The Effect of TGF-β₁ Polymorphisms on Pulmonary Disease Progression in Patients with Cystic Fibrosis

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Research

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Abstract

**Background**: Transforming Growth Factor-β₁ (TGF-β₁) is a genetic modifier in patients with cystic fibrosis (CF). Several single nucleotide polymorphisms (SNPs) of TGF-β₁ are associated with neutrophilic inflammation, lung fibrosis and loss of pulmonary function.

**Aim**: The aim of this study was to assess the relationship between genetic TGF-β₁ polymorphisms and pulmonary disease progression in CF patients. Furthermore, the effect of TGF-β₁ polymorphisms on inflammatory cytokines in sputum were investigated.

**Methods**: 56 CF-patients and 62 controls were genotyped for three relevant SNPs in their TGF-β₁ sequence using the SNaPshot® technique. Individual slopes in forced expiratory volume in 1 second (FEV₁) for all patients were calculated by using documented lung function values of the previous five years. The status of Pseudomonas aeruginosa (Pa) infection was determined. Sputum concentrations of the protease elastase, the serine protease inhibitor elafin and the cytokines IL-1β, IL-8, IL-6, TNF-α were measured after a standardized sputum induction and processing.

**Results**: The homozygous TT genotype at codon 10 was associated with a lower rate of chronic Pa infection (p<0.05). The heterozygous GC genotype at codon 25 was associated with lower lung function decline (p<0.05). Patients with homozygous TT genotype at the promotor SNP showed higher levels of TNF-α (p<0.05). Higher levels of TGF-β₁ in plasma were associated with a more rapid FEV₁ decline over five years (p<0.05).

**Conclusions**: Our results suggest that polymorphisms in the TGF-β₁ gene have an effect on lung function decline, Pa infection as well as levels of inflammatory cytokines. Genotyping these polymorphisms could potentially be used to identify CF patients with higher risk of disease progression. TGF-β₁ inhibition could potentially be developed as a new therapeutic option to modulate CF lung disease.

Background

Cystic fibrosis (CF) is an autosomal recessive, genetic disorder that affects approximately 85,000 individuals worldwide [1]. This multisystemic disorder is caused by mutations affecting the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in the epithelial membrane of exocrine glands, which lead to dysfunctional fluid and ion-transport causing a production of thickened mucus [2]. Pathological mucociliary clearance leads to CF lung disease, which over time becomes the major life-shortening factor for CF patients [1]. Chronic plugging of bronchioles with secretions, recurring bacterial infections and pulmonary exacerbations instigate the development and retention of a hostile inflammatory environment in the lungs, leading to tissue breakdown and irreversible lung damage [3]. The most relevant microorganism in CF lungs, Pseudomonas aeruginosa (Pa), provokes a vigorous inflammatory response with neutrophilic infiltration of airways and subsequent damage by the release of proteases and oxidants [4]. This dysregulated chronic state of inflammation in CF airways is sustained by
a variety of proinflammatory mediators including TNF-α, IL-1β, IL-6 and IL-8 and leads to a decline in lung function caused by bronchiectasis and irreversible fibrotic remodeling of lung tissue [4]. 98% of all CF patients die of progressive respiratory insufficiency [5].

Whilst prevalent CFTR mutations are an important determinator for the severity of CF lung disease, the genotype-phenotype correlation between the genetically determined loss of CFTR function and lung function decline is approximately 60% [6, 7]. This suggests that other non-CFTR related factors, such as genetic modifiers with a regulatory effect on the inflammatory response in CF lungs, may also have a significant impact on lung function decline in CF patients.

TGF-β₁ has been identified as such a genetic modifier for CF lung disease [6]. Produced by bronchial epithelial cell, this growth factor acts with a localized, modulatory role in the recruitment and activation of neutrophilic granulocytes within a complex network of inflammatory and anti-inflammatory cytokines, thereby regulating inflammatory processes, specifically in context of chronic pulmonary disease [8]. TGF-β₁ inhibits the degradation of extracellular matrix by stimulating protease-inhibitors leading to fibrotic reconstruction of lung tissue [9]. Furthermore, it promotes smooth muscle cell hypertrophy and hyperplasia [4, 10].

In a recent study, Sagwal et al have shown that levels of serum TGF-β₁ were increased in pulmonary exacerbation phases, in infection with Pa and in subjects with a ΔF508 mutation [11]. TGF-β₁ levels decreased significantly after antibiotic treatment of pulmonary exacerbations [11].

