The influence of genotype and chronic infection on pulmonary phenotypes in patients with cystic fibrosis

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Abstract

Influența geneticii și a infecției asupra fenotipurilor pulmonare la pacienții cu fibroză chistică

Fibroza chistică (CF) este o boală ereditară autosomal-recesivă care evoluează cu un conținut ridicat de cloruri de transpirație, boală pulmonară cronică, insuficiență pancreatică cu maldigestie, malabsorbție și scădere în greutate. Sunt analizate interrelațiile dintre fenotipul modificărilor bronhopulmonare la 3 pacienți cu fibroză chistică și colonizarea infecțioasă cu germeni patogeni. Diagnosticarea CF la copiii evaluați a fost confirmată prin cercetarea ADN moleculară pentru a determina mutațiile CFTR. Sa raportat că infecția cu Pseudomonas aeruginosa accelerează procesele distructive ale parenchimului pulmonar și contribuie la răspândirea fibrozei pulmonare, bronhiectazei, emfizemului, distrugerii pulmonare, fibrozei focale sau difuze, pleurezii, toate fiind vizualizate și diagnosticate în examinările imagistice.
Relația dintre genotip și fenotip a fost investigată la pacienții cu CF și infecții pulmonare cu diferite etiologii. Asocierea a două mutații CFTR și a infecției cu P. aeruginosa au fost asociate cu fenotipurile clasice severe ale CF.

Cuvinte cheie: fibroză chistică, CFTR, infecție, fenotip, copii

Abstract

Cystic fibrosis (CF) is an autosomal-recessive hereditary disease that evolves with high content of perspiration chlorides, chronic pulmonary disease, pancreatic insufficiency with maldigestion, malabsorption and weight loss.
The interrelations between phenotype of bronchopulmonary changes in 3 patients with cystic fibrosis and infectious colonization with pathogenic germs are analyzed. Diagnosis of CF in evaluated children was confirmed by molecular DNA research to determine CFTR mutations. It has been reported that *Pseudomonas aeruginosa* infection accelerates the destructive processes of pulmonary parenchyma and contributes to the spread of pulmonary fibrosis, bronchiectasis, emphysema, pulmonary destruction, focal or diffuse fibrosis, pleurisy, all of which are visualized and diagnosed in imaging examinations.
The genotype-phenotype relationship was investigated in patients with CF and pulmonary infection of different etiologies. The association of two CFTR mutations and *Ps.aerugenosa* infection were associated with the classical severe phenotypes of CF.

Keywords: cystic fibrosis, CFTR, infection, phenotype, children

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Introduction

CFTR gene mutations and their role in CF pathogenesis remain challenging for both researchers and physicians, so more information on genetic variation would help to study the effect on the phenotype of the disease [12]. CF is the most common autosomal recessive disease in the Caucasian population with very high mortality and is caused by mutations in the CFTR gene [8]. This is a hereditary disease that evolves with high content of perspiration chlorides, chronic pulmonary disease, pancreatic insufficiency with maldigestion, malabsorption and weight loss. The CF phenotype is very heterogeneous even among siblings carrying identical CFTR mutations, suggesting that the severity of the disease is affected by the CFTR genotype and other causal factors [13]. More than 2000 mutations and polymorphisms have been identified on the CFTR gene [9]. An association between the various severe genotypes and CF pulmonary phenotype has been reported in various international studies [2]. The term diagnosis of the disease may be useful in the prognosis of the cystic fibrosis phenotype. Thus, a late diagnosis age may be the result of an unsatisfactory system of health system administration that could lead to a substantial progression of the disease due to inadequate treatment, creating a negative association between age to diagnosis and survival. The findings of various investigators reveal significant deviations between diagnostic term and mode. There are children diagnosed early by prenatal screening without meconial ileus, early with manifest manifestations of the disease or late progression of the disease due to absent treatment [5, 10].

Aim. Evaluation of the influence of CFTR genetic mutation and pulmonary infection on the phenotype of bronchopulmonary changes in patients with cystic fibrosis.

