FEATURES OF CYSTIC FIBROSIS COURSE IN CHILDREN DEPENDING ON INTERLEUKIN-4 GENE MUTATION

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Abstract. Cystic fibrosis (CF) course features depend not only on the difference in CF transmembrane conductance regulator gene mutations, but also on other gene modifiers. Interleukin-4 (IL-4) gene is a one of gene modifiers. The aim of the present study was to improve medical care for patients with CF by clarifying the pathogenic role of IL-4 gene polymorphism in the course of the disease. The study involved examination of 42 children with CF and 54 practically healthy children as control group. Patient examination was carried out by the standard methods in the remission period. Determination of C589T polymorphism of IL-4 gene was carried out using polymerase chain reaction in real time. DNA was isolated from buccal epithelium cells. Patients with IL-4 gene mutation (C589T) were characterized by more frequent primary manifestation of the respiratory signs, Staphylococcus aureus predominance within respiratory pathological microorganisms, the incidences of bronchiectasis and lung fibrosis, more severe liver lesions, elevated total serum immunoglobulin E level, elevated levels of the CD25 lymphocytes, circulating immune complex and reduced immunoglobulin A levels. The data demonstrate features of CF phenotype associated with IL-4 gene polymorphism.

Key words: *children, cystic fibrosis, clinical and paraclinical features, interleukin-4 gene mutation.*

Introduction. Cystic fibrosis (CF) is a hereditary disease characterized by variety of clinical manifestation depending not only on the difference in CF transmembrane conductance regulator gene mutations, but also on other gene modifiers [3, 13, 14, 22].

CF is characterized by lifelong inflammation [10]. The study of the influence of inflammatory factors on genetic defects is of great scientific interest. Scientists have identified a number of genes modifiers that influence CF severity (interleukin (IL)-1B, IL-4, IL-10 genes, tumor necrosis factor-α (TNF-α), transforming growth factor β1, mannose-binding lectin, α-1-antitrypsin etc.) [4, 5, 8, 13, 21, 24]. For example, IL-10 gene polymorphism (G1082A) is associated with *Aspergillus fumigatus* colonization, which has a significant role on the bronchopulmonary inflammation development [13]. Conversely, TNF-α gene polymorphism is associated with a milder course of CF and later colonization of *Pseudomonas aeruginosa (P. aeruginosa)* [13].

According to the scientists, C589T polymorphism of IL-4 gene is involved in the modification of various diseases. IL-4 is an anti-inflammatory cytokine synthesized by activated T-helper type 2, basophils, mast cells, switches B-lymphocytes to immunoglobulin (Ig) class E production [15]. Replacement of C allele with T allele leads to a significant increase of the IgE, which takes an important part not only in the development of allergic reactions, but also in protecting of the organism from infectious agents, which is very relevant for CF patients with the chronic bronchopulmonary inflammation [2, 9, 12].

The authors note to the significant influence of the cytokines polymorphism on the CF course, depending on the presence of which the disease acquires distinctive features with the same mutation CFTR [7].

Genetic defects that are harmless for general population and not accompanied by pathological manifestations can have a significant effect on CF course [1, 6, 13].

Determination of additional allelic genes that directly affect the CF phenotype provides an opportunity not only to expand scientific

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knowledge about the CF nature, but also to improve an individualized approach to compiling the patient's algorithm [16, 20].

2. PURPOSES, SUBJECTS and METHODS:

2.1 Purpose. To improve medical care for patients with CF by clarifying the pathogenic role of IL-4 gene polymorphism in the course of the disease.

Tasks of study:

1. To determine the clinical features of children with CF depending on IL-4 gene polymorphism.

2. To determine the features of the immune status of children with CF depending on IL-4 gene polymorphism.

2.2 Subjects & Methods

The research was conducted in the pulmonology department of Kharkiv Regional Clinical Children's Hospital No. 1 in 2015–2017. Clinical and paraclinical examination of patient with CF was carried out according to the Order of Ministry of Healthcare of Ukraine of 15 July 2016 No. 723 "On approval of the unified clinical protocol of primary, secondary (specialized) and tertiary (highly specialized) medical care "Cystic fibrosis", Order of Ministry of Healthcare of Ukraine of 29 January 2013 No. 59 "On approval of unified clinical protocols of medical care for children with diseases of the digestive system". Forty-two children with CF were observed. The control group (for assessment of immune analysis) consisted of 54 practically healthy children who had been randomized according to the age.

