

## The Effect of TGFB1 and CD14 Gene Polymorphisms on the Clinical Findings of Cystic Fibrosis in Turkish Patients

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**ABSTRACT** Significant effects of several modifying genes on the clinical features of cystic fibrosis (CF) have been reported. In the present study, the researchers investigated the effects of transforming growth factor beta 1 (TGFB1) and cluster of differentiation 14 (CD14) polymorphisms on the clinical status of patients with CF. The present study included sixty-five patients with CF and eighty five healthy controls with no pulmonary disease. Single-nucleotide polymorphisms in the TGFB1 gene (rs1800469, rs1800470, rs8179181) were studied using DNA sequence analyses; the CD14 gene polymorphism rs2569190 was evaluated using restriction fragment length polymorphism analysis. The frequency of rs1800469 (TT genotype) was significantly higher in the healthy controls than in the patients with CF. Thus, the TT genotype may be protective against CF. Although rs8179181 (CT genotype) may have an overall negative effect, this genotype may have a favourable effect on growth parameters. However, these results should be confirmed in larger studies.

### INTRODUCTION

Cystic fibrosis (CF) is a chronic, progressive, multisystem single-gene disease that is caused by mutations of the gene encoding the CF transmembrane conductance regulatory (CFTR) protein. CF is inherited autosomal recessively and is observed frequently in Caucasians (Drumm et al. 2005). Although the genotype-phenotype relationship in patients with CF is known, differences in the clinical course of patients with the same mutation have been observed (Kerem et al. 1990). Specifically, patients carrying the same variants in CFTR exhibit substantial variation in the severity of lung disease, although 45 percent of this variation can be explained by non-CFTR genetic variations (Corvol et al. 2015).

There is no strong relationship between the clinical status of patients with CF and the CFTR genotype, particularly concerning lung disease, the major cause of morbidity and mortality in these patients (Bremer et al. 2008).

The observation of differences in the clinical status of CF in patients who carry the same mutation and live in similar environments implies the importance of secondary genetic factors. Genes that affect the clinical course of CF are known as 'CF-modifying genes'. It is unknown whether the modifying genes have an effect in healthy people (Bartlett et al. 2009; Faria et al. 2009; Frangolias et al. 2003; Gabolde et al. 2001; Henrion-Caude et al. 2002; Tug et al. 2003). Candidate modifiers of clinical phenotypes for pulmonary function that have been confirmed in studies include immunology and/or inflammation factors (Brennan et al. 2016). Interest in investigating transforming growth factor beta 1 (TGFB1) as a potential modifier of CF-associated pulmonary disease stemmed from its identification as a modifier of asthma and chronic obstructive pulmonary disease (Weiller

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et al. 2013). TGFB1, encoded on chromosome 19q13.1-q13.3, is a member of the growth/differentiation factor family. This cytokine regulates the proliferation and differentiation of a wide variety of cell types (Guillot et al. 2014). TGFB1 stimulates the production of extracellular matrix and the progression of fibrinogenesis; thus, TGFB1 plays a critical role in the chronic process. Tissue fibrosis in the involved organ after progression of the disease has also been observed (Brazova et al. 2006). Cluster of differentiation 14 (CD14) is another CF-modifying gene. The CD14 gene is located on chromosome 5q31.1 (Guillot et al. 2014). The CD14 protein, as a CD14 gene product expressed on the surfaces of macrophages, monocytes and neutrophils, is present on the outer membrane of gram (negative) bacteria such as *Pseudomonas aeruginosa* and functions as a receptor for lipopolysaccharides with immunologic effects. Identification of the effect of modifying genes would allow us to understand the genotype–phenotype relationship and CF pathogenesis for developing treatments (Faria et al. 2009).

### Objectives

The aim of this study was to elucidate the possible relationship between candidate CF-modifying genes, specifically the TGFB1 single nucleotide polymorphisms (SNP) rs1800469, rs1982073 and rs8179181 and the CD14 promoter area –159 SNP (rs2569190), with the clinical status of the disease.

