

Sabina Więcek, Halina Woś, Bożena Kordys-Darmolińska, Magda Sankiewicz-Szkółka, Urszula Grzybowska-Chlebowczyk

DOES THE MUTATION OF THE SERPINA1 GENE CONTRIBUTE TO LIVER DAMAGE AND CHOLESTASIS IN PATIENTS WITH DIAGNOSED CYSTIC FIBROSIS? PRELIMINARY STUDY

CZY MUTACJA GENU *SERPINA1* MA WPŁYW NA WYSTĄPIENIE USZKODZENIA WĄTROBY I CHOLESTAZĘ U PACJENTÓW Z ROZPOZNANĄ MUKOWISCYDOZĄ? BADANIA WSTĘPNE

The Department of Paediatrics, Medical University of Silesia, Katowice, Poland

Abstract

Mutation of the SERPINA1 gene is present in about 2% of patients with cystic fibrosis but is more common and accounts for about 5% in patients with cystic fibrosis and co-existing liver lesions. The SERPINA1 gene is responsible for the synthesis of a serine protease inhibitor. The protein related with this gene is accumulated within the endoplasmic reticulum of hepatocytes causing their damage, inflammation and cirrhosis.

***The aim** was to assess the presumable effect of the SERPINA1 mutation gene in patients with diagnosed cystic fibrosis on damage to the liver and/or cholestasis.*

***Material and method:** The analysis included 30 children, 13 girls (43.3%) and 17 boys (56.6%), aged from 6 months to 18 years (the average age was 5.5 years) with diagnosed cystic fibrosis. All the patients have undergone a genetic test of the mutation of the SERPINA1 gene. The analysis included age, sex, clinical symptoms, type of mutation of the CFTR protein, abnormalities in laboratory tests (the activity of aminotransferases, GGTP, alkaline phosphatase , protein, the indicator of acid steatocrit, the rate of APRI) and abdominal ultrasonography.*

***Results:** Symptoms of damaged liver were concluded in 9/30 patients (30%) with diagnosed cystic fibrosis. Most commonly observed were increased activities of aminotransferases in 9/30 patients (30%) and of gamma glutamyl transferase in 6/30 (20%) of the assessed patients. In 4/30 patients the abdominal ultrasonography revealed an enlarged liver and increased echogenicity. Mutation within the SERPINA1 gene was observed only in 1/30 patients (3.3%) with diagnosed cystic fibrosis. As far as the patient is concerned, currently the activities of aminotransferases, GGTP and AF are normal, but there has been a considerable increase in the intensity of symptoms from the respiratory system. No co-relation between the mutation of the SERPINA1 gene and clinical symptoms, type of mutation of the CFTR protein, laboratory results of the functions and damage to the liver and the abdominal ultrasonography was observed.*

***Conclusions:** We did not find a more frequent occurrence of the SERPINA1 gene mutation in children with cystic fibrosis and coexisting features of damaged liver and cholestasis. The obtained results suggest the contribution of other than SERPINA1 gene mutation factors responsible for the development of changes in the liver in patients diagnosed with cystic fibrosis. The studies on the subject should be extended and performed on a larger group of patients.*

Key words: cystic fibrosis, damage to the liver, *SERPINA1 gene*

Streszczenie

Mutacja genu SERPINA1 występuje u około 2% pacjentów z mukowiscydozą, natomiast u około 5% pacjentów z mukowiscydozą i współistniejącymi zmianami wątrobowymi. Gen SERPINA1 odpowiedzialny jest

za syntezę inhibitora proteazy serynowej, a białko związane z tym genem jest gromadzone w obrębie retikulum endoplazmatycznego hepatocytów prowadząc do ich uszkodzenia, zapalenia i marskości.
***Celem pracy** było poszukiwanie wpływu mutacji genu SERPINA1 na wystąpienie uszkodzenia wątroby i/lub cholestazy u pacjentów z rozpoznaną mukowiscydozą.*
***Materiał i metoda:** Analizą objęto 30 dzieci, 13 dziewczynek (43,3%) oraz 17 chłopców (56,6%), w wieku od 6 miesięcy do 18 lat (średni wiek 5,5 lat) z rozpoznaną mukowiscydozą. U wszystkich pacjentów wykonano badanie genetyczne w kierunku mutacji genu SERPINA1. W analizie uwzględniono wiek, płeć, objawy kliniczne, rodzaj mutacji genu białka CFTR, nieprawidłowości w wynikach badania laboratoryjnych (aktywności aminotransferaz, GGTP, FA, białka, wskaźnik kwaśnego steatokrytu, współczynnik APRI) oraz badanie usg jamy brzusznej.*
***Wyniki:** Cechy uszkodzenia wątroby stwierdzono u 9/30 pacjentów (30%) z rozpoznaną mukowiscydozą. Najczęściej obserwowano podwyższone aktywności aminotransferaz – u 9/30 (30%), natomiast gammaglutamylotransferazy u 6/30 (20%) badanych pacjentów. U 4/30 pacjentów stwierdzono powiększoną wątrobę o podwyższonej echogeniczności w badaniu usg. Mutację w obrębie genu SERPINA1 wykazano jedynie u 1/30 (3,3%) pacjenta z rozpoznaną mukowiscydozą. U pacjentki tej aktualnie stwierdza się prawidłowe aktywności aminotransferaz, GGTP, FA, natomiast obserwuje się znaczne nasilenie objawów ze strony układu oddechowego. Nie wykazano korelacji pomiędzy występowaniem mutacji genu SERPINA1 a objawami klinicznymi, rodzajem mutacji białka CFTR, wynikami badań laboratoryjnych funkcji i uszkodzenia komórki wątrobowej oraz badaniem ultrasonograficznym jamy brzusznej.*
***Wnioski:** Nie wykazano częstszego występowania mutacji genu SERPINA1 u dzieci z rozpoznaną mukowiscydozą i współistniejącymi cechami uszkodzenia wątroby i cholestazy. Uzyskane dane sugerują udział innych, niż mutacje genu SERPINA1 czynników odpowiedzialnych za rozwój zmian obrębie wątroby u pacjentów z rozpoznaną mukowiscydozą. Wskazane byłoby przeprowadzenie badań na większej grupie pacjentów.*

