# The effect of TGF-beta1 polymorphisms on pulmonary disease progression in patients with cystic fibrosis

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# Abstract

***Background:*** Transforming Growth Factor-$b_1$ (TGF-$b_1$) is a genetic modifier in patients with cystic fibrosis (CF). Several single nucleotide polymorphisms (SNPs) of TGF-$b_1$ are associated with neutrophilic inflammation, lung fibrosis und loss of pulmonary function.

***Aim:*** The aim of this study was to assess the relationship between genetic TGF-$b_1$ polymorphisms and pulmonary disease progression in CF patients. Furthermore, the effect of TGF-$b_1$ polymorphisms on inflammatory cytokines in sputum were investigated.

***Methods:*** 56 CF-patients and 62 controls were genotyped for three relevant SNPs in their TGF-$b_1$ sequence using the SNaPshot® technique. Individual “slopes” in forced expiratory volume in 1 second ($FEV_1$) 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-1b, 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-$b_1$ in plasma were associated with a more rapid $FEV_1$ decline over five years ($p<0.05$).

***Conclusions:*** Our results suggest that polymorphisms in the TGF-$b_1$ 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-$b_1$ 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-$\beta_1$ 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-$\beta_1$ 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-$\beta_1$ levels decreased significantly after antibiotic treatment of pulmonary exacerbations [11].

Moreover, it has been shown that TGF-$\beta_1$ 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-$\beta_1$ 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-$\beta_1$ was associated with CFTR inhibition and prevention of functional rescue in human epithelial cells [10].

However, *in vivo* levels of TGF-$\beta_1$ are dependent on specific polymorphisms in the TGF-$\beta_1$ gene [13]. So far, few studies have investigated the effects of genetic polymorphisms of TGF-$\beta_1$ on lung function. In the context of CF, three single nucleotide polymorphisms (= SNPs) have previously been investigated. Each of these polymorphisms, i.e. rs1800469 located in the promotor region and both rs1800470 and rs1800471 located in Exon 1 of the TGF-$\beta_1$ gene, result in a change in the primary amino acid sequence of the TGF-$\beta_1$ [6, 13, 14].

In previous studies it was shown that some of these TGF-$\beta_1$ polymorphic genotypes are associated with higher TGF-$\beta_1$ expression, a steeper decline in pulmonary function ($FEV_1$) 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-$\beta_1$ polymorphism-related dysregulation of the signal pathway of TGF-$\beta_1$ 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-$\beta_1$ 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-$\beta_1$ phenotypes with the individual $FEV_1$ slopes of CF patients. $FEV_1$ 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-$\beta_1$ polymorphisms are associated with higher TGF-$\beta_1$ expression, higher *Pa* infection rates and elevated levels of proinflammatory cytokines in sputum.

## Materials And Methods

# Study population

The TGF-$\beta_1$ 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 $FEV_1$ value for every year was obtained for $FEV_1$ 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 $FEV_1$ slope.

# TGF-$\beta_1$ polymorphism genotyping

DNA was extracted from whole blood and diluted to a standard concentration of 1ng/$\mu$l. 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 didesoxyribonucleosid-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, CAGCGGTAGCAGGAGC(G/A) for Codon 10 SNP rs1800470 and GTGCTGACGCCTGGCC(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 Figure A of Supplementary Files. 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 $FEV_1$ slopes, different patient subgroups were created to investigate patients with a steeper decline in $FEV_1$ (more than − 1.0% / -2.0% $FEV_1$ change per year), a relatively steady $FEV_1$ with only little decline or even small improvement (ranging from >-1.0% to < + 1.0% / >-2.0% to < + 2.0% $FEV_1$ change per year) and patients with clear improvements in $FEV_1$ ( > + 1.0 / >+2.0% change per year). According to their absolute $FEV_1$ progression over time, patients were also analyzed in different $FEV_1$ subgroups in order to investigate patients who finished their 5-year $FEV_1$ slope in a "normal" $FEV_1$ group (> 80% predicted), an "intermediate" $FEV_1$ group (40–80% predicted) or a "low" $FEV_1$ group (< 40% predicted).