### METHODOLOGY

The study group consisted of 65 patients who had been diagnosed with CF, followed up at the Pediatric Allergy and Pulmonology Outpatient Clinic and referred to the Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University between 31 August 2010 and 1 July 2013. Patient demographic information was obtained from the hospital records, including age at onset, age at diagnosis, sex, average sweat test findings, respiratory function test data, radiologic findings, weight and height, throat culture results and pharmacologic treatment. The clinical severity of the disease in the patient group was evaluated using the Shwachman–Kulczycki scoring system (Shwachman et al. 1958; Stollar et al. 2011). The patients underwent CFTR gene mutation analyses. The con-

trol group consisted of 85 healthy children and adolescents who had no acute and/or chronic pulmonary disease. Local Ethical Committee approval was received from the Istanbul University Istanbul Faculty of Medicine, Istanbul University Ethical Assessment Commission (24 April 2010, no. 305).

Peripheral venous blood was drawn from the patients and controls into 5-ml tubes containing EDTA. Genomic DNA was obtained from leukocytes in peripheral blood using the spin column method in accordance with the manufacturer's protocol (Roche High pure PCR Template Preparation Kit). Isolated DNA was stored at –20°C.

The Tn polymorphism and 57 mutations in the CFTR gene were studied using the reverse dot blot method (INNO-LiPA CFTR17 + Tn, CFTR19 and CFTR Italian). Genomic DNA was subjected to three multiplex polymerase chain reactions (PCRs) using the method defined in the kit's protocol. The PCR products were submitted to electrophoresis on 2% agarose gel at 85 V for 1.5 h. Mutations were assessed using reverse hybridization with amplification via agarose gel electrophoresis, and oligonucleotides specific for alleles were located on three different strips.

Analysis of the TGFB1 gene SNPs rs1800469, rs1982073 and rs8179181 was performed using the Sanger sequencing method. Analysis of the CD14 gene polymorphism rs2569190 was performed using the restriction fragment length polymorphism method. PCR was performed using the *AvaII* enzyme (Table 1).

Statistical analysis was performed using the Statistical Package for Social Sciences version 21.0. Descriptive values were presented as the mean and standard deviation or median and range. Categorical variables were presented as the number of cases and percentage. Comparisons of the categorical variables were performed using the chi-squared test and Fisher's exact probability tests. Statistical significance was accepted as  $p < 0.05$ .

### RESULTS

The study included 65 patients with CF and 85 controls (CG). According to the Shwachman–Kulczycki scoring system for clinical status, 44, 15 and 6 patients had a *good*, *mild* and *moderate* clinical status, respectively. There were no patients with an *excellent* or *severe* disease status (Table 2). In terms of age, statistical significance was detected between the good and mod-

**Table 1: PCR amplification and sequencing programme for the rs1800469, rs1800470 and rs8179181 polymorphisms and PCR amplification and restriction fragment length polymorphism programme for rs2569190**

| Gene region |        | PCR* amplification |                       | PCR sequence          |           |
|-------------|--------|--------------------|-----------------------|-----------------------|-----------|
| rs1800469   | 95°C   | 95°C-10 s          | 35 cycles 72°C—10 min | 95°C-1 min            | 25 cycles |
| rs1800470   | 10 min | 52°C-30 s          |                       | 96°C-10s              |           |
| rs8179181   |        | 72°C-1 min         |                       | 50°C-5s               |           |
|             |        |                    | 60°C-4 min            |                       |           |
| Gene region |        | PCR amplification  |                       | Restriction of enzyme |           |
| rs2569190   | 95°C   | 95°C—10 s          | 35 cycles 72°C-10 min | 37°C-30 min           |           |
|             | 3 min  | 55°C—30 s          |                       |                       |           |
|             |        | 72°C—1 min         |                       |                       |           |

\*PCR, polymerase chain reaction.