Moreover, it has been shown that TGF-β₁ has an inhibitory effect on the biogenesis of CFTR and prevents the functional rescue of delF508-CFTR [10]. In a recent study by Mitash et al., TGF-β₁ has been associated with degradation of CFTR mRNA in human bronchial epithelial cells via recruitment of microRNAs to an RNA-induced silencing complex [12]. Snodgrass et al. have shown that TGF-β₁ was associated with CFTR inhibition and prevention of functional rescue in human epithelial cells [10].

However, in vivo levels of TGF-β₁ are dependent on specific polymorphisms in the TGF-β₁ gene [13]. So far, few studies have investigated the effects of genetic polymorphisms of TGF-β₁ on lung function. In the context of CF, three single nucleotide polymorphisms (= SNPs) have been investigated. Each of these polymorphisms, i.e. rs1800469 located in the promoter region and both rs1800470 and rs1800471 located in Exon 1 of the TGF-β₁ gene, result in a change in the primary amino acid sequence of the TGF-β₁ [6, 13, 14].

In previous studies it was shown that some of these TGF-β₁ polymorphic genotypes are associated with higher TGF-β₁ expression, a steeper decline in pulmonary function (FEV₁) as well as increased pulmonary fibrosis [6, 13, 15, 16]. However, some of the results among these studies are contradictory, as different genotypes were associated with a decrease in pulmonary function and worse clinical status. Furthermore, very little is known about the impact of a TGF-β₁ polymorphism-related dysregulation of the signal pathway of TGF-β₁ on the complex inflammatory response of the CF airways. It has to be noted, however,
that immunological factors contributing to or perhaps even enabling the onset of bacterial infection with Pa, one of the major predictors for mortality and morbidity for CF patients, could not yet be identified [17].

The primary aim of this study was to investigate whether TGF-β1 SNP genotypes, as modifiers of CF lung disease, can be associated with a faster decline in pulmonary function. To our knowledge, there is no data correlating TGF-β1 phenotypes with the individual FEV₁ slopes of CF patients. FEV₁ correlates with morbidity and mortality of CF-patients and is a gold standard outcome parameter in routine diagnostics to assess disease progression as well as in clinical studies to investigate the efficacy of new drugs [18]. Furthermore, we wanted to investigate whether TGF-β1 polymorphisms are associated with higher TGF-β₁ expression, higher Pa infection rates and elevated levels of proinflammatory cytokines in sputum.

**Materials And Methods**

**Study population**

The TGF-β₁ genotypes for all three SNPs were determined in 56 CF-patients and 62 healthy controls. All CF patients had a confirmed diagnosis of CF according to the consensus guidelines of the Cystic Fibrosis Foundation [19]. Inclusion criteria were a signed informed consent and the ability of patients to expectorate sputum. The genomic DNA of CF patients was isolated from a whole blood sample obtained during the routine yearly blood sample collection. The DNA samples of 62 randomly chosen, healthy controls were obtained from paternity test samples at the Institute of Legal Medicine, to which the individuals had given their consent when these samples were obtained [20]. Patients with an acute pulmonary exacerbation at the study visit were excluded. All pulmonary function tests performed during the 5-year interval between 2010 and 2014 were reviewed. The best FEV₁ value for every year was obtained for FEV₁ slope calculation. 15 patients underwent changes in CF therapy (e.g. start of CFTR-modulatory therapy) or lung transplantation within this time period. For these, a different five-year time span, prior to their new therapy, was chosen for calculation of the individual FEV₁ slope.

**TGF-β₁ polymorphism genotyping**

DNA was extracted from whole blood and diluted to a standard concentration of 1ng/μl. The DNA was then amplified using Polymerase Chain Reaction (PCR) with specific primers designed to amplify two separate targets of the genome, containing the relevant SNPs, using Primer3Plus, BLAST and NCBI Electronic PCR-Software [21, 22]. Primers were produced and shipped by biomers.net [23]. PCR primer sequences used are available on request. Agarose gel electrophoresis tests of the amplicons were performed to monitor the correct amplification of the two targets. Enzymatic purification of samples followed using Exonuclease and Shrimp Alkaline Phosphate (SAP). According to instructions of the SNaPshot™ Multiplex Kit (Applied Biosystems), a Single Base Extension (SBE) with dideoxyribonucleosid-triphosphates (ddNTPs), marked with four different fluorescent signals, QIAGEN Mastermix (containing DNA Polymerase AmpliTaq®, reaction puffer) was performed in a thermocycler (Gene AMP PCR System 2720 thermocycler, Applied Biosystems) [24, 25, 26]. SNP typing primer
sequences were GGCAACAGGACACCTGA(A/G) for SNP rs1800469, CACCGGTCACAGGAGC(G/A) for Codon 10 SNP rs1800470 and GTGCTGACGCTGGCC(G/C) for Codon 25 rs1800471.

