

# Effects of Single Nucleotide Polymorphisms 869 T/C in the Exon 1 Locus of Transforming Growth Factor- $\beta$ 1 Gene on Susceptibility and Airflow Restriction of Chronic Obstructive Pulmonary Disease

Jie Liu

Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University

Wenjing Zhu (✉ [409901587@qq.com](mailto:409901587@qq.com))

Qingdao Municipal Hospital

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## Research

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# Abstract

**Background:** Chronic obstructive pulmonary disease (COPD) is the most common chronic respiratory disease which is expected to become the third leading cause of death worldwide in 2030. Series of susceptibility genes and single nucleotide polymorphism (SNPs) play an important role in the occurrence and development of COPD.

**Methods:** In our study, 98 COPD patients and 90 healthy volunteers were enrolled. The +869 SNP (SNP, Single Nucleotide Polymorphisms) of TGF- $\beta$ 1 was detected in 98 COPD patients and 90 healthy volunteers by PCR-DNA sequencing. The effects of different genotypes of +869 locus on the susceptibility of COPD, pulmonary function and airflow limitation of COPD patients were analyzed.

**Results:** Allele C of +869 locus was associated with the susceptibility of COPD (OR:1.913, 95% CI: 1.251-2.926). The predicted value of FEV1% (FEV1, Forced Expiratory Volume in One Second) in patients with CC of +869 locus was significantly lower than that in patients with TT ( $P < 0.05$ ). The genotype frequencies of CC, CT and TT were 6.5%, 58.7% and 34.8% in Mild-to-Moderate airflow restriction patients. In severe airflow restriction patients, the genotype frequencies were CC 23.1%, CT 57.7% and TT 19.2%. The distribution of CC genotype in severe airflow restriction COPD patients was significantly higher than that in Mild-to-Moderate airflow restriction COPD patients ( $P = 0.037$ ). Moreover, the frequency of C allele was significantly higher in patients with severe airflow restriction than that patients with Mild-to-Moderate airflow restriction ( $P = 0.024$ ).

**Conclusions:** The SNP of +869 T/C in TGF- $\beta$ 1 is closely related to the susceptibility of COPD and the airflow restriction of COPD patients.

## Background

Chronic obstructive pulmonary disease (COPD) is a serious chronic respiratory disease characterized by incomplete reversibility and progressive exacerbation of airflow restriction. As the most common chronic respiratory disease, it is expected to become the third leading cause of death worldwide in 2030<sup>[1]</sup>. The prevalence of COPD increased by 44.2% from 1990 to 2015, and 3.2 million people died from COPD worldwide in 2015 with an increase of 11.6% compared to 1990<sup>[2]</sup>.

It is well known that COPD is associated with abnormal inflammatory response of the lung to harmful gases or particles<sup>[3]</sup>. Smoking is considered to be the most important factor for the occurrence and development of COPD, nearly 80% - 90% of COPD patients are smokers<sup>[4]</sup>. However, only 10%-20% of chronic severe smokers develop into symptomatic COPD, and there are significant individual differences in airway obstruction caused by smoking<sup>[5]</sup>. This indicates that there are differences in the susceptibility of different individuals to cigarette injury, and smoking can only cause those susceptible to COPD. Moreover, a small proportion of nonsmokers also develop COPD, and a number of people diagnosed with airway limitation in childhood may also present with COPD later in life<sup>[4]</sup>. All of these studies showed that

COPD is a complex disease, influenced by genetic and environmental factors acting in a developmental context<sup>[6]</sup>. Therefore, how to screen the genetic susceptible genes of COPD and take effective preventive and therapeutic measures for the susceptible population in the early stage has been the main focus of international attention.

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a pleiotropic cytokine that regulates a wide range of biological functions including proliferation and differentiation of cells, extracellular matrix formation, gene transcription, protein biosynthesis, and tissue repair<sup>[7]</sup>. Transforming growth factor  $\beta$  (TGF- $\beta$ ) has a pivotal role in initiating mechanisms of tissue fibrosis, and it is normally released in response to injury and stimulates cell differentiation and wound healing<sup>[8]</sup>. High levels of TGF- $\beta$  are consistently observed in fibrotic lung diseases, in turn promoting excessive repair processes leading to organ dysfunction<sup>[9]</sup>. More specifically, TGF- $\beta$  attracts and induces the differentiation of resident or circulating fibroblasts into contractile myofibroblasts in the lung, which migrate to sites of injury and produce extracellular matrix (ECM)<sup>[8]</sup>. Furthermore, TGF- $\beta$  promotes alveolar epithelial cells transformed into migratory fibroblastic cells by epithelial-to-mesenchymal transition (EMT)<sup>[10]</sup>.