**Table 2: Demographic characteristics of the patients and healthy controls**

| Patients (n = 65)                       |              |                  |               |               |                |             |
|-----------------------------------------|--------------|------------------|---------------|---------------|----------------|-------------|
| Clinical severity*                      | Whole group  | Moderate (n = 6) | Mild (n = 15) | Good (n = 44) | HCG** (n = 85) | p           |
| Age (years)                             |              |                  |               |               |                |             |
| Mean ± SD                               | 10.1 ± 6.11  | 16.17 ± 5.91     | 13 ± 5.75     | 8.27 ± 5.40   | 8.3 ± 4.67     | p > 0.05*** |
| Median                                  | 10           | 16.5             | 12            | 7.5           | 8              |             |
| Min-max                                 | (1-25)       | (7-25)           | (2-21)        | (1-20)        | (1-18)         |             |
| Sex                                     |              |                  | p < 0.05      |               |                |             |
| Female                                  | 32 (49.23%)  | 5 (83.33%)       | 8 (53.33%)    | 23 (52.27%)   | 39 (45.88%)    | p > 0.05    |
| Male                                    | 33 (50.77%)  | 1 (16.67%)       | 7 (46.67%)    | 21 (47.73%)   | 46 (54.12%)    |             |
| Age at Diagnosis (Months)               |              |                  |               |               |                |             |
| Mean ± SD                               | 8.3 ± 4.67   | 10.83 ± 18.29    | 16.80 ± 17.64 | 26.68 ± 42.97 |                | p > 0.05    |
| Median                                  | 7.8          | 16.5             | 8             | 6             | -              |             |
| Min-max                                 | (1-168)      | (7-25)           | (3-48)        | (1-168)       |                |             |
| The Average Sweat Test (mmol/L)         |              |                  |               |               |                |             |
| Mean ± SD                               | 95.7 ± 15.37 | 100.5 ± 14.11    | 89.60 ± 19.96 | 96.95 ± 16.43 |                | p > 0.05    |
| Median                                  | 98           | 108              | 100           | 95            |                |             |
| Min-max                                 | (50-140)     | (80-112)         | (50-108)      | (55-140)      | -              |             |
| Mutations n (%)                         |              |                  |               |               |                |             |
| No mutations                            | 18 (27.69%)  | 1 (16.67%)       | 3 (20%)       | 14 (31.82%)   | -              | p > 0.05    |
| Heterozygous mutation                   | 13 (20%)     | 1 (16.67%)       | 5 (33.33%)    | 7 (15.91%)    | -              |             |
| Compound heterozygous mutation          | 14 (21.54%)  | 1 (16.67%)       | 0 (0%)        | 13 (29.55%)   | -              |             |
| Homozygous mutation                     | 16 (24.62%)  | 3 (50%)          | 5 (33.33%)    | 8 (18.18%)    | -              | p > 0.05    |
| Major deletion                          | 4 (6.15%)    | —                | 2 (13.33%)    | 2 (4.55%)     | -              |             |
| No ΔF508 n (%)                          | 44 (67.69%)  | 2 (33.33%)       | 11 (73.33%)   | 30 (68.18%)   | -              |             |
| ΔF508 Heterozygous                      | 10 (15.38%)  | 1 (16.67%)       | 2 (13.33%)    | 8 (18.18%)    | -              | p > 0.05    |
| ΔF508 Homozygous                        | 11 (16.92%)  | 3 (50%)          | 2 (13.33%)    | 6 (13.64%)    | -              |             |
| Weight n (%)                            |              |                  |               |               |                |             |
| 3 percentile and less than 3 percentile | 20 (30.77%)  | 3 (50%)          | 8 (53.33%)    | 9 (20.45%)    | -              | p < 0.05    |
| More than 3 percentile                  | 45 (69.23%)  | 3 (50%)          | 7 (46.67%)    | 35 (79.55%)   | -              |             |
| Height n (%)                            |              |                  |               |               |                |             |
| Third percentile and below              | 21 (32.31%)  | 2 (33.33%)       | 5 (33.33%)    | 14 (31.82%)   | -              | p > 0.05    |
| Over the third percentile               | 44 (67.69%)  | 4 (66.67%)       | 10 (66.67%)   | 30 (68.18%)   | -              |             |

\*Clinical severity was evaluated according to the Shwachman-Kulczycki score.

