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STAT3 gene polymorphism in chronic obstructive pulmonary disease

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Abstract. – OBJECTIVE: The aim of this study was to explore the correlation between rs8069115, rs41289087, and rs11079042 polymorphisms of the signal transducer and activator of transcription 3 (STAT3) gene and chronic obstructive pulmonary disease (COPD).

PATIENTS AND METHODS: A total of 200 patients diagnosed with COPD were enrolled in the disease group. Meanwhile, 200 normal subjects were selected as the control group. Peripheral blood was collected from subjects in the disease group and control group. Subsequently, nucleated cells were isolated for determination of STAT3 gene polymorphisms. Quantitative Polymerase Chain Reaction (qPCR) was utilized to detect the expression level of STAT3. Samples from 12 patients with differences in STAT3 haplotypes and 12 cases with no difference were collected and treated with transcriptome sequencing to analyze pathways enriched with differentially expressed genes.

RESULTS: There were statistically significant differences in allele distributions at rs8069115 between the disease group and control group (p=0.000), and the allele frequency of G was higher in disease group. Genotype distributions of rs8069115 (p=0.000) and rs41289087 (p=0.000) of the STAT3 gene in disease group were significantly different in comparison with the control group. The frequency of rs8069115 GG genotype was remarkably higher, while the frequency of rs41289087 TG genotype was lower in the disease group (p<0.05). In addition, compared with the control group, the distributions of the dominant model (p=0.002) and recessive model (p=0.004) of rs8069115 of the STAT3 gene were markedly different in the disease group. A significantly higher frequency of dominant model GG+GA and lower frequency of recessive model GA+AA were observed at rs8069115 in the disease group (p<0.05). Moreover, the haplotype distributions of AGC (p=0.002), ATC (p=0.001), GTA (p=0.010), and GTC (p=0.035) at rs8069115, rs41289087, and rs11079042 were different between the disease group and control group. Besides, rs8069115 locus and rs11079042 locus were linked to each other (D'=0.523). There was a remarkable association between rs11079042 polymorphism of the STAT3 gene and gene expression (p<0.05). STAT3 was highly expressed in patients with genotype CC (p<0.05). Furthermore, changes in transcriptome levels among different haplotype populations (haplotype with different distributions *vs.* haplotype with no difference in distribution) were analyzed. The results demonstrated that multiple pathways, such as ECM-receptor interactions, cell cycle checkpoints, and protein processing were notably enriched (p<0.05).

CONCLUSIONS: According to our results, we confirmed that the polymorphisms (rs8069115, rs41289087, and rs11079042) of STAT3 gene are noticeably correlated with the occurrence and progression of COPD.

Key Words:
STAT3, Gene polymorphism, Chronic obstructive pulmonary disease (COPD), Enrichment analysis.

Introduction

Chronic obstructive pulmonary disease (COPD), a long-term respiratory disease, ranks among the top five causes of death. It can be secondary to chronic pulmonary and cardiovascular diseases[1,2]. Some studies[3,4] have found that COPD is mainly induced by long-term smoking and inhalation of industrial dust, chronic infection, and the lack of the body's own alpha-1-anti-trypsin. COPD is mostly characterized by tissue damage and adhesion, as well as fibrous connective tissue and scar tissue hyperplasia caused by chronic respiratory inflammation. These can eventually result in gradual decrease of pulmonary respiratory function[5]. Thus, it is of great significance to search for susceptible factors of COPD, so as to prevent the occurrence of the disease.

Gene polymorphism serves as one of the factors that cause changes in susceptibility to various diseases[6]. It has been demonstrated that many diseases are all associated with genetic polymorphisms, such as Parkinson's disease[7], chronic

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Chagas' disease[8], and coronary heart disease[9]. As a vital transcriptional activator in intracellular signaling transmission, STAT3 affects multiple biological functions. Therefore, in this study, we compared the polymorphisms (rs8069115, rs41289087, and rs11079042) of STAT3 gene between COPD patients and healthy people and analyzed their haplotypes from the perspective of gene polymorphism. Moreover, the correlation between polymorphisms (rs8069115, rs41289087, and rs11079042) of STAT3 gene and COPD was investigated in combination with STAT3 gene expression and transcriptome sequencing analysis.