Many SNPs have been found in TGF -  $\beta$  1 gene. Among of them, the effects of polymorphisms 869 T/C in the exon 1 locus of transforming growth factor- $\beta$ 1 gene on COPD are controversial<sup>[11]</sup>. In Caucasian COPD patients<sup>[12]</sup> and New Zealand COPD patients<sup>[13]</sup>, COPD susceptibility is closely related to single nucleotide polymorphisms 869 T/C of TGF -  $\beta$ 1, and "TT" is the susceptible genotype of COPD. However, COPD patients from Canada<sup>[14]</sup>, Korea<sup>[15]</sup> and Hong Kong<sup>[16]</sup>, the susceptibility to COPD was not associated with the + 869T / C polymorphism of TGF -  $\beta$ 1 gene.

Therefore, the main purpose of this study is to elucidate the effect of the 869 T/C single nucleotide polymorphisms in TGF -  $\beta$ 1 on the susceptibility and airflow limitation of COPD patients.

## Methods

### Study participants

98 cases of COPD patients were enrolled in the test group, including 92 males and 6 females, with an average age of (74.6  $\pm$  8.0) years and a smoking index of (669.8  $\pm$  365.5) years. All patients were confirmed by detailed history, physical examination, pulmonary function test and chest high resolution CT (HRCT). The diagnosis was in accordance with the worldwide guidelines for chronic obstructive pulmonary disease<sup>[17]</sup>. Patients with pulmonary diseases such as bronchial asthma, bronchiectasis, pulmonary interstitial fibrosis, chest trauma, surgery or deformity, and negative  $\alpha$  1-antitrypsin antibody were excluded.

90 cases of healthy volunteers were included in the control group, including 81 males and 9 females, with an average age of (73.4  $\pm$  9.6) years and a smoking index of (483.3  $\pm$  98.3) years. This study was approved by Institutional Review Board of Qingdao Municipal Hospital. The number of the approval of

this study by the ethical committee is No.121. And the approval document was approved on December 7th, 2019.

### **Extraction of genomic DNA and PCR amplification of the target fragment**

DNA was extract from blood by Whole blood genomic rapid extraction kit (Aidlad Biotechnologies Co., Ltd., DN0112) and stored at - 80°C.

PCR amplification: Primers of the target fragment were synthesized and provided by Nanjing Jinsirui Biotechnologies Co., Ltd. The upstream primer was 5'-CCCCTATTCAAGACCCAC-3', and the downstream primer was 5'-TCCGCTTCAC-3'. The length of the amplified DNA fragment was 379bp. Total volume of PCR reaction system is 25μL, including 12.5μL 2×ES Taq Master Mix (provided by Beijing ComWin Biotech Co., Ltd.), 1μL upstream primer (10mmol/L), 1μL downstream primer (10mmol/L), 2μL template DNA and 8.5μL ddH<sub>2</sub>O. PCR reaction conditions were as follows: pre-denaturation at 94°C for 2 min; denaturation at 94°C for 30s, annealing at 55°C for 30s, extending for 1 min at 72°C; after 30 cycles, and then 72 °C extending again for 5 min to the end of PCR reaction. PCR products were examined by DNA electrophoresis on agarose gel.

Then, PCR products were sequenced and analyzed by Nanjing Jinsirui Biotechnologies Co., Ltd.

### **Pulmonary Function Test**

The pulmonary function was measured by MedGrapics profiler. COPD patients were divided into 4 grades according to the degree of airflow restriction: GOLD 1 (mild): FEV1 ≥ 80% predicted value, GOLD 2 (moderate): 50% ≤ FEV1 < 80% predicted value, GOLD 3 (severe): 30% ≤ FEV1 < 50% predicted value, GOLD 4 (extremely severe): FEV1 < 30% predicted value.