Lastly, after enzymatic purification of the Single Base Extension reaction (SBE) reaction products, capillary electrophoresis (using ABI Prism 3130 Genetic Analyzers) was performed to determine the genotype of each SNP for all patients and controls using the software Genemapper 4.0 (Applied Biosystems). An exemplary capillary electrophoresis result of one patient’s genotype for all three polymorphisms is shown in Fig. 1. Materials, concentrations, PCR primers sequences and exact reaction conditions for PCR and Single Base Extension (SBE) are available on request.

Spirometry

Spirometric measurements were performed according to the ATS guidelines using GLI references and assessed before any other study assessment with Master Screen Body (Jaeger, Heidelberg, Germany) and SentrySuite™ version 2.19 software (Carefusion, Becton Dickinson, Franklin Lakes, New Jersey, USA) [27]. For each measurement, the best FEV$_1$ value was used for analysis. The best yearly FEV$_1$ value was used in a linear regression model to calculate individual FEV$_1$ slope values for every patient. Patient results were also analyzed within different FEV$_1$ groups and FEV$_1$ slope groups, according to FEV$_1$ progression over time.

Pseudomonas aeruginosa (Pa) infection

The status of Pa infection, defined by clinically established Leeds criteria, was obtained from the patients’ files and is described according to the following three groups: Pa positive (= chronic infection), Pa naïve (= never infected) or Pa negative (infected in the past, currently not infected after eradication therapy) [28].

Sputum analysis of TGF-$\beta_1$ and other cytokines

As part of the regular outpatient visits, patients induced their sputum by inhalation of hypertonic saline during a routine physiotherapist session. This sputum was processed according to the standard operating procedure (SOP) of the TDN (Therapeutic Drug Development Network, USA). Concentrations of elastase and elafin in sputum were determined by specific ELISA assays (EnzChek® Elastase Assay Kit, - Molecular Probes Europe, Leiden, Netherlands; Elafin/Skalp Human ELISA Kit - abcam, Cambridge, UK). Pro-inflammatory cytokine concentrations in sputum were assessed using a human inflammatory cytokine ELISA-kit (BD Cytometric Bead Array Humane Inflammatory Cytokine Kit, San Jose, CA, USA). TGF-$\beta_1$ levels in sputum and plasma were determined by a TGF- $\beta_1$ specific ELISA-kit (Quantikine®ELISA Human TGF-$\beta_1$, R&D systems, Minneapolis, MN, USA).

Statistical Analysis

IBM SPSS Statistics 24 was used for statistical analysis. To compare two metric variables, we correlated using Pearson’s test. For correlation between one metric and one discontinuous variable, we used the
Kruskal-Wallis test. For tests correlating two discontinuous variables we used cross-classified tables with exact Fisher’s test. For all tests, a p-value < 0.05 was considered statistically significant.

For a detailed analysis of FEV\textsubscript{1} slopes, different patient subgroups were created to investigate patients with a steeper decline in FEV\textsubscript{1} (more than \(-1.0\% / -2.0\%\) FEV\textsubscript{1} change per year), a relatively steady FEV\textsubscript{1} with only little decline or even small improvement (ranging from \(>-1.0\%\) to \(<+1.0\% / >-2.0\%\) to \(<+2.0\%\) FEV\textsubscript{1} change per year) and patients with clear improvements in FEV\textsubscript{1} (\(>+1.0\% / >+2.0\%\) change per year). According to their absolute FEV\textsubscript{1} progression over time, patients were also analyzed in different FEV\textsubscript{1} subgroups in order to investigate patients who finished their 5-year FEV\textsubscript{1} slope in a “normal” FEV\textsubscript{1} group (>80% predicted), an “intermediate” FEV\textsubscript{1} group (40–80% predicted) or a “low” FEV\textsubscript{1} group (<40% predicted).

For more detailed analysis of TGF-\(\beta\textsubscript{1}\) genotypes, for some statistical investigations we used subgroups of combined genotypes to explore the impact of a heterozygous genotype when compared to homozygous genotypes (e.g. CT vs TT/CC).