Materials and Methods

Three patients with CF were evaluated in the study, who performed periodic stationary treatment at the Cystic Fibrosis Center in the Pneumology Clinic, Mother and Child Institute.

The positive diagnosis of CF was confirmed based on anamnestic data, clinical examination, paraclinical investigations, automated welding test (Macroduct, USA, Exudose, France). Through informed consent, molecular DNA research was performed to determine CFTR mutations. Pathogenic germs were examined bacteriologically from bronchial secretions and identified by cultural methods using the „WalkAway-96" device, Siemens, Germany.

The imaging examination included ultrasound results, pulmonary radiography. The imaging evaluation of bronchopulmonary lesions was performed by thoracic HRCT (Aquilion 32, Toshiba, Japan). Spiral pulmonary computed tomography (CT) is a radiologically binding exploratory, highly informative exploratory examining and detects structural changes in the bronchopulmonary system. Pulmonary CT provides high information in the assessment of the bronchopulmonary substrate, thus advanced lung bronchial pulmonary emphysema, atelectasis, bronchiectasis, fibrosis, pulmonary sclerosis have been identified in CF.

Results and Discussions

It is believed that the determinant in manifestation of phenotypic expression in patients with CF are inherited mutations. In the Pneumology Clinic, several studies have been conducted to diagnose and monitor several CF patients. Three children with CF mixed with different severity of the disease were evaluated in current research. The CF diagnosis was established based on the results of two sweat chloride tests (>60 mmol/l). The history of the disease, CF diagnostic age, CFTR genotype, body mass index (BMI), pulmonary function test results in children older than 5 years, exocrine pancreatic status were evaluated in the study children. Bacteriological results were obtained from each patient during hospitalization with the identification of pathogenic airway disease.

All patients exhibited characteristic CF symptoms with manifest respiratory and digestive signs. All children with CF have been found to have elevated concentrations of chloride in sweating. The diagnosis of this disease was confirmed in all infants during the first 6 months of life with signs of recurrent bronchopulmonary infections (severe obstructive bronchitis, tremors, recurrent pneumonia, nutrition disorders). Early diagnosis induces suspicion that at the onset of the disease, the children make a severe form or a "severe" phenotype of cystic fibrosis may be assessed for a shorter survival. Alternatively, an early diagnosis age could be the result of prenatal/neonatal positive screening, which generally identifies infants with cystic fibrosis at pre-symptomatic stages, and could thus lead to better survival [5, 10].

However, an early diagnosis was made, but the presence of severe mutation conditioned an evolution with severe respiratory symptoms over time and a nutritional compromise attested at the time of assessment. From confirmation of diagnosis, children were periodically monitored with enzyme replacement therapy and antibacterial treatment for respiratory infections.

The most severe general state was found in the girl who is 8 years old. During last staying in the hospital she had purulent wet productive caugh, hardly expectorated, loss of appetite, fatigue, diffuse bilateral submatity, bilateral diminuated vesicular murmur on

the background of wet medium rales. Respiratory frequence – 45/min, pulse – 150beats/minute, SaO_2 – 43%, FVC - 42%, FEV_1 - 29%, IT - 63%, and the weight of 17,5 kg (p 0-3), height – 116 cm (p 0-3), BMI – 12,96

The boy with the same age also presents a severe form of the disease. He is hospitalised with wet caugh with purulent expectorations, dyspnoea at physical effort, perioral cyanosis, bilateral medio-basal submatity and bilateral medium bulous rales. Respiratory frequence – 28/min, pulse – 95/min, SaO_2 – 96%, FVC - 54%, FEV_1 - 51%, IT - 85%, mass during hospitalisation – 22kg (p 10-25) , height 125 cm (p 25-50), BMI – 14,1. The boy who is 14 years old has a controlled state of the disease, being hospitalised with productive wet purulent caugh, loss of appetite, fatigue, bilateral diminuated vesicular murmur in the inferior segments on the background of wet fine rales. Respiratory frequence – 24/min, pulse – 93/min, SaO2 – 95%, FVC - 78%, FEV_1 - 70%, IT - 77%, weight – 42kg (p 10-25), height – 160 cm (p 25-50), BMI – 16,4