Determination of C589T polymorphism of IL-4 gene was carried out using polymerase chain reaction in real time. DNA was isolated from buccal epithelium cells using a special "DNA-express" kit ("Liteh" company).

Investigation of immune status was carried out by the standard methods in the remission period. Total immunoglobulin E (Ig E) in serum was determined by solid phase enzyme-linked immunosorbent assay (ELISA).

The study was conducted with respect to human rights in accordance with the current legislation in Ukraine, in compliance with international ethical requirements and did not violate ethical norms in science and standards for conducting biomedical research.

The results were processed by the IBM SPSS Statistics software according to the methods of variance statistics. Statistical significance was the difference between the indicators at $p < 0.05$.

Conflict of interests

There is no conflict of interests.

3. RESULTS AND DISCUSSION

The study involved examination of forty two children. CF was diagnosed by clinical and paraclinical characteristics and confirmed by the results of pilocarpine test. The examined patients were mainly boys (66.7 %). According to age, the majority of the children were of senior school age (*Table 1*).

Table 1

Distribution of children with CF by gender and age

Gender \ Age	1 year – 2 year 11 months 29 days		3 years – 6 years 11 months 29 days		7 years – 11 years 11 months 29 days		12 years – 17 years 11 months 29 days		Total	
	n	%	n	%	n	%	n	%	n	%
Boys	4	80	3	50	12	85,7	9	52,9	28	66,7
Girls	1	20	3	50	2	14,3	8	47,1	14	33,3
Total, n	5		6		14		17		42	

During the study of C589T polymorphism in IL-4 gene in children with CF allele T was determined in 15 cases. Homozygotes for allele T and heterozygotes among patients with CF were significantly more prevalent in comparison with the control group (*Table 2*).

The majority of patients were boys in all groups of children who were included in the study (*Fig. 1*).

Clinical features were studied in the group of children with IL-4 gene mutation (homozygous and heterozygous patients) compared to patients without IL-4 gene mutation because of children distribution and a small number of patients with TT phenotype.

Group with CT+TT genotype was characterized by a more frequent primary manifestation of the respiratory signs, but not gastrointestinal, unlike general population of patients (*Table 3*).

Assessment of CF severity showed that moderate course prevailed in all the groups. CC genotype frequency was significantly higher in

Table 2

Distribution of patients depending on C589T polymorphism of IL-4 gene

Genotype	CF patients group (n = 42)		Control group (n = 54)	
	n	%	n	%
CC	27	64.3*	45	83.3
CT	11	26.2*	8	14.8
TT	4	9.5*	1	1.9
Total, n	42	100	54	100

* compared with control group ($p<0.05$).