Patients and Methods

General Materials

A total of 200 patients with COPD admitted to our hospital during the past four years were enrolled as disease group. Meanwhile, 200 normal subjects were selected as control group. The selection of patients was based on the guideline proposed by The Global Initiative for Chronic Obstructive Lung Disease. General and clinical data (name, gender, age, past history, family history, smoking and drinking history, and drug allergy history) were collected from subjects in disease group and control group. Patients' previous diagnosis and treatment records were searched to observe and record changes in the routine blood tests, liver and renal functions, as well as pulmonary function. No significant differences were observed in general data between the two groups (p>0.05, t-test and chi-square test). Diagnostic criteria for COPD were as follows: a) persistent and progressive dyspnea, which can be exacerbated by activities, b) chronic cough and sputum, c) abnormal pulmonary function indicators, and d) forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) less than 0.7 after administration of bronchodilators. This study was approved by the Ethics Committee of Jinan Maternity and Child Care Hospital. Signed written informed consents were obtained from all participants before the study.

Sample Collection

About 5-7 mL of peripheral blood was extracted from 200 COPD patients and 200 normal subjects, respectively. After centrifugation at 3,000 rpm for 5 min, the middle-nucleated cell layer was collected and placed into new Eppendorf (EP; Hamburg, Germany) tubes. Next, genomic deoxyribonucleic acid (DNA) extraction, total ribonucleic acid (RNA) extraction, and sequencing were performed.

Genomic DNA Extraction

Peripheral blood genomic DNA was extracted from subjects in the disease group and control group using Genomic DNA Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA). All steps were strictly performed according to the instructions of the kit, as follows: 200-250 μL of proteinase K solution was added into the centrifuge tube according to the sample volume. Peripheral blood nucleated cell layer sample and 1 mL of GE buffer solution were added, followed by fully mixing using vortex oscillator for 1 min. Then, they were incubated at 65°C for 8 min. Next, 2 mL of ethanol was added, mixed evenly, and transferred to the adsorption column. Subsequently, 1.5 mL buffer was added into the adsorption column, followed by centrifugation at 3,000 rpm for 1 min. Then, 200 μL of elution buffer was added into the adsorption column, and the liquid obtained by the above methods was peripheral blood genomic DNA. Finally, DNA purity (1.8-2.0) was measured by a spectrophotometer.

PCR Amplification and STAT3 Gene Polymorphism Analysis

The polymorphic regions (rs8069115, rs41289087, and rs11079042) of STAT3 gene were amplified using a qPCR instrument under the following conditions: 95°C for 5 min, followed by 40 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 30 s, and 72°C for 5 min. The primers of polymorphic loci were as follows: STAT3 gene rs8069115: Forward: (5'→3') 'CTTCCTGCTAGTCTGTGTCCT', Reverse: (5'→3') 'ATTGGGGTAGACAGTCCAGGT'. Rs41289087: Forward: (5'→3') 'TGGACTGTCTACCCCAATGG', Reverse: (5'→3') 'CAGGGTGATGCGGAATACCG'. Rs11079042: Forward: (5'→3') 'GCAGCTTCACGACTGGATGT', Reverse: (5'→3') 'CTCTCTGCGGATCTGTTCCC'. qPCR products were sent to Heilongjiang Biotechnology Co., Ltd. (Qiqihar, China) for sequencing. Finally, rs8069115, rs41289087, and rs11079042 polymorphism distributions of STAT3 gene were analyzed in disease group and control group, respectively.

Detection of STAT3 Gene Expression

STAT3 gene expression was detected *via* Real-Time fluorescence quantitative-Polymerase Chain Reaction (qRT-PCR). Glyceraldehyde

Table I. Allele distributions of rs8069115, rs41289087 and rs11079042 of the STAT3 gene in disease group and control group.