**Results**

**Study population**

The mean age of CF-patients was 21 years (SD ± 11.1 years). The mean FEV\textsubscript{1} at the time of blood sampling was 74.26% predicted (SD ± 25.36% predicted). The mean FEV\textsubscript{1} slope of patients was \(-1.81\%\) FEV\textsubscript{1} change per year (SD ± 3.20%FEV\textsubscript{1} change). 33.9% of patients were chronically infected with Pa. The demographic results of our cohort are presented in Table 1. As shown in Table 2, there was no significant difference in the distribution of genotypes or alleles in our CF patients compared to healthy controls. The genotype results were successfully tested for conforming to expected distributions according to the Hardy Weinberg Equilibrium.
Table 2
Distribution of genotypes in CF-patients and controls

| Genotypes | Prevalence in CF-Patients [%] | Prevalence in controls [%] |
|-----------|------------------------------|--------------------------|
| Promotor  |                              |                          |
| CC        | 35.7                         | 44.1                     |
| CT        | 55.4                         | 47.5                     |
| TT        | 8.9                          | 8.5                      |
| Codon 10  |                              |                          |
| CC        | 12.5                         | 14.8                     |
| CT        | 55.4                         | 52.5                     |
| TT        | 32.1                         | 32.8                     |
| Codon 25  |                              |                          |
| GG        | 89.3                         | 88.7                     |
| GC        | 10.7                         | 9.7                      |
| CC        | 0.0                          | 1.6                      |

**TGF-β₁ polymorphisms and pulmonary status:**

Codon 10: The homozygous TT genotype at codon 10 was significantly associated with a lower Pa infection rate. 16.7% of patients with this genotype were infected with Pa, compared to 42.1% in the combined CC/CT genotype group (p = 0.047). Of all patients with chronic Pa infection in our cohort (n = 19), 15.2% showed the TT genotype.

No association between the average FEV₁ slope and this genotype at codon 10 was found.

Codon 25: For the polymorphism at codon 25, we found no significant association with Pa infection rate. However, we were able to show that the group of patients with the heterozygous GC genotype contained a significantly higher proportion of patients with positive FEV₁ slopes, with FEV₁ greater than 1.0% per year (p = 0.003). As shown in Table 3, of all patients with the GC genotype, 66.7%, exhibited an FEV₁ slope greater than +1.0% FEV₁ change/year, compared to only 10% of patients with the GG genotype. Furthermore, all of the patients with FEV₁ slopes between −1%/year and +1%/year and 93% of the patients with declining FEV₁ slopes <1%/year presented with a GG genotype at codon 25.
Table 3
Prevalence of FEV$_1$-slopes >1%/year for CF patients with different TGF-β$_1$ codon 25 genotypes

| Genotype codon 25 | Prevalence of FEV$_1$-Slope Group > 1.0%[%] |
|------------------|---------------------------------------------|
| GC               | 66.67                                       |
| GG               | 10.20                                       |

Promoter: We neither found a significant association between polymorphisms in the TGF-β$_1$ promoter and patients’ Pa infection status nor with their FEV$_1$ slopes.

TGF-β$_1$ concentration and FEV$_1$ slopes:

Higher TGF-β$_1$ concentrations in patients’ plasma significantly correlated with a steeper decline in FEV$_1$ slope (p = 0.045). This is demonstrated in Fig. 3. Patients with FEV$_1$ slopes that below than −2%/year showed a higher TGF-β$_1$ concentration (25,332 pg/ml) in plasma compared to patients with a positive FEV$_1$ slope of greater than +2%/year (16,754 pg/ml).

In contrast, a higher concentration of TGF-β$_1$ in the patients’ sputum significantly correlated with a more positive FEV$_1$ slope (p = 0.010). The median TGF-β$_1$ concentration in sputum was 66.0 pg/ml higher in patients with a positive FEV$_1$ slope (>0%/year) compared to patients with a negative FEV$_1$ slope (<0%/year).

However, patients with intermediate FEV$_1$ values (40–80% predicted) over five years showed significantly higher concentrations of TGF-β$_1$ in sputum compared to patients with normal (>80%) or low (<40%) FEV$_1$ values (p = 0.01). These results are demonstrated in Fig. 4a & 4b.