For more detailed analysis of TGF-$\beta_1$ 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 $FEV_1$ at the time of blood sampling was 74.26% predicted (SD ± 25.36% predicted). The mean $FEV_1$ slope of patients was − 1.81%$FEV_1$ change per year (SD ± 3.20%$FEV_1$ change). 33.9% of patients were chronically infected with *Pa*. The demographic results of our cohort are presented in Table 1.

Table 1
Clinical data of CF patients

| Parameter | Mean |
|---|---|
| Age[years] | 21 (SD ± 11.1) |
| Adult : Children ratio[%] | 57:43 |
| Sex ratio (m : f)[%] | 55:45 |
| $FEV_1$ [%predicted] | 74.26 (SD ± 25.36) |
| $FEV_1$ slope[%$FEV_1$ change/year] | -1.81 (SD ± 3.20) |
| Prevalence of chronic *Pa* infection[%] | 33.90 |

As shown in Table 2, there was no significant difference in the distribution of genotypes or alleles in the investigated cohort of 56 CF patients compared to 62 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 | *CF patients* | | *Controls* | |
|---|---|---|---|---|
| | n | % | n* | % |
| Promotor | 20 | 35.7 | 26 | 44.1 |
| CC | 31 | 55.4 | 28 | 47.5 |
| CT | 5 | 8.9 | 5 | 8.5 |
| TT | | | | |
| Codon 10 | 7 | 12.5 | 9 | 14.8 |
| CC | 31 | 55.4 | 32 | 52.5 |
| CT | 18 | 32.1 | 20 | 32.8 |
| TT | | | | |
| Codon 25 | 50 | 89.3 | 55 | 88.7 |
| GG | 6 | 10.7 | 6 | 9.7 |
| GC | 0 | 0.0 | 1 | 1.6 |
| CC | | | | |

* As shown in Table 2, in our control group, the total “n” for Promotor and Codon 10 genotype results were 59 and 61 respectively, due to unclear technical problems in genotyping of singular SNPs of 3 and 1 control samples, respectively.

# TGF- $\beta_1$ polymorphisms and pulmonary status:

Codon 10: The homozygous TT genotype at codon 10 was significantly associated with a lower *Pa* infection rate, as demonstrated in Fig. 1. 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 $FEV_1$ 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 $FEV_1$ slopes, with positive $FEV_1$ change 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 $FEV_1$ slope greater than + 1.0% $FEV_1$ change/year, compared to only 10% of patients with a GG genotype. Furthermore, all of the patients with $FEV_1$ slopes between − 1%/year and + 1%/year and 93% of the patients with declining $FEV_1$ slopes <-1%/year presented with a GG genotype at codon 25.

Table 3
Prevalence of $FEV_1$-slopes > + 1%/year for patients with different Codon 25 genotypes

| Genotype codon 25 | Prevalence in $FEV_1$-Slope Group > 1.0% $FEV_1$ change/year | |
|---|---|---|
| | n | % |
| GC | 6 | 10.20 |
| GG | 50 | 66.67 |

Promotor: We neither found a significant association between polymorphisms in the TGF-$\beta_1$ promotor and patients' *Pa* infection status nor with their $FEV_1$ slopes.

TGF-$\beta_1$ concentration and $FEV_1$ slopes:

Higher TGF-$\beta_1$ concentrations in patients' plasma significantly correlated with a steeper decline in $FEV_1$ slope (p = 0.045). This is demonstrated in Fig. 2. Patients with $FEV_1$ slopes that below than − 2%/year showed a higher TGF-$\beta_1$ concentration (25,332 pg/ml) in plasma compared to patients with a positive $FEV_1$ slope of greater than + 2%/year (16,754pg/ml).