CF is a complex disease with multisystemical involvement. Correlation between the genotype and the severity of disease varies in function of affected systems and organs. Phenotype expression in respiratory system varies a lot and there were found few correlations between the genotype fenotype and pulmonary fenotype. An exception is the fact that patients with pancreatic insufficiency have, generally, a pulmonary disease which is more severe than in patients with normal pancreatic function [3].

The main purpose of our study was to investigate if the mutations had an impact over pulmonary modifications in CF and if chronic colonisation with gram negative infection with *Pseudomonas aeruginosa* differs between patients (table 1).

***Table 1*. Infectious disease in children with CF at the last admissions for 2 years**

Girl, 8 years old - F508del/F508del	**Boy, 8 years old - F508del/W1282X**	**Boy, 14 years old - F508del/F508del**
Pseudomonas aeruginosa 10^5	*Pseudomonas aeruginosa* 10^5	*Kl. pneumoniae* 10^5
BNGN 10^6	*Staphylococcus aureus* 10^6	*Corynebacterium spp* 10^5
Flavobacillus spp 10^4	*Candida albicans* 10^3	*Staphylococcus aureus* 10^4
Staphylococcus aureus 10^6		
Candida albicans 10^3		
Candida glabrata 10^3		

In every child there was at least one mutation F508del. The same CFTR mutation in homosigous state F508del/F508del was found in the girl with the most severe form of CF and in the boy with controlled form of the disease (with rare hospitalisation). In the 8 years old boy who has a more severe variant of the disease was found the F508del/W1282X genotype.

There is presented the result of an international study Geborek A., et al. 2011 , where the authors find the role of CFTR genotype upon the ailing phenotype of the patients with CF (table 2, 3). This way heterozygous mutations F508del are more severe than homozygous ones [6]. Patients with 2 mutations of the first class had inferior pulmonary function (FEV_1 și FVC) than the ones who had a combination between mutations of the first and second class, or two mutations of the second class.

In conclusion, patients with CF who have 2 mutations of the first class risc to develop a more severe pulmonary disease than the patients who have at least one mutation of the second class [6].

The results of the patients involved in our study show us the fact that the genotype CFTR F508del/F508del leads to a severe pulmonary disease if associated with chronic colonizaton with *Pseudomonas aeruginosa*. With all these said, in a study based on Register of Cystic Fibrosis HuiChuan J. Lai et al. 2004 compared with the "homozygous Δ*F508*" group, "severe genotype other than homozygous Δ*F508*" was associated with a significantly lower risk of shortened survival, whereas the "mild genotype" group had lower risks of shortened survival and *P. aeruginosa* acquisition (table 4). No differences in the risk of having poor lung function were found among all genotype groups [7].

Table 2. **The different CFTR mutations according to functional class** [6]

Class I	Class II	Class III	Class IV	Class V
1717-1 G- > A	F508del	G551D	297 C- > A	2789 + 5 G- > A
3659delC	S945L	R560T	R117C	3849 + 10 kb C > T
394delTT			R347P	A455E
R553X			T 3381	3849 + 10 kb C-T
621 + 1 G- > T				
E60X				
G542X				
W79R				
W1282X				

Table 3. **The different CFTR mutations combinations of class I and class II mutations** [6]

Class I/class I		*Class I/class II*		*Class II/class II*	
1717-1 G- > A/1717-1 G->A	n = 1	3659delC/S945L	n = 1	F508del/F508del	n = 165
3659delC/3659delC	n = 5	3659delC/F508del	n = 23	F508del/S945L	n = 5
3659delC/394delTT	n = 7	394delTT/F508del	n = 38		
394delTT/394delTT	n = 4	621 + 1 G->T/F508del	n = 6		
R553X/E60X	n = 1	E60X/F508del	n = 4		
		G542X/F508del	n = 1		
		R553X/F508del	n = 2		
		W79R/F508del	n = 1		
		W1282X/F508del	n = 1		
		1717-1 G->A/F508del	n = 1		
Total	**18**		**78**		**170**