Locus	Allele	Control group	Disease group	OR value	95% CI	χ^2	p
rs8069115	G	195 (0.487)	270 (0.675)	0.45	0.34-0.61	28.88	0.000
	A	205 (0.512)	130 (0.325)				
rs41289087	T	218 (0.545)	231 (0.578)	0.87	0.66-1.15	0.85	0.354
	G	182 (0.455)	169 (0.422)				
rs11079042	A	176 (0.440)	196 (0.490)	1.22	0.92-1.61	2.01	0.156
	C	224 (0.560)	204 (0.510)				

3-phosphate dehydrogenase (GAPDH) was used as the internal reference in the quantitative analysis of the STAT3 gene expression. The primers were designed using Prime Premier 5.0 and synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. STAT3 gene: Forward: (5'→3') 'TACCTCATCCATTGCAGACATCT', Reverse: (5'→3') 'CTCCTGGGGTGATTGTCCAAG'; GAPDH: Forward: (5'→3') 'CGCTCTCTGCTCCTCCTGTTC', Reverse: (5'→3') 'ATCCGTTGACTCCGACCTTCAC'. The reaction system of qRT-PCR was as follows: 25 μL as a total, therein, 1 μL of forward primer, 1 μL of reverse primer, 0.5 μL of template cDNA, 12.5 μL of SYBR premix Taq, and 10 μL of double-distilled water. qRT-PCR was performed under the following conditions: 94°C for 2 min, followed by 45 cycles of 95°C for 30 s, 55°C for 40 s and 72°C for 30 s, and 72°C for 5 min.

RNA Sequencing

Total RNA was extracted from peripheral blood nucleated cells among 12 patients with different distributions in STAT3 haplotypes (AGC, ATC, GTA, and GTC) and 12 subjects with no difference (AGA, ATA, GGA, and GGC). Subsequently, extracted RNA was sent to the company for transcriptome sequencing, so as to analyze gene expressions among the above populations. Enrichment analysis was performed as well.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was adopted for statistical analysis. *t*-test and Hardy-Weinberg equilibrium test were utilized for comparison of measurement data. SHEsis website online analysis was selected for haplotype analysis. Furthermore, GO and KEGG enrichment analyses were used for differential genes. $p<0.05$ was considered statistically significant.

Results

Allele Distributions of Rs8069115, Rs41289087 and Rs11079042 of the STAT3 Gene in Disease Group and Control Group

As shown in Table I, there were statistically significant differences in allele distributions at rs8069115 between the disease group and control group ($p=0.000$), and the allele frequency of G was higher in disease group ($p<0.05$).

Genotype Distributions of Rs8069115, Rs41289087 and Rs11079042 of the STAT3 Gene in Disease Group and Control Group

As shown in Table II, genotype distributions of rs8069115 ($p=0.000$) and rs41289087 ($p=0.000$)

Table II. Genotype distributions of rs8069115, rs41289087 and rs11079042 of the STAT3 gene in disease group and control group.

Locus	Genotype	Control group	Disease group	χ^2	p
rs8069115	GG	55 (0.275)	98 (0.490)	24.48	0.000
	GA	85 (0.425)	74 (0.370)		
	AA	60 (0.300)	28 (0.140)		
rs41289087	TT	54 (0.270)	85 (0.425)	24.55	0.000
	TG	110 (0.550)	61 (0.305)		
	GG	36 (0.180)	54 (0.270)		
rs11079042	AA	50 (0.250)	51 (0.255)	4.71	0.094
	AC	76 (0.380)	94 (0.470)		
	CC	74 (0.370)	55 (0.275)		

Table III. Polymorphism analysis of rs8069115, rs41289087 and rs11079042 of the STAT3 gene in disease group and control group.