**TGF-β$_1$ polymorphism and concentration of inflammatory markers:**

We found a significantly higher concentration of sputum TNF-α in patients with a homozygous TT genotype at the TGF-β$_1$ promoter polymorphism (p = 0.019), as shown in Fig. 5. For all other inflammatory parameters there was no significant correlation to TGF-β$_1$ concentration.

**Discussion**
The aim of this study was to investigate whether TGF-β1 SNP genotypes, as modifiers of CF lung disease, can be associated with a faster decline in pulmonary function, higher TGF-β1 expression, higher Pa infection rates and elevated levels of proinflammatory cytokines in sputum. As expected, we found no difference in the genotype frequency distributions of TGF-β1 polymorphisms between CF patients and healthy controls. The genotype frequencies for each TGF-β1 polymorphism corresponded with known allele frequencies for these polymorphisms as documented following a PubMed dbSNP search [29]. This distribution of the various genotypes demonstrates that the patient cohort is a representative for a randomly selected population sample.

We showed that the codon 10 TGF-β1 polymorphism genotypes (CC or CT) are associated with a higher rate of Pa infection (p = 0.047). The observed significant correlation between TGF-β1 genotype TT in codon 10 and less chronic Pa infection is a finding of special interest. However, it contrasts the findings of Arkwright et al., who found no significant difference between Pa infection and TGF-β1 codon 10 TT genotype [15]. As shown in studies by Emerson et al. and Thomassen et al., chronic Pa infection leads to an activation of profibrotic inflammatory mediators in CF patients and increased pulmonary inflammation resulting in higher mortality rates. [17, 30]. In a recent study by Sagwal et al., increased levels of TGF-β1 in serum were found in all types of bacterial infections, with comparably more increase in patients infected with Pa [11]. In our cohort, only 16.7% of patients with the homozygous codon 10 TT genotype were chronically infected with Pa, compared to a 42.1% infection-rate in the group of patients with either the CT or CC genotype. Specifically, the high chronic infection rate of 57.1% in the CC group was noticeable at this context. Of all the patients with chronic PA infection in our cohort (n = 19), only 15.2% showed the TT genotype. There might be an unclear mechanism, by which the immunological protection against this pathogen is affected. The noticeably higher infection rate of patients with the CC genotype for this polymorphism might be supported by Drumm et al’s observed association of this genotype with a worsened lung function, as it also correlates with increased gene expression and circulating TGF-β1 levels in their study [13]. Whether TGF-β1 with a TT genotype at codon 10 has protective antimicrobial properties, e.g. by contributing to a generally more controlled pulmonary inflammation that significantly lowers the rate of Pa infection in this subgroup, or whether the TGF-β1 CC-genotype at codon 10 acts as an immunological predisposition for onset of Pa infection, is yet to be investigated.

We also found a significant association between the TGF-β1 polymorphism genotypes at the promotor SNP of TGF-β1 and the concentration of TNF-α in the patients’ sputum (p = 0.019). Interestingly, the concentration of TNF-α for patients with the homozygous TT promotor genotype at this polymorphism was four times higher than in patients with other genotypes. TNF-α acts as a signal cytokine, that activates the acute phase proteins [31]. Yang et al. hypothesized that colonization with flagellated bacteria, such as Pa, may lead to a higher expression of TGF-β1 via MAP kinases [32]. In addition to this, Eickmeier et al. found a higher co-expression of TGF-β1 and TNF-α in patients with microbiological evidence of at least one type of bacterial infection [3]. Our results correspond with the hypothesis of TGF-
β1/TNF-α co-expression, as the homozygous TGF-β1 promotor genotype TT was significantly associated with a higher expression of TNF-α.

We also found, that the GC genotype of the TGF-β1 codon 25 polymorphism correlates significantly with better FEV1 slopes in CF patients (p = 0.003). 66.6% of patients with this genotype showed a positive FEV1 slope > 1% FEV1 change/year, compared to merely 10.2% of the patients in the group with homozygous GG genotypes. This correlation has not been described previously in other studies before. Although Arkwright’s study on TGF-β1 polymorphisms included the combined TT/GG genotype (Codon 10/Codon 25) in the “high-producer” categorization of patients, no correlation was found between codon 25 genotype and measures of survival (age of death/transplantation), Pa infection or lung function [15].