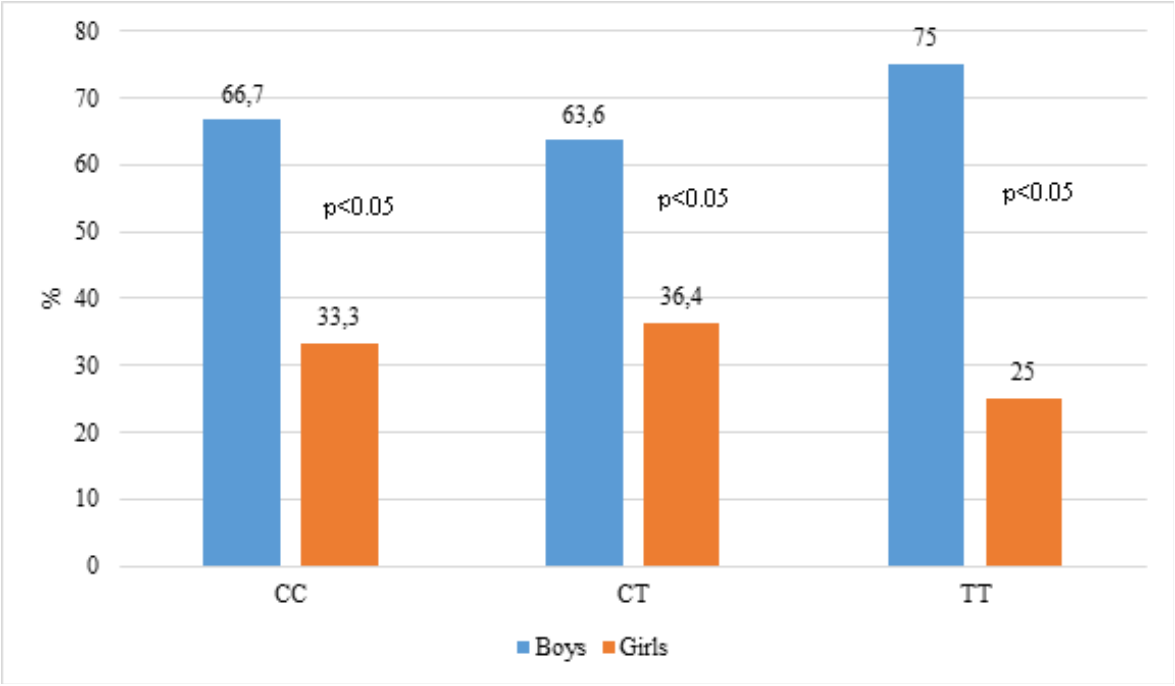

Fig. 1. Distribution of patients with CF by gender depending on C589T polymorphism of IL-4 gene

patients with mild course. CT+TT genotype group was characterized by significantly higher frequency in patients with severe course (*Fig. 2*).

The incidence of bronchiectasis and lung fibrosis was significantly higher in the CT+TT genotype group (66.7 % and 100 % vs 25.9 % and 65.9 % respectively).

The study of hepatic disorders showed that cirrhotic changes in liver parenchyma were more common in patients with IL-4 gene mutation (*Table 4*).

Bacteriological tests showed Staphylococcus aureus (S. aureus), P. aeruginosa, Candida albicans (C. albicans) were identified as predominant pathological microorganisms in the CT + TT genotype group (*Fig. 3*).

More frequent primary manifestation of the respiratory signs, S. aureus predominance within respiratory pathological microorganisms, more severe respiratory system and the liver lesions were found in the study of clinical features depending on C589T polymorphism of IL-4 gene.

Table 3

Manifestation of CF signs depending on C589T polymorphism of IL-4 gene

Genotype / Signs	CC (n=27)	CT+TT (n=15)
	%	%
gastrointestinal	74,07	53,4
respiratory	25,93	46,6*

* compared with CC genotype group ($p<0.05$).