	Locus	Genotype	Control group	Disease group	χ^2	*p*
Dominant model	rs8069115	GG+GA	140 (0.700)	172 (0.860)	12.34	0.002
		AA	60 (0.300)	28 (0.140)		
	rs41289087	TT+TG	164 (0.820)	146 (0.730)	3.09	0.213
		GG	36 (0.180)	54 (0.270)		
	rs11079042	AA+AC	126 (0.630)	145 (0.725)	3.04	0.219
		CC	74 (0.370)	55 (0.275)		
Recessive model	rs8069115	GG	55 (0.275)	98 (0.490)	11.23	0.004
		GA+AA	145 (0.725)	102 (0.510)		
	rs41289087	TT	54 (0.270)	85 (0.425)	2.7	0.259
		TG+GG	146 (0.730)	115 (0.575)		
	rs11079042	AA	50 (0.250)	51 (0.255)	3.6	0.165
		AC+CC	150 (0.750)	149 (0.745)		
Heterozygous model	rs8069115	GG	55 (0.275)	98 (0.490)	2.19	0.335
		GA	85 (0.425)	74 (0.370)		
	rs41289087	TT	54 (0.270)	85 (0.425)	2.8	0.247
		TG	110 (0.550)	61 (0.305)		
	rs11079042	AA	50 (0.250)	51 (0.255)	3.56	0.169
		AC	76 (0.380)	94 (0.470)		
Homozygous model	rs8069115	GG	55 (0.275)	98 (0.490)	3.4	0.183
		AA	60 (0.300)	28 (0.140)		
	rs41289087	TT	54 (0.270)	85 (0.425)	3.6	0.165
		GG	36 (0.180)	54 (0.270)		
	rs11079042	AA	50 (0.250)	51 (0.255)	4.33	0.115
		CC	74 (0.370)	55 (0.275)		

of STAT3 gene in disease group were significantly different in comparison with the control group. The frequency of rs8069115 GG genotype was higher, while the frequency of rs41289087 TG genotype was lower in the disease group ($p<0.05$).

Polymorphism Analysis of Rs8069115, Rs41289087 and Rs11079042 of the STAT3 Gene in Disease Group and Control Group

As shown in Table III, compared with control group, the distributions of dominant model ($p=0.002$) and recessive model ($p=0.004$) of rs8069115 of STAT3 gene were remarkably different in the disease group. A higher frequency of dominant model GG+GA and lower frequency of recessive model GA+AA were observed at rs8069115 in disease group ($p<0.05$).

Haplotype Analysis and Linkage Disequilibrium Analysis on Rs8069115, Rs41289087 and Rs11079042 of the STAT3 Gene in Disease Group and Control Group

As shown in Table IV and V, the haplotype distributions of AGC ($p=0.002$), ATC ($p=0.001$), GTA ($p=0.010$) and GTC ($p=0.035$)

Table IV. Haplotype analysis on rs8069115, rs41289087 and rs11079042 of the STAT3 gene in disease group and control group.

Haplotype	Control group	Disease group	OR value	95% CI	χ^2	*p*
AGA	40.25 (0.101)	27.96 (0.070)	0.672	0.406-1.112	2.423	0.120
AGC	54.86 (0.137)	27.62 (0.069)	0.467	0.289-0.754	10.031	0.002
ATA	43.25 (0.108)	37.99 (0.095)	0.866	0.547-1.371	0.379	0.538
ATC	66.64 (0.167)	36.43 (0.091)	0.501	0.326-0.771	10.159	0.001
GGA	51.14 (0.128)	63.92 (0.160)	1.297	0.872-1.930	1.658	0.198
GGC	35.74 (0.089)	49.50 (0.124)	1.439	0.914-2.267	2.485	0.115
GTA	41.35 (0.103)	66.13 (0.165)	1.718	1.133-2.605	6.598	0.010
GTC	66.76 (0.167)	90.45 (0.226)	1.458	1.026-2.074	4.442	0.035

Table V. Linkage disequilibrium analysis on rs8069115, rs41289087 and rs11079042 of the STAT3 gene in disease group and control group.