### **Statistical analysis**

Hardy-Weinberg law of balance was used to test the balance of genetic, and chi square test was carried out for genotype frequency of the two groups. The formula was:  $\chi^2 = \sum (\text{actual value} - \text{theoretical value})^2 / \text{theoretical value}$ , degree of freedom (v) = 1 (genotype number - allele gene number). *P*-value > 0.05 indicating that the two groups were in genetic balance, which was consistent with Hardy-Weinberg genetic balance, and the research objects were representative of the population.

SPSS17.0 was used for statistical analysis. Measurement data were expressed by  $\bar{x} \pm s$ , and mean values between the two groups were compared by t test. The genotype and allele frequency were compared by chi square test. Differences were considered statistically significant when the *P*-value was < 0.05.

## **Results**

### **General characteristics**

The study population consisted of 98 patients with COPD and 90 control subjects, and their characteristics are described in Table 1. The COPD cases and control subjects did not differ significantly in sex, age, body mass index (BMI) and smoking index. However, the parameters of forced expiratory volume in 1 second (FEV1)/predicted and FEV1/forced vital capacity (FVC) were significantly decreased in the COPD case subjects compared with the controls (Table 1,  $P < 0.001$ ).

Table 1. the characteristics of the study participants

<b>Variables</b>	<b>COPD group (n=98)</b>	<b>Control group (n=90)</b>	<b>P Value</b>
Gender (Male/Female)	92/6	81/9	>0.05
Age (years)	74.6±8.0	73.4±9.6	>0.05
BMI (kg/m <sup>2</sup> )	19.87±4.12	20.15±3.6	>0.05
Smoking Index*	537.5±140.8	483.3±98.3	>0.05
FEV1 / Predicted (%)	51.74±18.54	92.48±5.20	<0.001
FEV1 / FVC (%)	58.17±7.25	89.57±7.59	<0.001

\*Number of packs of cigarettes smoked per day × number of years of smoking.

### Genotyping for +869 T/C of TGF-β1 polymorphisms

DNA was extracted from whole blood of COPD patients and healthy volunteers. Then, we amplified the target fragment including +869 T/C of TGF - β1 by PCR, and PCR products were verified by DNA electrophoresis on agarose gel (Figure 1).

In order to determine the genotype at +869 site of TGF-β1 gene, PCR products were sequenced and analyzed by Nanjing Jinsirui Biotechnologies Co., Ltd. The results of DNA sequence assays showed that there exist three genotypes, including “CC”, “TC” and “TT” polymorphic genotypes, at +869 site of TGF - β1 gene (Figure 2).

### Hardy-Weinberg Genetic equilibrium test

Hardy-Weinberg equilibrium test was adopted to verify the characteristic of group representation in the genotypic distribution of the cases and the controls, and chi square test was carried out for genotype frequency of the two groups. The formula was:  $\chi^2 = \sum (\text{actual value} - \text{theoretical value})^2 / \text{theoretical value}$ , degree of freedom ( $\nu$ ) = 1 (genotype number - allele gene number).  $P$ -value > 0.05 indicating that the two groups were in genetic balance, which was consistent with Hardy-Weinberg genetic balance, and the research objects were representative of the population.

The number of CC, CT and TT genotypes in COPD patients and healthy volunteers was calculated by sequencing. The distribution of the three genotypes in COPD patients and healthy volunteers was consistent with Hardy Weinberg genetic balance (Table 2,  $P > 0.05$ ). And, the genotypic distribution of the COPD patients and healthy volunteers has the characteristic of group representation.

Table 2. Hardy-Weinberg Genetic equilibrium test

Genotype	COPD group			Control group		
	Actual value	Theoretical value	$\chi^2$	actual value	theoretical value	$\chi^2$
CC	15	19.32		4	7.78	
CT	57	48.39	3.108*	45	37.36	3.731*
TT	26	30.30		41	44.86	

### 869 T/C genotype of TGF- $\beta$ 1 associate with the susceptibility of COPD

The frequency of genotype C/C, C/T and T/T in COPD patients are 15 cases (15.3%), 57 cases (58.2%) and 26 cases (26.5%), respectively. The probabilities of allele gene C and T in COPD patients were 44.4% and 55.6%, respectively. However, there were 4 cases of C/C genotype (15.3%), 45 cases of C/T genotype (58.2%) and 41 cases of T/T genotype (26.5%) in healthy volunteers.