***Table 4.* Associations of baseline risk factors with survival and lung disease outcomes in US patients reported to the 1986–2000 Cystic Fibrosis Foundation Patient Registry** [7]

Baseline risk factor	Survival (*n* = 13,690)		FEV_1† <70% (*n* = 3,320)		*Ps. aeruginosa* acquisition (*n* = 5,290)	
	OR†	95% CI†	OR	95% CI	OR	95% CI
Diagnostic group (compared with SCREEN†)						
MI†	2.59**	1.28, 5.25	1.18	0.78, 1.80	1.18*	1.01, 1.36
FH†	2.21*	1.06, 4.61	0.99	0.57, 1.73	1.25*	1.01, 1.56
SYMPTOM†	2.63**	1.31, 5.29	1.15	0.77, 1.71	1.13	0.98, 1.30
Female sex	1.27***	1.14, 1.41	1.11	0.95, 1.28	1.10**	1.02, 1.17
Genotype (compared with Δ*F508*/Δ*F508*)						
Severe genotype other than Δ*F508*/Δ*F508*‡	0.76***	0.67, 0.86	0.88	0.74, 1.05	1.03	0.95, 1.11
Mild genotype§	0.51***	0.37, 0.70	1.16	0.55, 1.33	0.65*	0.42, 1.00

* $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$.
† FEV_1, forced expiratory volume in 1 second; OR, odds ratio; CI, confidence interval; SCREEN, group of patients without meconium ileus and identified via prenatal/neonatal screening; MI, group of patients with meconium ileus; FH, group of patients with a positive family history without symptoms; SYMPTOM, group of patients with symptoms other than meconium ileus.
‡ Includes Δ*F508*/non-Δ*F508*-I, II, III and non-Δ*F508*-I, II, III/non-Δ*F508*-I, II, III; refer to table 1 for specific mutations categorized to classes I–V.
§ Includes Δ*F508*/IV, V; non-Δ*F508*-I, II, III/IV, V; and IV, V/IV, V. See table 1 for specific mutations categorized to classes I–V.

Patients presented in our study who have CF have the advantage of a health care system, which works well, with the same treatment in the country. However, the difference regarding the adjustment for colonisation with *Pseudomonas aeruginosa* is significant. In addition to it, in the protocol of the study, all the patients were evaluated in the same clinical condition, during a exacerbation, which required hospitalization for medication, this way standartizing the investigation even more. According to the study chronic infection with *Pseudomonas aeruginosa* affects the evolution of the disease in presented children. It seems that socio-economical status of the pacients is less likely to affect the severity of the disease because of the medical and social assistance of the medical system, which fully supports the costs for the medication, as well as health care.

Homozygous patients for the F508del mutation have a wide variety of phenotypic variations [1]. Another posibility is the aspect related to the environment, such as chronic infection in combination with modifying genes or by itself, may contribute to progressive evolution with aggravation. According to Navarro H., 2002 study, pancreatic insufficiency and worse nutritional state were common for patients with F508del mutation. Respitarory implication was variable, both for heterozygous and homozygous patients. Another severe mutations, such as W128X and G542X, determined clinical manifestation similar to those found in F508del mutation [11].

Another study reveals that the average age of establishing the diagnosis of CF is 34 months, and the majority of the patients (73%) had pancreatic insufficiency during the evolution of the disease. The presence of exocrine pancreatic insufficiency was significant in the group of homozygous patients with F508del mutation, also the death index was high for the group of people with this CFTR mutation [4].