Furthermore, we demonstrate that high TGF-β1 plasma levels are associated with a more rapid decline in lung function over a five year period (p = 0.045). The highest TGF-β1 plasma concentrations were found in patients with a more severe FEV1 decline over time. This correlation has also been described by Brazova et al [6]. However, an association of very low TGF-β1 concentration with a more deteriorated lung function was also demonstrated in their study which our results do not confirm. Interestingly, we found that the highest TGF-β1 concentrations were associated with intermediate FEV1 values (40–80% predicted) of CF patients over 5 years (p = 0.01), whereas for patients with both high (>80% predicted) or low (< 40% predicted) FEV1 values over 5 years, the TGF-β1 concentrations were significantly lower. In patients with normal and stable FEV1 values above 80% predicted, inflammatory processes might not be activated to the same level as in patients with intermediate FEV1 values showing a higher rate of decline. Patients with highly impaired lung function (FEV1 < 40% predicted) might show more activation in pulmonary tissue remodeling and pulmonary fibrosis and less active inflammation; this could explain the < 40% FEV1 group showing lower TGF-β1 concentrations in their sputa. Zemel et al. also showed that an initially high FEV1 value in CF patients is linked to worse FEV1 progression over time, which may be linked to a less aggressive anti-inflammatory and antimicrobial treatment in children with an initially higher FEV1 [33]. This might suggest that the impact of TGF-β1 on pulmonary function is significant, when chronic inflammation reaches its maximum during a phase of steady pulmonary function decline of 1–3% FEV1/year.

**Conclusions**

In conclusion our results demonstrate the relevance of the multifunctional cytokine TGF-β1 as a genetic modifier in patients with CF. We showed that genetic polymorphisms in the TGF-β1 sequence have an impact on pulmonary function, rates of chronic Pa infection as well as the concentration of inflammatory cytokines, such as TNF-α. TGF-β1 polymorphisms might therefore be used to identify patients with a high risk for disease progression. Furthermore, TGF-β1 inhibition could be used as a therapeutic target to prevent the effects of a dysregulated signal pathway leading to higher levels of pulmonary inflammation for certain TGF-β1 polymorphisms.
Abbreviations
ATS: American Thoracic Society; A: Adenine; C: Cytosine; CF: Cystic Fibrosis; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; ddNTP: dideoxynucleotide triphosphate; DNA: Deoxyribonucleic acid; FEV₁: forced expiratory volume in 1 second; G: Guanine; IL-1β: Interleukine 1 Beta; IL-8: Interleukine 8; IL-6: Interleukine 6; PA: Pseudomonas aeruginosa; PCR: Polymerase chain reaction; rs: RefSNP; SAP: shrimp alkaline phosphate; SBE: single base extension; SD: standard deviation; SNP: single nucleotide polymorphism; T: Thymine; TGF-β1: transforming growth factor beta 1; TNF-α: Tumor necrosis factor alpha

Declarations

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Authors’ contributions
MvM, GF, SvK collected sputum and performed sputum processing. MvM, GF, MA and JCT collected data regarding the analysis of sputum and plasma TGF-β1 levels. TT and PMS collected data regarding genotypes of TGF-β1 polymorphisms. TT and SvK collected data regarding clinical data of patients. TT, SvK, ER, MA and PMS designed the study and interpreted the data. SvK and ER contributed in manuscript writing. All authors read and approved the final manuscript.

Ethical approval and consent to participate
Human guidelines of good clinical practice and the declaration of Helsinki (1964) and Edinburgh (2000) were followed in the conduct of the trial. Ethical approval was obtained from the Medical Ethical Committee of the University Hospital Cologne. Written informed consent was obtained from all parents and all patients older than eight years of age, as well as from all healthy donors of control DNA samples.

Consent for publication
Not applicable

Availability of data and material
The datasets generated and analyzed during the current study are not publicly available due to further studies being conducted with the data. The datasets and materials used are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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**Figures**

**Figure 1**

Exemplary capillary electrophoresis result of CF-patient showing binding of fluorescent bases to sense DNA strand for promoter and Codon 10 genotyping and binding to antisense DNA strand for Codon 25 genotyping. (Promotor genotype = CT, Codon 10 genotype = CT, Codon 25 genotype = GG)
Figure 2

TGF-β1 codon 10 SNP genotypes and prevalence of Pa infection status within the genotype group
Figure 3

TGF-β1 plasma concentration and FEV1 slope of CF patients

Figure 4
(a) TGF-β1 sputum concentration and FEV1 slope group (b) TGF-β1 sputum concentration and FEV1 group over five years

Figure 5

TGF-β1 Promotor SNP genotype group and TNF-α concentration in sputum