There existed obviously different alterations in the distribution of CC, CT and TT at +869 T/C locus in COPD group ( $P < 0.05$ ) compared with the control group, and the frequency of CC genotype in COPD group (15.3%) was significantly higher than that in control group (4.4%) (Table 3). Compared with TT genotype, CC genotype significantly increased the risk of COPD (Table 3, OR:5.913, 95% CI: 1.916-18.245). However, there was no significant correlation between CT genotype and the occurrence of COPD (Table 3, OR: 1.997, 95% CI: 1.070-1.866).

The frequency of allele gene C and T in COPD patients were 87 cases (44.4%) and 109 cases (55.6%), and have a significant difference with control group. Especially, the frequency of allele gene C has a higher level in COPD patients. Further, logistic regression analysis was used to analyze the relation of allele gene with COPD. Results showed that allele C was associated with COPD (Table 3, OR:1.913, 95% CI: 1.251-2.926).

Table 3. Distribution of TGF- $\beta$ 1 +869 T/C genotype and allele gene (n, %)

		COPD group	Control group	$\chi^2$	<i>P</i> value	OR (95%CI)
<b>Genotype</b>	CC	15(15.3)	4(4.4)	10.831	0.004	5.913 (1.916-18.245)
	CT	57(58.2)	45(50)			1.997 (1.070-1.866)
	TT	26(26.5)	41(45.6)			
<b>Allele Gene</b>	C	87(44.4)	53(29.4)	8.964	0.003	1.913 (1.251-2.926)
	T	109(55.6)	127(70.6)			

### 869 T/C genotype of TGF- $\beta$ 1 associate with the airflow restriction of COPD

Generally, values of FEV1 (FEV1, Forced Expiratory Volume in One Second) and FEV1/FVC (FVC, Forced Vital Capacity) in the pulmonary function tests are used to confirm whether there is airflow restriction, and the most reliable index to judge the degree of airflow restriction is the predicted value of FEV1%<sup>[18]</sup>. Therefore, we usually use the predicted value of FEV1% to classify the degree of airflow restriction in patients with COPD.

In our study, we also examined the effect of +869 T/C genotype on FEV1 and FEV1/FVC of COPD patients (Table 4). The predicted value of FEV1% in patients with CC genotype was significantly lower than that in patients with TT genotype ( $P < 0.05$ ). This result suggested that the genotype of +869 T/C may have an impact on the degree of airflow restriction in patients with COPD.

Table 4. Relationship between +869 T/C genotype of TGF- $\beta$ 1 and predicted value of FEV1% in COPD Patients

Genotype	FEV1% predicted value
CC	43.67±16.13
CT	51.39±18.64
TT	57.19±18.40*

\*:  $P < 0.05$ , compared with CC genotype

Further, we compared the +869 T/C genotypes of TGF- $\beta$ 1 in COPD patients with different degrees of airflow restriction. The results showed that the genotype +869 CC of TGF- $\beta$ 1 was significantly higher in severe airflow restriction COPD patients than in mild-to-moderate airflow restriction COPD patients ( $P =$

0.037), and the C allele was significantly higher in severe airflow restriction COPD patients than in mild-to-moderate airflow restriction COPD patients ( $P = 0.024$ ).

Table 5. Relationship between +869 T/C genotype of TGF- $\beta$ 1 and predicted value of

Airflow Restriction	Genotype			P value	Allele Gene		P value
	CC	CT	TT		C	T	
Mild-to-Moderate	3(6.5)	27(58.7)	16(34.8)	0.037	33(35.9)	59(64.1)	0.024
Severe	12(23.1)	30(57.7)	10(19.2)		54(51.9)	50(48.1)	

## Discussion

As a developing disease, current treatments of COPD mainly focus on ameliorating symptoms induced by inflammatory pathways as opposed to curing disease<sup>[19]</sup>. COPD is heterogeneous in its molecular and clinical presentation, making it difficult to understand disease etiology and define robust therapeutic strategies, and most patients have entered the middle or late stage of the disease when they are diagnosed<sup>[20-22]</sup>. Therefore, early diagnosis and early intervention are the main strategies to improve the prognosis of COPD patients and reduce the social burden<sup>[23, 24]</sup>.