The character of expectorations in children with CF is related to the bacteriological spectre of the pulmonary infections : pyogenic germs determines production of abundant bronchial secretions, purulent ones, with fetid sputum in infectious exacerbation. It was determined thoracic deformation (emphysematous thorax, dorsal kyphosis) in 8 years old girl, which is a clinical expression of a severe broncho-pulmonary pathological process. In time progressive respiratory insufficiency develops, chronic severe persistent xypoxy determined the formation of digital hypocratism, wich is less observed in other two children.

Radiological pulmonary explorations (fig. 1, 2, 3) of the patients with CF show signs of bronchitis, broncho-obstructive syndrome, thoracic distension with emphysema, pulmonary hypertransparence expressed more in apical segments, segmentary opacities, regions of consolidation, alveolar opacities, bronchiectasis “in bouquet” (superior right lobe is frequently affected).

Separate description of latest radiological films of these children detects significantly different affection in them. A more severe affection is seen in girl's homozygous affection *F508del* (fig. 1) and boy's heterozygous mutation *F508del* (fig. 2), also having chronic infection with *P. aeruginosa*.

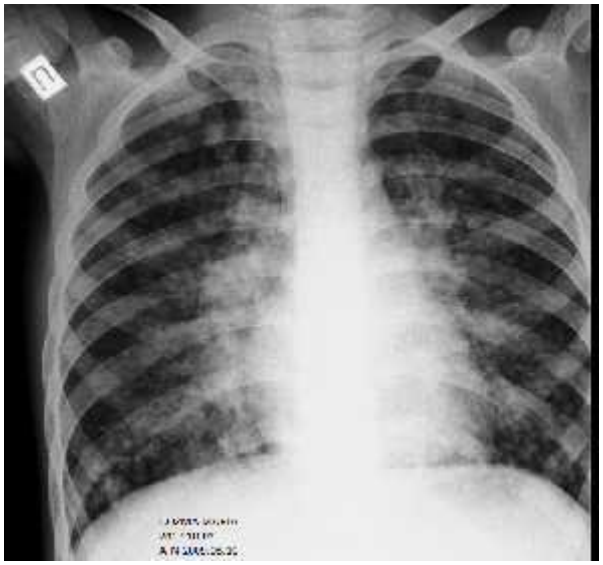

Fig.1 Thoracic Rx (2.10.2017) – aerated lungs. Homogenous hili, dilated on the background of enlarged lymph nodes. Pulmonary pattern presents cystic distensions of different dimensions. Mediobasal on the right – aeric bronchogram. CTI 0,42. Sinuses are clear.

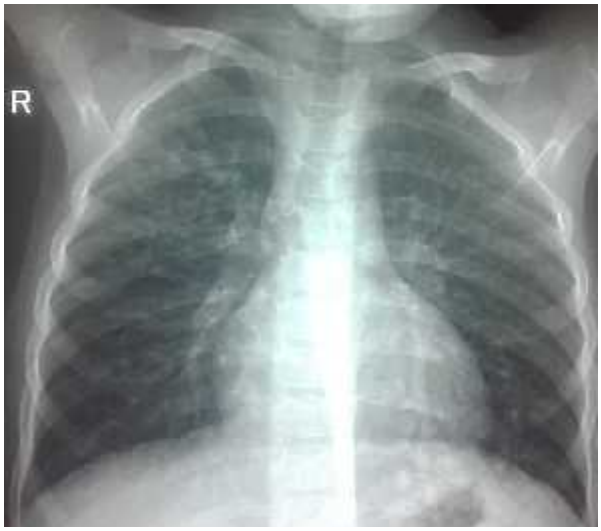

Fig. 2. Thoracic Rx (05-04-2017) – patchy pneumatised lungs. Fibrotic pulmonary pattern, distorted apically on the right and basal on the left. Enlarged homogenous hili. There are determined cystic heteromorphic deformations, heterogenous with erased contour, from 2.0-2.4 to 2.5-2.9 apically on the right S3 and basal on the left S5, S10. Mediobasal on the right and basal on the left – pneumonic infiltrations. CTI 0,44. Sinuses are clear.