D′	rs8069115	rs41289087	rs11079042
rs8069115	–	0.008	0.523
rs41289087	0.008	–	0.021
rs11079042	0.523	0.021	–

at rs8069115, rs41289087, and rs11079042 were significantly different between the disease group and control group. Besides, rs8069115 locus and rs11079042 locus were linked to each other (D'=0.523).

Relationships Between Rs8069115, Rs41289087 and Rs11079042 Polymorphisms of the STAT3 Gene and Gene Expressions

As shown in Figures 1-3, there was a remarkable association between rs11079042 polymorphism of STAT3 gene and gene expression ($p<0.05$). Moreover, significantly higher STAT3 expression was found in patients with genotype CC ($p<0.05$).

Relationships Between Rs8069115, Rs41289087 and Rs11079042 Polymorphisms of the STAT3 Gene and Changes in Cell Transcriptome Levels

As shown in Figures 4-5, the changes in transcriptome levels among different haplotype populations (haplotype with different distributions *vs.* haplotype with no difference in distribution) were analyzed. The results displayed that multiple pathways, such as ECM-receptor interactions, cell cycle checkpoints, and protein processing were notably enriched ($p<0.05$).

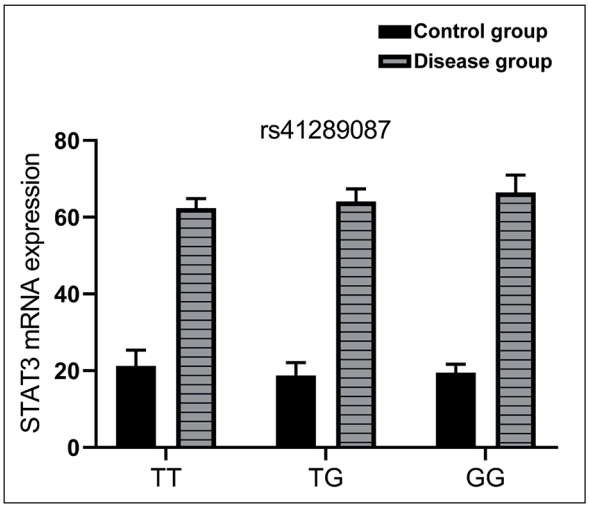

Figure 2. Relationship between rs41289087 polymorphism of STAT3 gene and gene expression (*$p<0.05$).

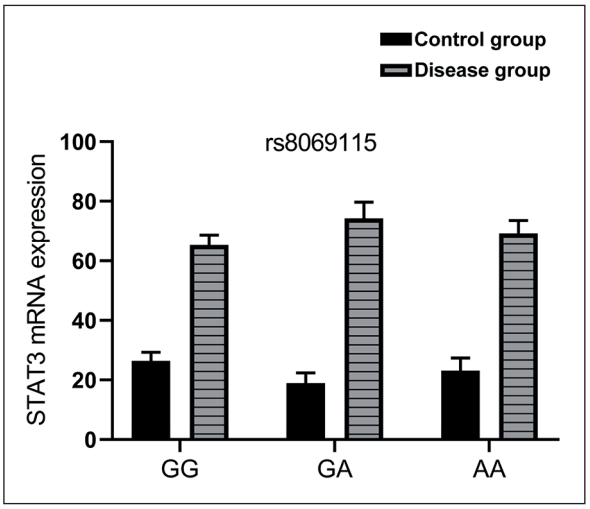

Figure 1. Relationship between rs8069115 polymorphism of STAT3 gene and gene expression (*$p<0.05$).