Figure 2: **TGF- $\beta_1$ plasma concentration and $FEV_1$ slope of CF patients**

In contrast, a higher concentration of TGF-$\beta_1$ in the patients' sputum significantly correlated with a more positive $FEV_1$ slope (p = 0.010). The median TGF-$\beta_1$ 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-$\beta_1$ in sputum compared to patients with normal (> 80%) or low (< 40%) $FEV_1$ values (p = 0.01). These results are demonstrated in Fig. 3a & 3b.

# TGF-$\beta_1$ 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-$\beta_1$ promotor polymorphism (p = 0.019), as shown in Fig. 4. For all other inflammatory parameters there was no significant correlation to TGF-$\beta_1$ concentration.

# Discussion

The aim of this study was to investigate whether TGF-$\beta_1$ SNP genotypes, as modifiers of CF lung disease, can be associated with a faster decline in pulmonary function, higher TGF-$\beta_1$ 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- $\beta_1$ polymorphisms between CF patients and healthy controls. The genotype frequencies for each TGF-$\beta_1$ 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- $\beta_1$ polymorphism genotypes (CC or CT) are associated with a higher rate of *Pa* infection (p = 0.047). The observed significant correlation between TGF- $\beta_1$ 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- $\beta_1$ 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- $\beta_1$ 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- $\beta_1$ levels in their study [13]. Whether TGF- $\beta_1$ 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- $\beta_1$ CC-genotype at codon 10 acts as an immunological predisposition for onset of *Pa* infection, is yet to be investigated.

Our results show a significant association between the TGF- $\beta_1$ polymorphism genotypes at the promotor SNP of TGF- $\beta_1$ 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- $\beta_1$ via MAP kinases [32]. In addition to this, Eickmeier et al. found a higher co-expression of TGF-$\beta_1$ and TNF-α in patients with microbiological evidence of at least one type of bacterial infection [3]. Our result therefore partially corresponds with the hypothesis of a TGF-β1/TNF-α co-expression, as the homozygous TGF-$\beta_1$ promotor genotype TT was significantly associated with a higher expression of TNF-α, despite there being no significant difference in TGF-$\beta_1$ concentration in sputum.

We also found, that the GC genotype of the TGF- $\beta_1$ codon 25 polymorphism correlates significantly with better $FEV_1$ slopes in CF patients ($p = 0.003$). 66.6% of patients with this genotype showed a positive $FEV_1$ slope > 1% $FEV_1$ 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-$\beta_1$ 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]. In ou r study, plasma and sputum levels of TGF-$\beta_1$ showed no significant difference between codon 25 genotype groups.

We were able to demonstrate that high TGF-$\beta_1$ plasma levels are associated with a more rapid decline in lung function over a five-year period ($p = 0.045$). The highest TGF-$\beta_1$ plasma concentrations were found in patients with a more severe $FEV_1$ decline over time. This correlation has also been described by Brazova et al [6]. However, an association of very low TGF-$\beta_1$ 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-$\beta_1$ concentrations were associated with intermediate $FEV_1$ 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) $FEV_1$ values over 5 years, the TGF-$\beta_1$ concentrations were significantly lower. In patients with normal and stable $FEV_1$ values above 80% predicted, inflammatory processes might not be activated to the same level as in patients with intermediate $FEV_1$ 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% $FEV_1$ group showing lower TGF-$\beta_1$ concentrations in their sputa. Zemel et al. also showed that an initially high $FEV_1$ value in CF patients is linked to worse $FEV_1$ progression over time, which may be linked to a less aggressive anti-inflammatory and antimicrobial treatment in children with an initially higher $FEV_1$ [33]. This might suggest that the impact of TGF- $\beta_1$ on pulmonary function is significant, when chronic inflammation reaches its maximum during a phase of steady pulmonary function decline of 1–3% $FEV_1$/year.