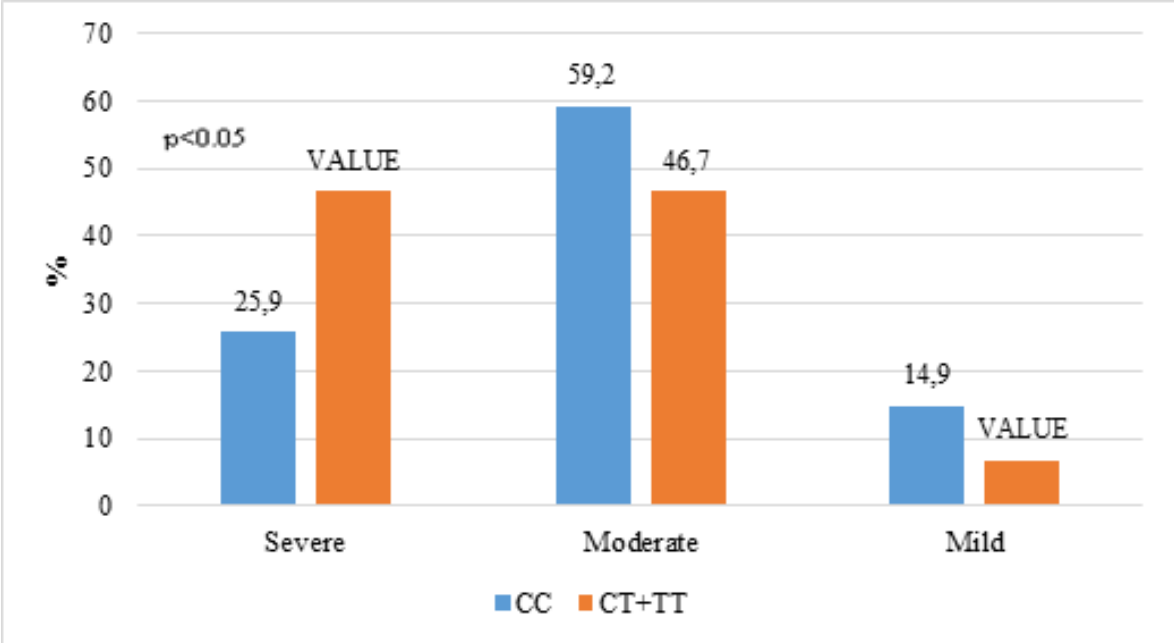

Fig. 2. The severity of CF course depending on C589T polymorphism of IL-4 gene

Elevated levels of CD25, circulating immune complex (CIC) and reduced IgA levels were found in patients of group with CT+TT genotype during the study of immune status (*Table 5*, *Fig. 4*).

The mean value of the total serum IgE was 344.6 (63.8; 670.3) IU / ml.

Elevated total serum IgE level frequency (80 %) was significantly higher in the CT + TT genotype group (446.9 (187.82; 884.72) IU/ml), when the CC genotype group was characterized by results within the age range (74%).

Discussion

C589T polymorphism of IL-4 gene affects the course of many diseases. For example, some authors described the negative effect of C589T polymorphism of IL-4 gene on the development of coronary artery disease [12].

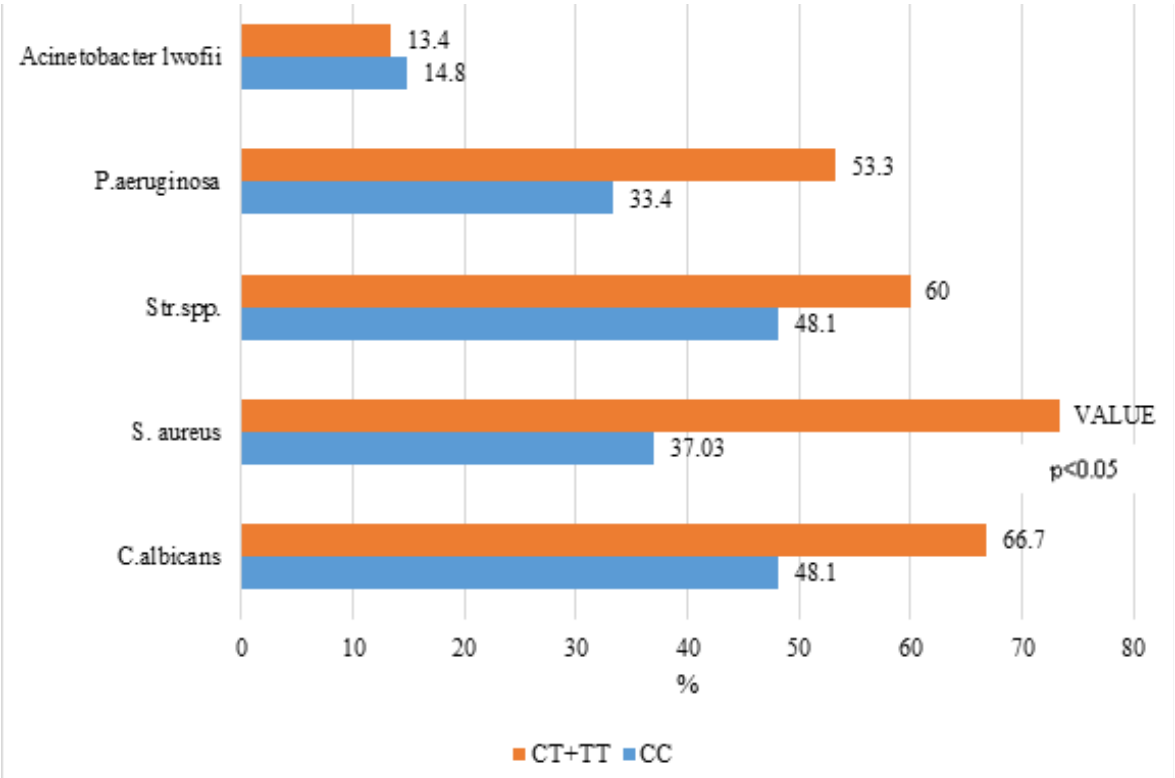

Fig 3. Indices of bacteriological tests depending on C589T polymorphism of IL-4 gene

Table 4

The degree of hepatic disorder depending on C589T polymorphism of IL-4 gene

Genotype / The degree of hepatic disorder	CC (n=27) %	CT+TT (n=15) %
Norm	29.6	6.6*
Moderate lesions	62.9	46.7*
Cirrhotic changes	7.5	46.7*

*compared with CC genotype group ($p<0.05$).