\*\*HCG, healthy control group.

\*\*\*Between the entire patient and healthy control groups.

erate groups and the good and mild groups (both  $p < 0.01$ ). In this study, the researchers observed that patients' pulmonary function decreased with age. There was no statistically significant difference in pulmonary functions according to sex, mean sweat test findings and mutation status (Table 3). Whereas CFTR mutations were not detected in 18 patients, 40 patients had heterozygous mutations, compound heterozygous mutations, or major deletions.

**Table 3: Distribution of cystic fibrosis transmembrane regulatory gene mutations in the patient group**

|                                       |                  |
|---------------------------------------|------------------|
| <i>Homozygous Mutation</i>            | <i>n: 16 (%)</i> |
| ΔF508                                 | 10 (62.5)        |
| 2183AA-G                              | 2 (12.5)         |
| G542X                                 | 2 (12.5)         |
| W1282X                                | 1 (6.25)         |
| 3849 + 10kbC-T                        | 1 (6.25)         |
| <i>Heterozygous Mutation</i>          | <i>n: 13 (%)</i> |
| ΔF508                                 | 6 (46.154)       |
| G542X                                 | 1 (7.692)        |
| R347P                                 | 1 (7.692)        |
| 2789 + 5G-A                           | 2 (15.4)         |
| G85E                                  | 1 (7.692)        |
| I148T                                 | 1 (7.692)        |
| N1303K                                | 1 (7.692)        |
| <i>Compound Heterozygous Mutation</i> | <i>n: 14 (%)</i> |
| 2789 + 5G-A/R1162X                    | 3 (21.43)        |
| W1282X/621 + 1G-A                     | 1 (7.143)        |
| R347P/F508                            | 1 (7.143)        |
| ΔF508/2183AA-G                        | 1 (7.143)        |
| N1303K/L346P                          | 1 (7.143)        |
| 621 + 1G-T/2789 + 5G-A                | 1 (7.143)        |
| ΔF508/R1070                           | 1 (7.143)        |
| ΔF508/C1408                           | 1 (7.143)        |
| G85E/G542X                            | 2 (14.286)       |
| ΔF508/R347P                           | 1 (7.143)        |
| R347P/621 + 1G-T                      | 1 (7.143)        |
| <i>Major Deletion</i>                 | <i>n: 4 (%)</i>  |
| 4-10 exon major deletion              | 1 (25)           |
| 16-17 exon major deletion             | 1 (25)           |
| 17-18 exon major deletion             | 2 (50)           |

In terms of rs1800469 (TT genotype), between the patient and control groups, statistical significance was detected in favour of the control group regarding the frequency of the TT genotype (odds ratio [OR] = 0.37; 95% confidence interval [CI] = 0.15–0.93,  $p = 0.035$ ). When the good and control groups were compared, there was a significant difference concerning the frequency of the TT genotype (OR = 0.31; 95% CI = 0.097–0.96;  $p = 0.036$ ) in favour of the control group; however, there was no significant difference in the frequency of the CT genotype (OR = 2.27; 95% CI = 1.07–4.8;  $p = 0.048$ ) in favour

of the patient group. In terms of the presence of CFTR mutations, there was no statistically significant difference regarding the frequencies of genotypes and allele SNPs ( $p > 0.05$ ) within the patient subgroups.

There was a significant difference in terms of the TGFB1 rs1800470 SNP (T allele) in patients with the ΔF508 homozygote mutation in comparison with the other patient groups (OR = 3.16; 95% CI = 1.09–9.17;  $p = 0.034$ ). However, this difference was not significant among the patient subgroups with good, mild and moderate disease (all  $p > 0.05$ ).