# Conclusions

In conclusion our results demonstrate the relevance of the multifunctional cytokine TGF-$\beta_1$ as a genetic modifier in patients with CF. We showed that genetic polymorphisms in the TGF-$\beta_1$ 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-$\beta_1$ polymorphisms might therefore be used to identify patients with a high risk for disease progression. Furthermore, TGF- $\beta_1$ 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-$\beta_1$ 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; $FEV_1$: forced expiratory volume in 1 second; G: Guanine;IL-1β: Interleukine 1 Beta; IL-8: Interleukine 8; IL-6: Interleukine 6; *Pa*: *Pseudomonas aeruginos*a; PCR: Polymerase chain reaction; rs: RefSNP; SAP: shrimp alkaline phosphate; SBE: single base extension; SD: standard deviation; SNP:single nucleotide polymorphism; T: Thymine; TGF-b1: transforming growth factor beta 1;TNF-α:Tumor necrosis factor alpha

# Declarations

### Ethical approval and consent to participants

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 to publication

Not applicable

### Data availability

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. The genetic data of the investigated, previously known TGF-$\beta_1$ polymorphisms can be found under the following web links of the dbSNP database:

rs1800469 - https://www.ncbi.nlm.nih.gov/snp/rs1800469?horizontal_tab=true

rs1800470 - https://www.ncbi.nlm.nih.gov/snp/rs1800470?horizontal_tab=true

rs1800471 - https://www.ncbi.nlm.nih.gov/snp/rs1800471?horizontal_tab=true

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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-$\beta_1$ levels. TT and PMS collected data regarding genotypes of TGF-$\beta_1$ 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.

### Conflict of interest

The authors declare that they have no conflicts of interests.