With the development of molecular biology, it has been clarified that there are a series of COPD susceptibility genes and single nucleotide polymorphism (SNPs) which play an important role in the occurrence and development of COPD<sup>[25, 26]</sup>. For example, the decreased levels of miR-146a-5p in COPD fibroblasts can contribute to chronic inflammation in COPD by inducing a more pro-inflammatory phenotype<sup>[27]</sup>. Pulmonary hypertension (PH), as a common complication for COPD, is an important indicator in the prognostic evaluation of COPD patients. However, "TC" genotype of TRPV1 SNP rs3744683 was a protective factor for PH in COPD patients compared with the genotype of "TT"<sup>[28]</sup>. Further, SNPs can lead to the decline of lung function through the modification of gene expression level, mRNA stability and structural protein<sup>[29, 30]</sup>.

Universally, TGF- $\beta$  is implicated as a major factor underlying fibrotic phenotypes, and polymorphisms promoting increased TGF- $\beta$  expression were identified as genetic modifiers of COPD and CF lung disease severity<sup>[31, 32]</sup>. Recent study showed that a raised production of total and active TGF- $\beta$ 1 were founded from airway cells of COPD patients, and the total TGF- $\beta$ 1 correlates with the severity of airway obstruction without evidence of a link with emphysema<sup>[33]</sup>. As the controversy of the effect of 869 T/C polymorphism at TGF- $\beta$ 1 on COPD was controversial<sup>[11]</sup>, we conducted further study.

In our experiment, we collected 98 COPD patients and 90 healthy volunteers as COPD group and control group. There were no differences in age, gender, BMI and smoking index between the COPD group and the control group. However, FEV1 and FEV1/FVC, which represent the presence of airflow limitation and the degree of restriction, were significantly lower in COPD patients than in healthy volunteers. Moreover, we

sequenced the +869 locus of TGF -  $\beta$ 1, and found that there were three genotypes including CC, TC and TT in the +869 locus. Hardy-Weinberg Genetic equilibrium test showed that the genotype of TGF -  $\beta$  1 gene + 869 locus was representative of population.

Further, we found that the frequency of CC genotype in COPD group was significantly higher than that in control group by comparing the genotype of +863 locus between COPD group and control group, and CC genotype significantly increased the risk of COPD compared with TT genotype (OR: 5.913, 95% CI: 1.916-18.245, Table 3). Logistic regression analysis showed that the C allele gene of +863 locus was closely related to the incidence of COPD (OR:1.913, 95% CI: 1.251-2.926, Table 3).

Values of FEV1 (FEV1, Forced Expiratory Volume in One Second) and FEV1/FVC (FVC, Forced Vital Capacity) in the pulmonary function tests are used to confirm whether there is airflow restriction, and the most reliable index to judge the degree of airflow restriction is the predicted value of FEV1%<sup>[18]</sup>. Therefore, we hypothesized that the +863 genotype may have an effect on FEV1 and FEV1/FVC in COPD patients. The results showed that the predicted value of FEV1% in patients with CC was significantly lower than that in patients with TT ( $P < 0.05$ ). Moreover, we compared the genotypes of +869 locus in COPD patients with different degrees of airflow restriction. The results showed that the genotype CC of +869 locus was significantly higher in severe airflow restriction COPD patients than in mild-to-moderate airflow restriction COPD patients ( $P = 0.037$ ), and the C allele was also highly expressed in the severe group ( $P = 0.024$ ). These results suggest that the +869 genotype of TGF -  $\beta$ 1 gene is not only related to the presence of airflow restriction in COPD patients, but also related to the degree of airflow restriction in patients with COPD.

In conclusion, we found that the allele C at +869 site of TGF -  $\beta$ 1 is highly expressed in COPD patients, and its expression is closely related to the airflow restriction and degree of airflow restriction in COPD patients. Our results suggest that TGF -  $\beta$ 1 +869 allele C may be a genetic locus and therapeutic target for COPD. This study provides the basis for early prevention, targeted intervention and gene targeted therapy for COPD susceptible population.

## Abbreviations

TGF- $\beta$ 1: Transforming Growth Factor-beta I; COPD: Chronic Obstructive Pulmonary Disease; FEV1: Forced Expiratory Volume in One Second; FVC: Forced Vital Capacity; PCR: Polymerase Chain Reaction; SNP: Single Nucleotide Polymorphism; EB: Elution Buffer; OR: Odds Ratio; 95%CI: 95% Confidence Interval.