When the patient groups were assessed for *P. aeruginosa* and *Staphylococcus aureus* colonization in throat culture, there was no statistically significant difference in the frequencies of colonization among the rs2569190, rs1800469, rs1800470 and rs8179181 genotypes and alleles (all  $p > 0.05$ ). When patients were assessed in terms of the age of onset for *P. aeruginosa* acquisition, patient age was lower for the CT genotype than for the TT genotype ( $p = 0.043$ ). When they were assessed in terms of the age of onset for *Staphylococcus aureus* acquisition, no statistically significant difference was detected ( $p > 0.05$ ).

The patients were divided into two groups regarding weight and height ( $d \leq$  third percentile and  $>$  third percentile regarding child standards in Turkey). There was a statistically significant difference in weight associated with the T allele in the first group (OR = 4.25; 95% CI = 1.29–13.96;  $p = 0.02$ ) as well as rs8179181 (CC genotype) (OR = 0.23; 95% CI = 0.06–0.86;  $p = 0.036$ ) in the latter group. In addition, in the first group, there was a statistically significant difference in height associated with the CT genotype (OR = 5; 95% CI = 1.27–19.7;  $p = 0.03$ ) and high statistically significant differences associated with the T allele (OR = 5.73; 95% CI = 1.65–19.9;  $p = 0.0047$ ) and rs8179181 (CC genotype) in the latter group (OR = 0.16; 95% CI = 0.041–0.63;  $p = 0.013$ ).

## DISCUSSION

CF is the only single-gene disease with a known genetic aetiology that is chronic, progressive and multisystemic. The relationships of the complicated pathogenesis of the disease and CFTR genotypes with survival have not yet been explained (Brazova et al. 2006; McKone et al. 2006; Shwachman et al. 1958; Stollar et al.

2011). Pulmonary involvement is an important factor associated with the long-term outcome of the disease. The CF database published in the United Kingdom in 2014 assessed pulmonary function in patients aged 6 years and older between 2008 and 2014, uncovering a decrease in pulmonary function with age (Cystic Fibrosis Trust Annual Data Report 2014).

Patients carrying similar CFTR mutations exhibit variability in pulmonary phenotypes (Dorfman et al. 2008; UK Clinical Molecular Genetics Society 2009). It is assumed that secondary genetic factors, known as CF-modifying genes, have a role in the course of the pulmonary disease that develops due to CF (El Gamel et al. 1998; UK Clinical Molecular Genetics Society 2009). SNPs in these genes, the effect of which in healthy individuals is unknown, may be modifying in CF. Defining the effect of the modifying genes may contribute to a better understanding of the genotype–phenotype relationship and the physiopathologic aspects. These genes exert their effects by regulating the expression and splicing of the CFTR gene or by modifying the sensitivity of the lungs to bacterial infection and inflammatory responses (Faria et al. 2009). As one of the modifying genes, the gene product of TGFB1 is a regulatory cytokine that has various functions including cell growth, differentiation, inflammation and tissue fibrosis (Bremer et al. 2008). CD14 is the product of another modifying gene. CD14 is expressed on the surfaces of macrophages, monocytes and neutrophils. The aim of this study was to investigate the modifying effect of SNPs in TGFB1 and CD14, which are believed to have an influence on clinical status in patients with CF.

In the present study, patients were divided into groups in accordance with the Shwachman–Kulczycki scoring system of disease severity, namely moderate, mild and good groups. Compatible with the United Kingdom's CF yearly data 2014, the researchers observed that patients' pulmonary function decreased with age.