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# Figures

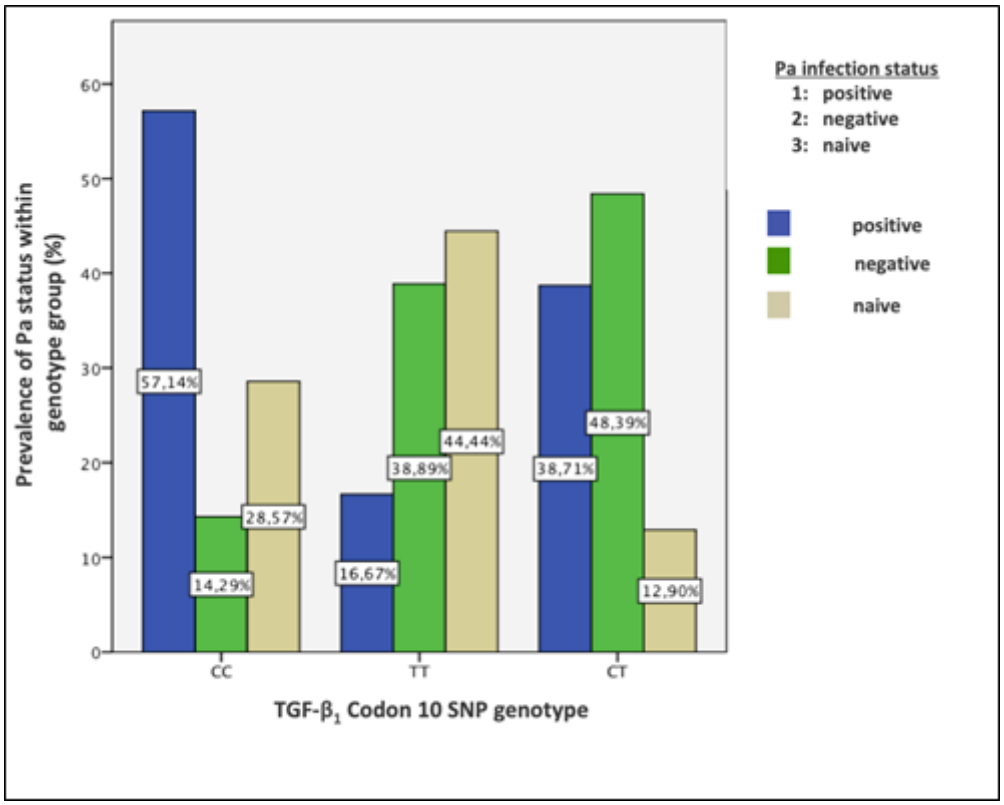


**Figure 1**

**TGF- $b_1$ codon 10 SNP genotypes and prevalence of *Pa* infection status within the genotype group**

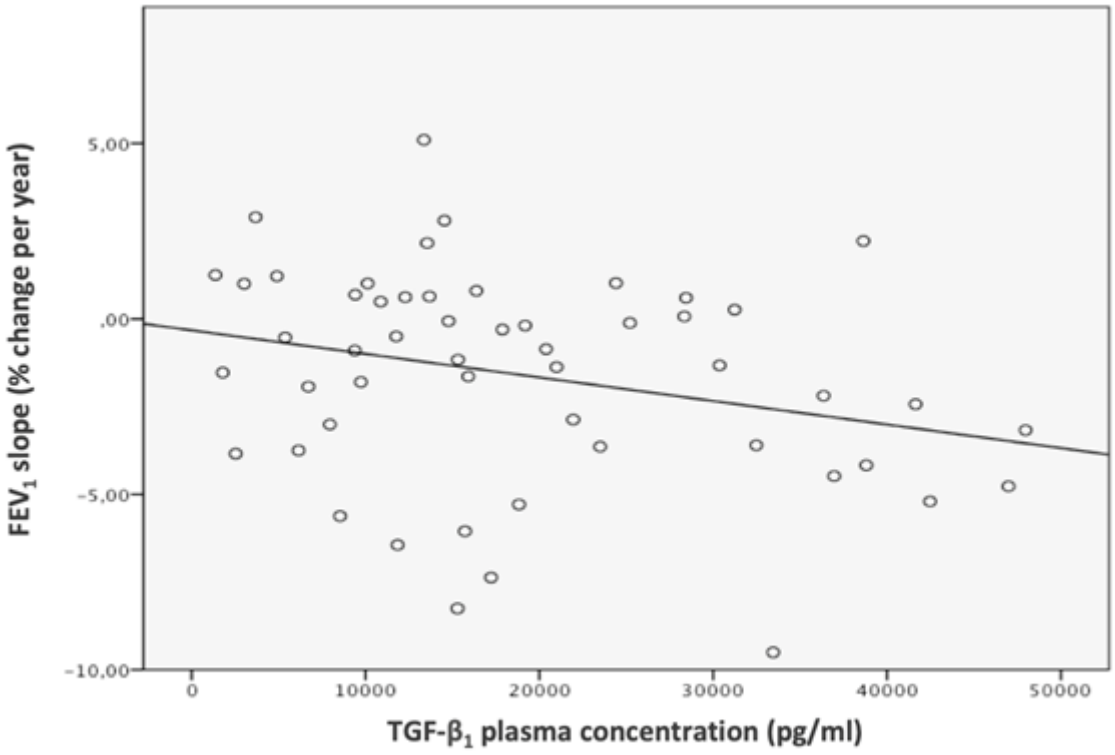


Figure 2

TGF- $b_1$ plasma concentration and $FEV_1$ slope of CF patients

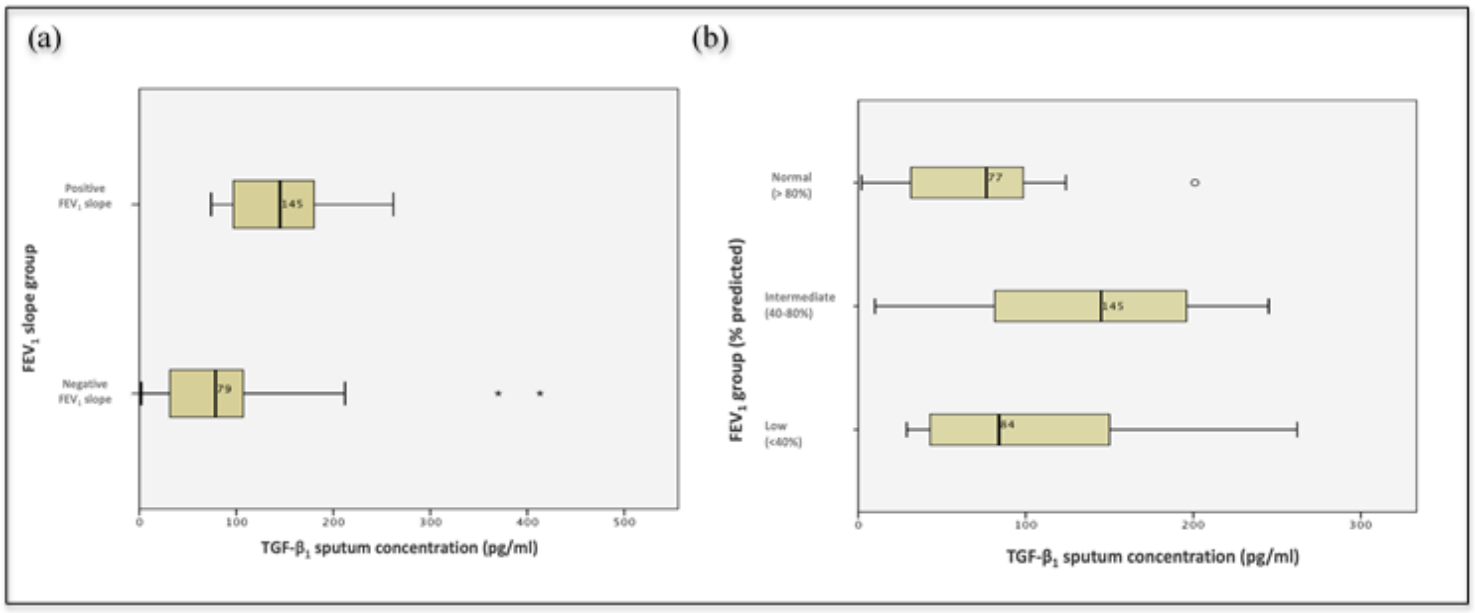