Table 5

Indices of immunological status of children with CF depending on C589T polymorphism of IL-4 gene

Indicator	IL-4 gene genotype			p_1	p_2	p_3
	CC (n=27)	CT+TT (n=15)	Control group (n=30)			
Leukocytes, $x10^9$/l	6.32 (5.5; 7.9)	6.5 (5.1; 7.5)	6.35 (5.47;7.0)	p >0.05	p >0.05	p >0.05
Neutrophils, %	49.0 (40.5; 61.0)	49.0 (42.0; 56.0)	51.0 (48.0;63.0)	p >0.05	p >0.05	p >0.05
Lymphocytes, %	50.0 (42.0; 61.0)	51.0 (44.0; 58.0)	43.0 (36.5;47.0)	p >0.05	p <0.05	p <0.05
CD 3, %	69.0 (65.5; 69.0)	66.0 (63.0; 70.0)	61.0 (58.7;69.0)	p >0.05	p >0.05	p >0.05
CD 4, %	40.0 (38.0; 40.0)	39.0 (37.0; 41.0)	44.0 (39.0;48.0)	p >0.05	p >0.05	p >0.05
CD 8, %	28.0 (27.0; 29.0)	27.0 (29.0; 29.0)	30.0 (29.0; 32.0)	p >0.05	p >0.05	p >0.05
CD 16, %	14.0 (10.0; 15.0)	12.0 (9.0; 16.0)	14.0 (13.75;15.0)	p >0.05	p >0.05	p >0.05
CD 22, %	19.0 (18.0; 20.5)	19.0 (17.0; 21.0)	18.0 (17.0; 20.0)	p >0.05	p >0.05	p >0.05
CD 25, %	21.0 (19.0; 37.0)	26.0 (17.0; 37.0)	17.0 (14.0;22.0)	p <0.05	p <0.05	p <0.05
Phagocytosis of latex, %	63.0 (59.0;68.0)	61.0 (54.0; 70.0)	60.0 (54.0; 65.0)	p >0.05	p >0.05	p >0.05
Phagocytic number	3.8 (3.7;4.1)	3.7 (3.54; 4.2)	3.5 (3.3;3.7)	p >0.05	p >0.05	p >0.05
Total complement (CH 50)	64.0 (61.0;65.0)	61.0 (62.0; 68.0)	48.0 (45.5; 52.0)	p >0.05	p >0.05	p >0.05
CIC with 3.5% PEG, units	7.6 (6.4;9.3)	8.3 (7.0; 9.9)	7.95 (6.8; 9.5)	p <0.05	p <0.05	p <0.05
Spontaneous nitroblue tetrazolium (NBT) tests, %	24.0 (18.0;42.0)	28.0 (17.0; 47.0)	30.0 (22.0; 32.0)	p >0.05	p >0.05	p >0.05
Spontaneous index of activated neutrophils (IAN) test, units	0.54 (0.26;0.81)	0.53 (0.27; 0.88)	0.58 (0.46; 0.8)	p >0.05	p >0.05	p >0.05
Stimulated NBT test, %	63.0 (52.0;68.0)	67.0 (62.0; 72.0)	55.0 (49.7; 70.0)	p >0.05	p >0.05	p >0.05
Stimulated IAN test, units	1.33 (1.12;1.49)	1.38 (1.22; 1.49)	1.18 (0.87; 1.29)	p >0.05	p >0.05	p >0.05
Lysosomal cationic proteins, units	1.18 (1.01;1.22)	1.19 (1.08; 1.27)	1.12 (0.93; 1.17)	p >0.05	p >0.05	p >0.05
Ig A, g/l	1.38 (1.02;1.54)	1.23 (0.99; 1.39)	1.18 (0.83; 1.33)	p <0.05	p <0.05	p <0.05
Ig M, g/l	1.02 (0.83;1.22)	0.98 (0.85; 1.08)	0.97 (0.64;1.2)	p >0.05	p >0.05	p >0.05
Ig G, g/l	10.38 (10.28;10.98)	10.29 (9.66; 10.64)	10.09 (8.53;10.9)	p >0.05	p >0.05	p >0.05

p_1 – CC genotype compared with the CT+TT genotype, p_2 – CC genotype compared with the control group, p_3 – CT+TT genotype compared with the control group.