## Declarations

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NA

### Authors' contributions

JL and WJZ designed the experiments. JL performed the experiments. JL and WJZ analyzed the experimental data. WJZ wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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Not applicable.

## **Availability of data and materials**

The datasets used and/or analysed during the current study are available

from the corresponding author on reasonable request.

## **Ethics approval and consent to participate**

All patients consented to the institutional review board which allows comprehensive analysis of tumor specimens (Ethics committee of the Qingdao Municipal Hospital, Ref.: 2019 LSZ No.121).

## **Consent for publication**

Not applicable

## **Competing interests**

The authors declare that they have no competing interests.

## **Author details**

<sup>1</sup>Department of Emergency, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University

<sup>2</sup>Department of Pharmacy, Qingdao Municipal Hospital, School of Medicine, Qingdao University, Qingdao, Shandong Province, China.

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## Figures

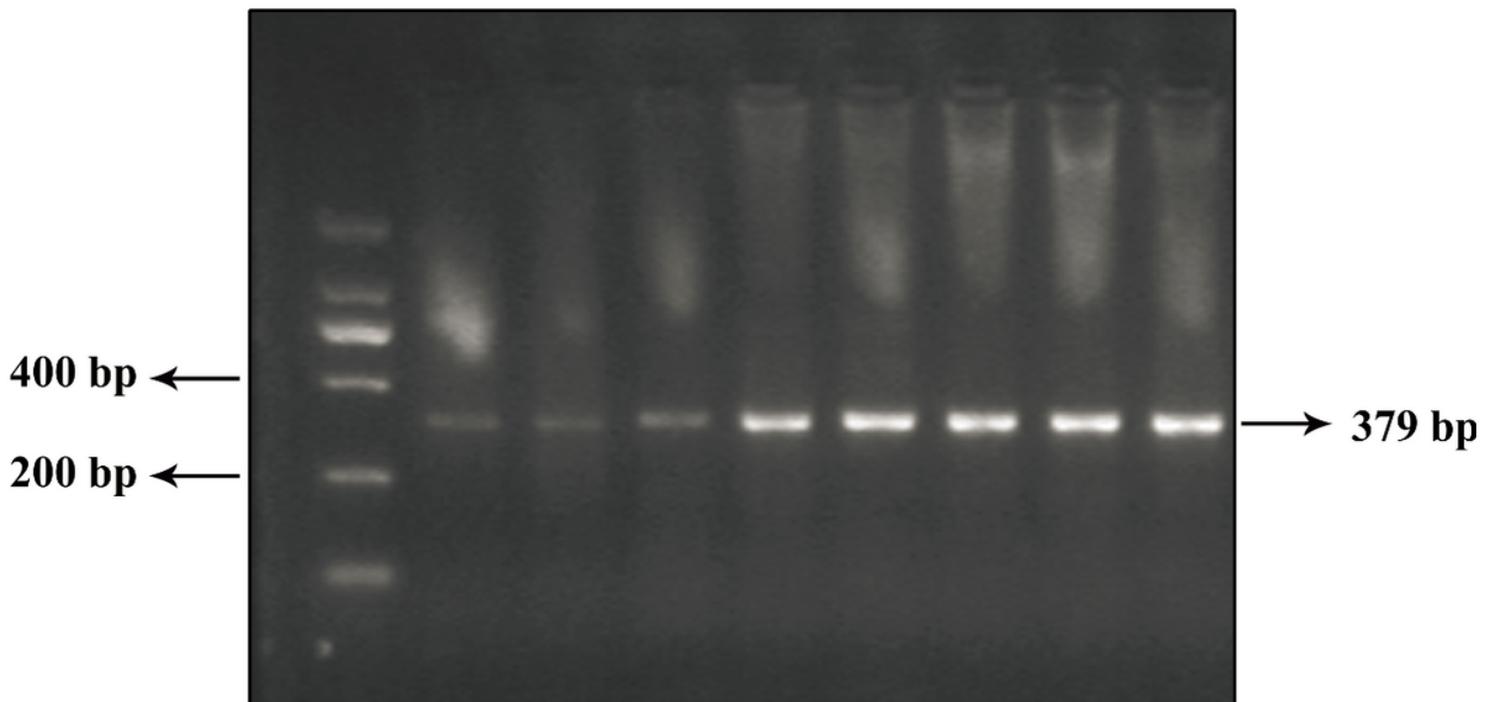
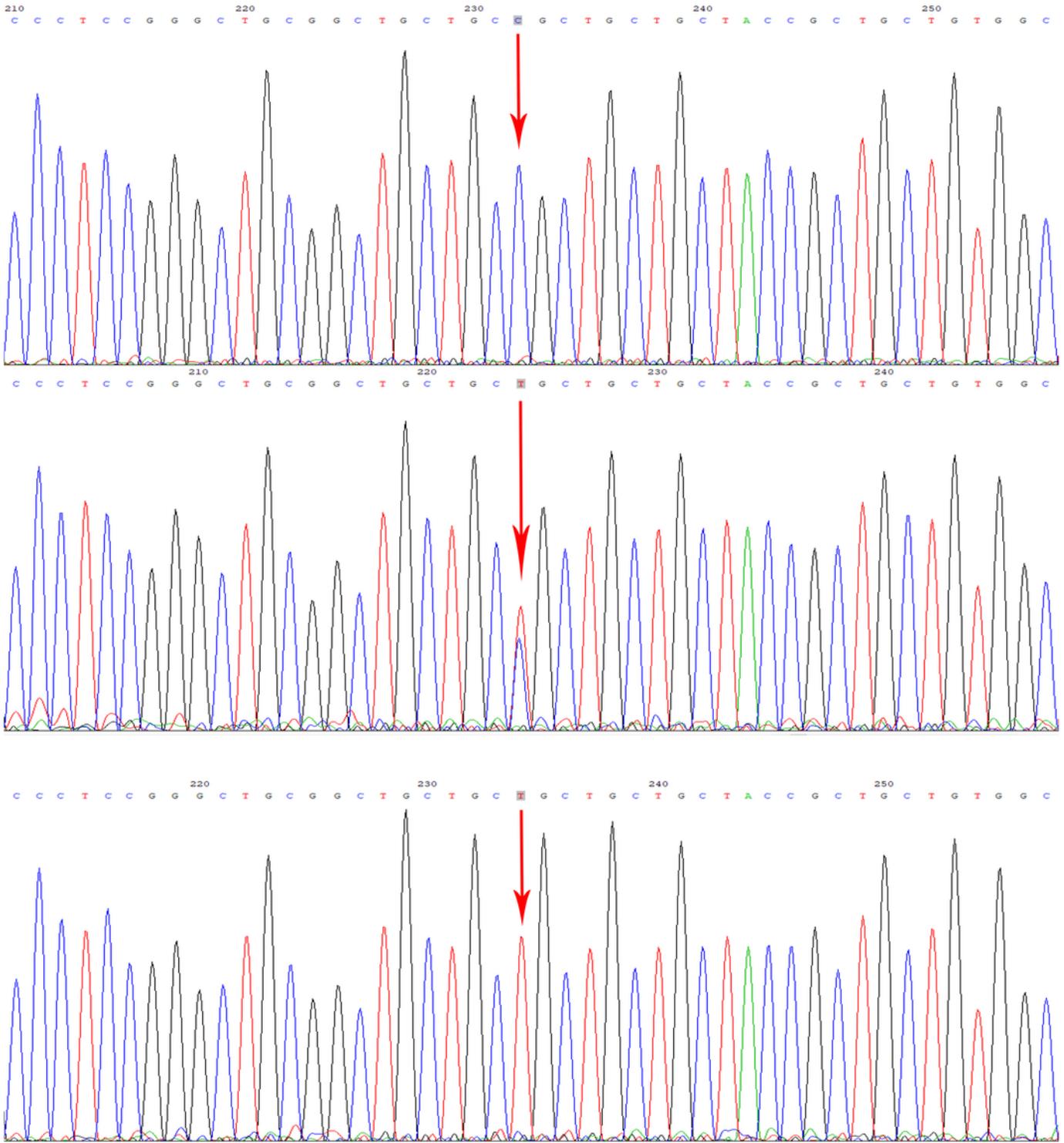


Figure 1

PCR products of target fragment including +869 site of TGF -  $\beta$ 1



**Figure 2**

DNA sequence assays showed that three genotypes exist at +869 site of TGF -  $\beta$ 1. (A) CC genotype, (B) CT genotype, (C) TT genotype.