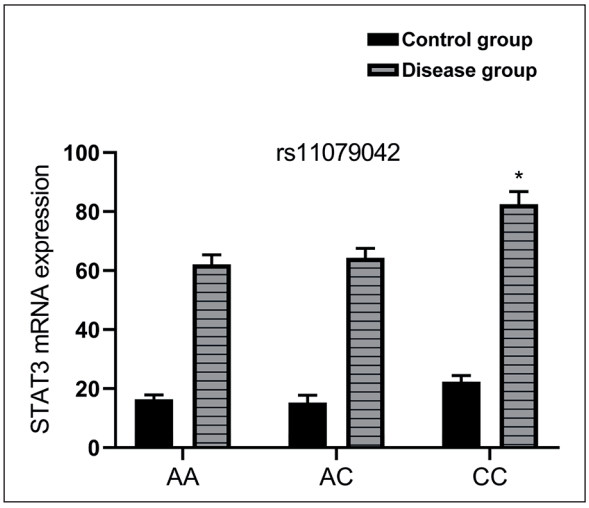

Figure 3. Relationship between rs11079042 polymorphism of STAT3 gene and gene expression (*$p<0.05$).

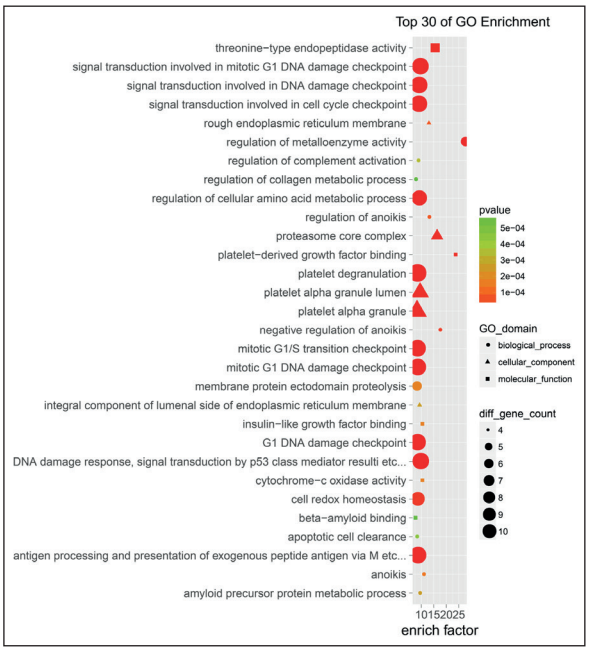

Figure 4. GO enrichment analysis on differential genes.

Discussion

COPD, a chronic respiratory disease characterized by persistent airflow limitation, mainly occurs in the middle-aged and elderly. It is often secondary to cardiovascular diseases, such as pulmonary heart disease and pulmonary hypertension[10,11]. COPD greatly impacts patient's quality of life, and socio-economic resources have also been wasted. The main reason of COPD is long-term exposure to harmful substances, such as cigarettes, chemical dust or other harmful substances. These factors may eventually lead to airway damage and chronic airway inflammation[12,13]. Additionally, COPD can also be induced by familial factors[14]. It has been confirmed that COPD can be affected by gene polymorphism (including the heme oxygenase 1[15] and HIF1A gene[16]). This is considered as the simplest and most common cause to influence gene base sequences and lead to genetic information changes. Notably, these susceptible factors are conducive to the prevention of the disease and general survey of disease in a wide population. Therefore, it is of significance to search for novel susceptible genes of COPD.

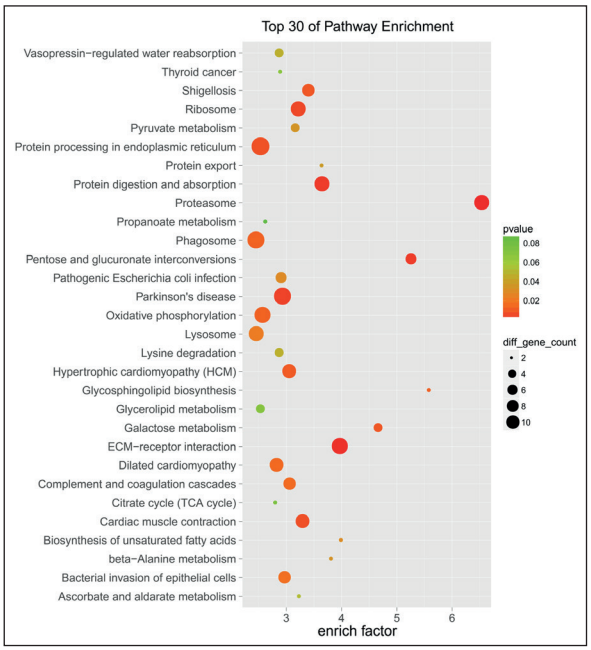

Figure 5. KEGG enrichment analysis on differential genes.