TGFB1 has an important role in the pathophysiology of CF with pulmonary involvement as it does in inflammation and tissue rearrangement. Many studies investigated the modifying effect of TGFB1 on CF. Li et al. (2007) investigated the relationship between the rs1800469 SNP of the TGFB1 gene and susceptibility to asthma and reported a high increase in asthma risk in patients carrying the T allele. Drumm et al. (2005)

and Bremer et al. (2008) reported that rs1800469 (T allele) in patients with CF was associated with better pulmonary function. Although Drumm et al. (2005) found a significant relationship between the TT genotype and the course of the pulmonary disease, whereas Brazova et al. (2006) detected no relationship. In this study, the frequency of the TGFB1 gene rs1800469 SNP (TT genotype) was significantly higher in the control group than in the patient group. The TT genotype was also significantly more common in the control group than in the patient group among patients with good lung function and disease activity. These results demonstrated that the TT genotype may be protective against CF with pulmonary involvement. Manjari et al. (2015) reported that rs1800469 can be considered a biomarker of the aetiology of the disease, and it may contribute to the pathogenesis of chronic pancreatitis by increasing the proliferation of pancreatic stellate cells. The researchers also observed that the CT genotype was statistically more frequent in patients with better pulmonary function and clinical activity than in those with worse pulmonary function and disease activity. The CT genotype may have a favourable effect on the course of the pulmonary disease (Bremer et al. 2008; Cutting et al. 2010).

Faria et al. (2009) described rs1800470 (CT genotype) as a risk factor in patients with CF and detected a relationship between the CT genotype and the moderate pulmonary disease. Dorfman et al. (2008) investigated the individual effects of the CC, CT and TT genotypes of rs1800470 on pulmonary function failure in 511 Canadian patients with CF and reported that the CC genotype may be protective owing to the significant difference observed in the frequency of the CC genotype in patients with good pulmonary function. Arkwright et al. (2000) performed a study of 171  $\Delta$ F508 homozygous patients and reported that pulmonary failure developed more rapidly in patients carrying the rs1800470 polymorphism (TT genotype). Arkwright et al. (2003) was unable to demonstrate the same relationship in 194  $\Delta$ F508 homozygous patients. Drumm et al. (2005) demonstrated the beneficial effect of the T allele on pulmonary function and the harmful effect of the CC genotype in rs1800470 in their study of 808  $\Delta$ F508 homozygous patients (263 and 545 patients with severe and moderate disease, respectively) from 44 different CF clinics in North America. Bremer

et al. (2008) examined 472 patients with CF and their families and found that the rs1800470 SNP (T allele) had a positive effect on pulmonary function. Brazova et al. (2006) found no relationship between rs1800470 and severe clinical presentation in their study of 118 patients with the  $\Delta$ F508 homozygous CFTR genotype and 268 controls. In this study, consistent with the results of Brazova et al. (2006), the researchers identified no significant difference in pulmonary function failure associated with any genotype for rs1800470 (Arkwright et al. 2000; Arkwright et al. 2003; Dorfman et al. 2008; Faria et al. 2009).

Li et al. (2007) investigated the relationship between the TGFB1 gene rs8179181 SNP and susceptibility to asthma and detected no difference associated with this polymorphism. Bremer et al. (2008) investigated the rs1800469, rs1800470 and rs8179181 SNPs in the TGFB1 gene in a haplotype base in a study of 472 patients with CF and their families. The authors reported that the C-T-C haplotype in patients who were not  $\Delta$ F508 homozygous was related with a good clinical course of pulmonary disease, and the C-T-T haplotype was associated with a severe clinical course. When we compared the patient and control groups in terms of rs8179181, no statistically significant relationship was uncovered.

In the CD14 gene promoter area, the rs2569190 polymorphism has an allele frequency of approximately 50 percent in Europe (Bobadilla et al. 2002). Similarly, we identified an allele frequency of 52.9 percent. Faria et al. detected TT genotype (rs2569190) predominance in patients with CF with respect to healthy controls, but they found no relationship of the SNP with lung disease severity. Other researchers also found no relationship between rs2569190 and the course of pulmonary disease (Faria et al. 2009).