There are seen irreversible bronchopulmonary modification in boy with homozygous mutation *F508del* (fig. 3), but modifications are less expressed and there is no chronic infection with *P.aeruginosa* which favors a better quality of life.

Fig. 3. Thoracic Rx (1.12.2017) – fibrotic, endured and deformed pulmonary pattern. Cylindric and varicous expansions of the bronchi, predominant in superior pulmonary areas. Thick-walled bronchi. Pulmonary hili are structured. Infiltrative and nodular opacities are not seen. "In drop" cord . CTI 0,42. Sinuses are clear

Computed tomography examination (CT) of the thoracic cavity (fig. 4, 5, 6) is a particular and highly informative method for assessment of bronchopulmonary changes in CF patients, which reveals signs of bronchopulmonary alterations in these children. Important CT findings are evident in a girl with a homozygous mutation in *F508del* (fig. 4). There are bilateral diffuse distributed bronchiectasis (cylindrical and varicose), of moderate grade (bronchi size increased 2-3 times compared to adjacent vessel), predominantly affecting upper lobes, associated with moderate peribronchial thickening. Intraluminal mucus deposits are detected bilateral in segmental and subsegmental bronchi. Centrilobular lung opacity of Y- or V-shaped structure represents mucus deposits in terminal bronchioles, which is suggestive for bronchiolitis. There are ground glass opacities with bilateral perihilar distribution. Multiple pleuropulmonary adhesions bilaterally distributed (predominantly in upper lobes). Diffuse accentuated vascular pattern. Moderate bilateral hilar

enlargement. Minimal bilateral pleural effusion. Multiple mediastinal lymph nodes (paracaval, subcarinal), ovoid and round in shape, homogeneous structure, well-defined contour, soft-tissue density (+35+40 Uh), without calcification.

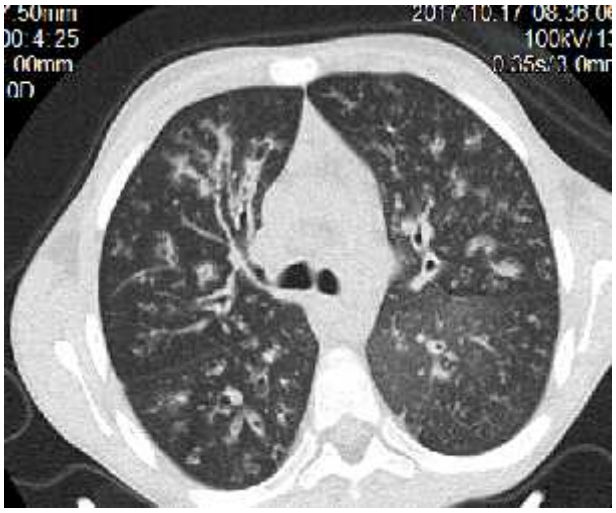

Fig. 4. *F508del/F508del*. CT of the thoracic cavity (17.10.17) bronchopulmonary changes with cylindrical and varicose bronchiectasis, bronchiolitis. Mediastinal lymphadenopathy, probably reactive. Minimal left pleural effusion.

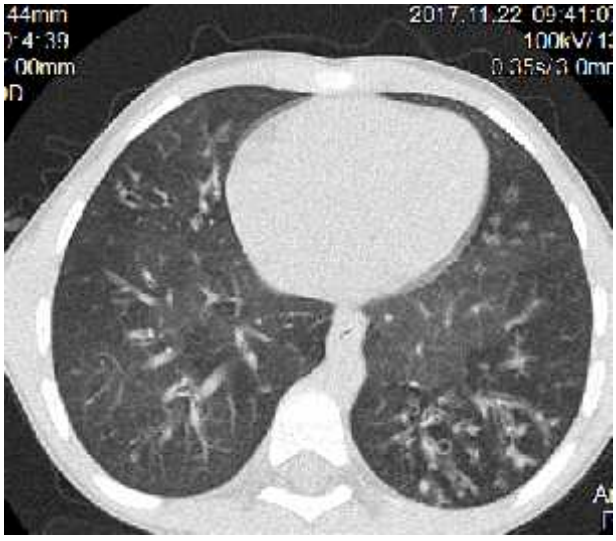

Fig. 5. *F508del/W1282X*. CT of the thoracic cavity (22.11.2017) cylindrical and varicose bronchiectasis in S_1, S_2, S_4, S_5, S_6 of the right lung and in S_5, S_9, S_{10} of the left lung. Minimal left pleural effusion..