STAT3 functions as an important intracellular signaling transmission molecule. It mainly transmits extracellular signals into the nucleus and triggers various effects *via* inducing transcription and expression of target genes[17,18]. After the signal molecule receptor is paired, gp130 will form a dimer, which causes phosphorylation of JAK linked to gp130. Then, the C-terminal tyrosine residue (Y705) of STAT3 molecule is phosphorylated, activated by forming a dimer in its SH2 region, and transferred to the nucleus. This may bind to the promoter of the corresponding target gene, thereby inducing the transcription of target genes[19]. Given that STAT3 is one of the key signaling molecules in various cells, its genetic polymorphisms exert a wide range of effects on various diseases[20]. The rs8069115, rs41289087, and rs11079042 polymorphisms of STAT3 gene in COPD populations and healthy people were analyzed in this study. The results displayed that there were statistically significant differences in allele distributions at rs8069115 between disease group and control group (p=0.000), and the allele frequency of G was higher in disease group (p<0.05). Genotype distributions of rs8069115 (p=0.000) and rs41289087 (p=0.000) of STAT3 gene in disease group were different in comparison with the control group. The frequency of rs8069115 GG genotype was significantly higher, while the frequency of rs41289087 TG genotype was lower in the disease group (p<0.05). The results indicated that susceptibility of COPD was markedly influenced by STAT3, serving as one of significant susceptibility factors. Therefore, rs8069115, rs41289087, and rs11079042 polymor-

phisms of STAT3 gene could be utilized as screening indicators among population in clinical prevention.

In addition, compared with control group, the distributions of dominant model (p=0.002) and recessive model (p=0.004) of rs8069115 of STAT3 gene prompted significant differences in disease group. A higher frequency of dominant model GG+GA and lower frequency of recessive model GA+AA were observed at rs8069115 in the disease group (p<0.05). Moreover, the haplotype distributions of AGC (p=0.002), ATC (p=0.001), GTA (p=0.010), and GTC (p=0.035) at rs8069115, rs41289087 and rs11079042 were remarkably different between the disease group and control group. Besides, rs8069115 locus and rs11079042 locus were linked to each other (D'=0.523). These results illustrated that the effect of STAT3 on the susceptibility of COPD might be caused by not only a single locus or genotype, but also a combination of multiple factors. Our findings need to be verified through a larger-sample analysis.

Subsequent analysis on the relationships between STAT3 gene polymorphisms and gene expressions showed that there was a remarkable association between rs11079042 polymorphism and gene expression (p<0.05). STAT3 expression was significantly higher in patients with genotype CC (p<0.05). This suggested that the effect of gene polymorphism on COPD might be achieved *via* affecting on gene expression, a mediating factor. Furthermore, the changes in transcriptome levels among different haplotype populations (haplotype with different distributions *vs.* haplotype with no difference in distribution) were analyzed. The results displayed that multiple pathways, such as ECM-receptor interactions, cell cycle checkpoints, and protein processing were notably enriched (p<0.05). The fact that pathways are enriched by these differential genes demonstrates more strongly that STAT3 gene polymorphism influences COPD *via* affecting on STAT3 gene expression and expressions of downstream target genes and key molecules in other pathways.

Conclusions

We showed that the polymorphisms (rs8069115, rs41289087, and rs11079042) of STAT3 gene are noticeably correlated with the occurrence and progression of COPD, which could provide a basis for the susceptibility study of COPD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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