Figure 3

(a) TGF- $b_1$ sputum concentration and $FEV_1$ slope group (b) TGF- $b_1$ sputum concentration and $FEV_1$ group over five years

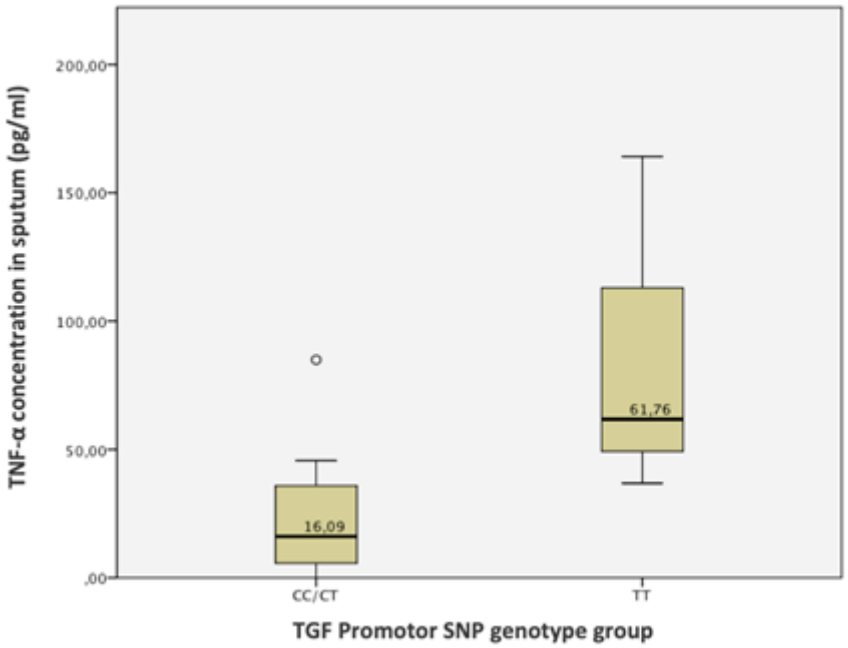


Figure 4

TGF-$b_1$ Promotor SNP genotype group and TNF-α concentration in sputum

# Supplementary Files

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- SupplementaryFiles.pdf