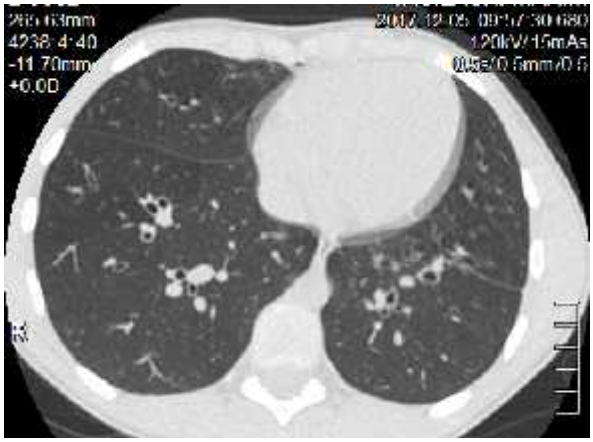

Fig. 6. *F508del/F508del.* CT of the thoracic cavity (14.01.2016) broncho-pulmonary changes in the context of moderate grade cystic fibrosis, in exacerbation. Bronchiolitis. Pulmonary infiltration with atelectatic component in the right lung S5

CT of the thoracic cavity (fig. 5) in a boy with a heterozygous mutation in F508del reveals following bronchopulmonary changes: bilateral bronchiectasis (cylindrical and varicose) distributed in S_1, S_2, S_4, S_5 and S_6 of the right lobe, and S_5, S_9, S_{10} of the left lung, of moderate grade (bronchi size increased 2-3 times compared to adjacent vessel). Moderate peribronchial thickening. Few pleuropulmonary adhesions with bilateral distributions. Minimal bilateral pleural effusion. Diffuse accentuated vascular pattern. Calcification of the arterial ligament. Moderate bilateral hilar enlargement. Irreversible bronchopulmonary changes, with less important CT findings (fig. 6) compared to the previously described patients, in a boy with a homozygous mutation in F508del are limited to bronchiectasis (predominantly cylindrical, several varicose) distributed diffuse in both lung fields, of moderate grade (bronchi size increased 2-3 times compared to adjacent vessel). Minimal peribronchial thickening (bronchial wall thickness is equivalent to the adjacent vessel diameter). Intraluminal mucus deposits are detected bilateral in segmental and subsegmental bronchi. Centrilobular lung opacity of Y- or V-shaped structure represent mucus deposits in terminal bronchioles, which is suggestive for bronchiolitis. Pulmonary infiltration with atelectatic component in the right lung S_5. Multiple pleuropulmonary adhesions distributed bilateral in apical and basal segments. Diffuse accentuated vascular pattern; moderate bilateral hilar enlargement.

A big potential in medication of patients with CF has the implementation of neonatal screening programme, and a double-check programme of diagnosis in screening children in precocious states of the disease for preventing pleuro-pulmonary complications, improvement of the prognostic and lifespan.

Conclusions

1. Pulmonary involvement in infectious episodes with aggresive germs in children of the same age with CFTR F508del/F508del and F508del/non-F508del genotypes determines bronchopulmonary chronicity. Progressive evolution of pathologic pulmonary process in these children was determined by such resistant germs as *P. aeruginosa*, *S.aureus*, which accelerates the destructive process of lung parenchyma and contributes to the extension of pulmonary fibrosis and formation of bronchiectasis

2. Patients with severe mutations of cystic fibrosis present a serious respiratory, pancreatic and nutritional involvement. The frequence and severity of clinical manifestations of bronchopulmonary system is correlated with F508del genotype.

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