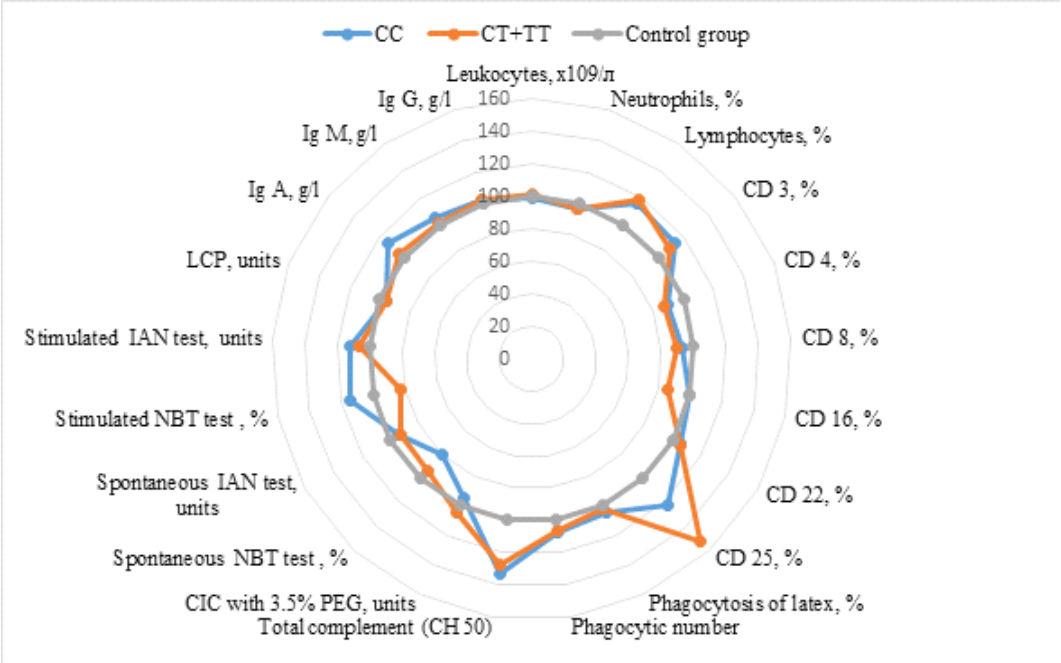

Fig. 4. Indices of immunological status of children with CF depending on C589T polymorphism of IL-4 gene

C589T polymorphism of IL-4 gene is associated with an elevated level of total IgE and bronchial hyperactivity in patients with bronchial asthma, which is an aggravating factor [17, 18].

There are not numerous studies regarding the influence of the IL-4 gene polymorphism the on CF. In this way, Sergienko DF and co-authors noted the relationship of 3'-UTR G/C mutation of IL-4 gene with the course severity of the bronchopulmonary inflammatory process [19].

Gembitskaya T.E. and co-authors studied the influence of IL-4 gene (C589T) mutation and its receptor (?-chain gene-IL-4R?) on the course severity of CF in the adult population (37 patients). Significant associations were not identified between polymorphic variants of IL-4 receptor gene and CF course, but the dependence of the clinical features on the IL-4 gene mutation was determined. It was found that homozygotes for T allele were only women [11].. According to our study, the influence of the C589T polymorphism of the IL-4 gene on the CF course severity was confirmed, but boys were dominated among patients with TT genotype.

Further research of the inflammatory genes modifiers will provide an opportunity to broaden knowledge about the peculiarities of the CF pathogenesis and mark new factors, which take part in the disease modification.

3. Conclusion.

Features of the CF phenotype associated with the polymorphism of the IL-4 gene were analyzed. More frequent primary manifestation of the respiratory signs, S. aureus predominance within respiratory pathological microorganisms, the incidence of bronchiectasis and lung fibrosis, more severe liver lesions, elevated total serum IgE level, elevated levels of the CD25 lymphocytes, CIC and reduced IgA levels were found in children with CF and C589T polymorphism of IL-4 gene.

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