lower median FEV1 values than non-colonized patients (Table 2). However, considering that colonized patients showed a higher median age than non-colonized subjects, this result may be affected by the age decline in lung function FEV1 values in CF. Efforts have been made to

understand how IL-10-mediated suppressor pathways may be relevant in the regulation of the inflammatory response to microbial agents in CF [4–7]. Some authors have shown that airway epithelial cells from patients with CF are deficient in IL-10 production [6]. However, Casaulta et al. [18] demonstrated that peripheral blood mononuclear cells of CF patients secrete more IL-10 than healthy individuals after polyclonal T-cell stimulation. We found that the percentage of colonized patients was higher among homozygous for GG at -1082 compared to subjects carrying the -1082AA genotype, and that the high-IL-10-producing haplotype GCC was associated with a greater risk of *P. aeruginosa* airway colonization (Table 4). This seems to be consistent with the results of Brouard et al. [19], who showed a significant relationship between the -1082GG genotype and colonization with *Aspergillus fumigatus* in CF patients. Furthermore, other association studies of IL-10 gene polymorphisms with infectious lung diseases have shown a correlation between the same -1082GG genotype and the severity of community-acquired pneumonia and pneumococcal infection in hospitalized patients [20,21]. Unexpectedly, IL-10 haplotypes did not related to median FEV1 values in our population and there are not similar studies in literature that may confirm our results in CF patients. We speculate that IL-10 haplotypes may influence the susceptibility to pulmonary infection, but not lung function performances. In agreement with our results, no association was found between IL-10 SNPs and the rate of decline in FEV1 in chronic obstructive pulmonary disease [22]. Contrarily, Lyon et al. [23] documented that the GCC haplotype was positively and the ATA haplotype negatively associated with FEV1 in asthmatic children. However, different mechanisms of inflammation are involved in asthma and CF lung infection. It is noteworthy that IL-10 haplotypes were affected by CFTR genotypes in our cohort (Table 5). Although our preliminary findings warrant confirmation by other groups, the data emerging from the present study suggest that IL-10 promoter haplotypes may represent a genetic factor, which may interact with mutations within CFTR and the environment, in determining susceptibility to *P. aeruginosa* infection and affecting prognosis in CF.

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