The  $\Delta$ F508 mutation is the most frequently observed mutation that causes CF. This mutation causes phenylalanine loss in the 508<sup>th</sup> position of the CFTR polypeptide, which results in changes to three base pairs (Castellani et al. 2008; Cohn et al. 1998; Zielenski et al. 2000). Its frequency is approximately 66 percent among Caucasian patients, but it varies in different ethnic groups (Moskowitz et al. 2008). Consistent with the other study from Turkey (Bobadilla et al. 2002), the researchers found a  $\Delta$ F508 mutation frequency of 24.6 percent.

The most important sign of CF disease with pulmonary involvement is the development of

chronic infections by specific pathogens that cause chronic inflammatory damage in lung tissue. In our study, 46 (71%) of 65 patients were infected with *P. aeruginosa*, and 45 patients (69%) were infected with *Staphylococcus aureus*. No significant correlation of CD14-159, rs1800469, rs1800470 and rs8179181 genotypes and alleles with bacterial growth in the patients' throat cultures was found.

The age of onset for colonization by *P. aeruginosa* is considered an important risk factor in terms of CF clinical status (Arkwright et al. 2003). Thus, the genetic factors affecting the susceptibility to infection are extremely important. Faria et al. (2009) reported a significant relationship between rs2569190 (CC genotype) and early colonization by *P. aeruginosa* in children with CF. The researchers did not detect such a relationship. To the researchers' knowledge, a relationship between TGFB1 and the age of onset for colonization by *P. aeruginosa* has not yet been reported. In this study, the age of onset for colonization by *P. aeruginosa* was younger in the presence of rs1800469 (CT genotype). Further studies are needed to support this finding. The researchers identified no statistically significant relationship between SNPs and the age of onset for colonization by *Staphylococcus aureus*.

The main aims of care for patients with CF are maintaining a normal growth rate in children and a normal body mass index for adults. A relationship between CF pulmonary involvement and nutrition has not been reported in previous studies (Faria et al. 2009; Milla et al. 2004). However, 23 percent of pediatric patients and 22 percent of adult patients were underweight, even in developed countries (Stallings et al. 2008). In this study, 30.8 percent and 32.3 percent of the patients were in the third percentile or lower for weight and height, respectively. Bremer et al. (2008) found no relationship between TGFB1 SNPs and nutrition in their study. When the patients in this study were assessed in terms of weight and height according to the presence of SNPs, the researchers detected a significant difference for rs8179181 (CC genotype) in terms of protectiveness. Bradley et al. (2012) reported that nutritional status is not well correlated with the CFTR genotype, which suggests the additional influence of environmental, genetic, or stochastic factors. The researchers found that the rs8179181 SNP (CT genotype) was an inhibiting factor for development concerning weight and the T allele was inhibiting for height. No significant difference was detected for other geno-

types and alleles in terms of their effects on height and weight.

### CONCLUSION

In conclusion, these findings suggest that the rs1800469 SNP (TT genotype) in the TGFB1 gene may be protective against disease development and that the CT genotype has positive effects on the course of the disease. The observation of a younger onset of age for *P. aeruginosa* colonization in patients carrying the TGFB1 gene rs1800469 SNP (CT genotype) demonstrated that this genotype may have a negative effect on the clinical course of the disease. Regarding rs8179181, it appears that the CT genotype is related with failure in weight gain, the T allele affects height and the CC genotype protects against growth deficiency.

### LIMITATIONS

The results of this paper contradict those of other studies in the literature on SNPs in TGFB1 and CD14, the 'modifying genes in CF pulmonary disease.' A limitation of this study was the inclusion of a patient group with high levels of CFTR gene heterogeneity caused by ethnic differences between populations. This heterogeneity may be considered disadvantageous when investigating the effect of TGFB1 and CD14 gene SNPs on the clinical status of the disease. Performing the study on patients with the same mutation would be more reliable because the presence of mutations with different effects on the CFTR gene may alter the modifying effect of SNPs. A second limitation of our study was the heterogeneous age distribution of the patients.

### RECOMMENDATIONS

New studies including more patients within certain age ranges and exhibiting homogenous genetic structures to determine the complicated pathogenesis of CF may resolve the differences in study results.

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