Słowa kluczowe: mukowiscydoza, uszkodzenie wątroby, *gen SERPINA1*

DEV PERIOD MED. 2015;XIX,1:92-97

INTRODUCTION

Liver lesions in the course of cystic fibrosis are a group of complex interactions of the processes of fibrosis, inflammation, remodelling, apoptosis and cholestasis. The complexity of the processes happening in the liver and bile ducts in the course of the disease have not yet been satisfactorily explained [1, 2]. Despite the fact that they affect only 5-20% of patients with diagnosed cystic fibrosis, they increase the mortality rate, shorten the survival rate and worsen the quality of life [3]. In the etiopathogenesis of liver lesions in the course of cystic fibrosis, the role of pathological and physiological changes to bile acids, genetic and immunological factors is highlighted. Due to the hindered expression of the CFTR protein on the apical membranes of cholangiocytes within the epithelium of the bile ducts and the epithelium of the gall bladder, the composition of bile changes, its transport also becomes abnormal with the retention of toxic bile acids (mainly taurocholic acid), which induces chemokines responsible for inflammatory processes and fibrosis [4]. So far, a specific mutation relating solely to liver damage in the course of cystic fibrosis has not been discovered. Most often these are so called "serious mutations" of the CFTR gene (F508del, G524X, N1303K, dele2,3(21kb), 1811+1G> C) [5, 6]. However, the clinical course in patients with diagnosed cystic fibrosis and the same mutation of the CFTR gene is varied and there is no obvious phenotype-genotype correlation. Other mutations of, for example, the SERPINA1 gene, plasminogen activator inhibitor-1, metalloproteinase gene and glutathione s-transferase P1 may also play a role [7, 8, 9]. Mutation of the SERPINA1 gene is present in about 2% of patients with cystic fibrosis but it is more common, accounting for 5%, in patients with cystic fibrosis and concomitant lesions of the liver. It is responsible for the synthesis of serine protease inhibitor and the protein linked with Z allele is accumulated within the endoplasmic reticulum of hepatocytes, causing their damage, inflammation and cirrhosis. (Figure 1) In about 10% of allele Z homozygotes the accumulation of the protein of SERPINA1 leads to neonatal hepatitis and in 2-3% to fibrosis and cirrhosis. A high risk of developing portal hypertension in patients with present allele Z of the SERPINA1 gene has been proved. Moreover, its presence is linked to the risk of developing pulmonary emphysema and chronic obstructive pulmonary disease [10, 11, 12, 13, 14].

AIM

The aim was to assess the effect of the mutation of the SERPINA1 gene on damage to the liver and cholestasis in patients with diagnosed cystic fibrosis.

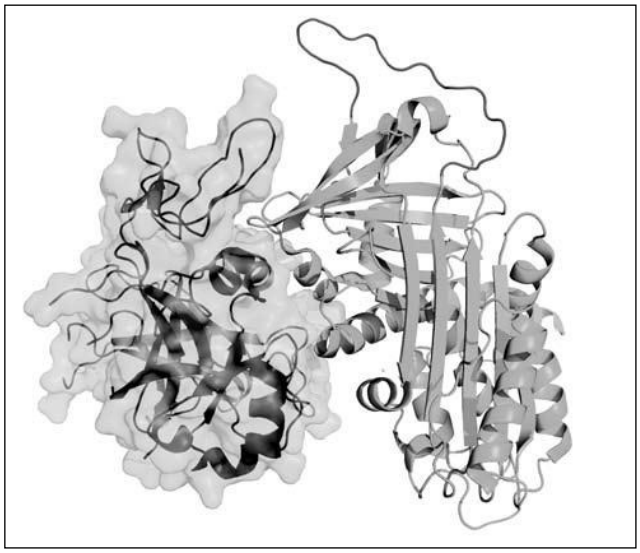

Fig. 1. The structure of the serpin protein Z-dependent inhibitor in complex with protein Z. (according to Wei) [15].

Ryc. 1. Schemat budowy inhibitora białka serpiny w połączeniu z białkiem Z. (według Wei) [15].

MATERIAL AND METHOD

30 children were included in the analysis:

- 13 girls (43,3%) and 17 boys (56,6%),
- Aged from 6 months to 18 years (average age – 5.5y-ears)
- with diagnosed cystic fibrosis selected from the screening programme (n=14) or based on the clinical picture (clinical symptoms, results of laboratory tests and sweat tests and the mutation of the *CFTR gene*).

The most common mutation of the CFTR protein in the patients analysed was the F508del/F508del mutation which concerned 16/30 children (53.1%). Other mutations were less frequent (Table I).

All the patients underwent a genetic test for the mutation of the *SERPINA1 gene* – identification of the mutation p.Glu288Val (allele S, E264V) and p.Glu366Lys (allele Z, E342K) in the *SERPINA1 gene*. The tests were conducted in the NZOZ GENOMED in Warsaw. The analysis included age, sex, clinical symptoms, type of mutation of the CFTR protein, abnormalities in laboratory tests (the activity of aminotransferases, gamma glutamyl transferase, alkaline phosphatase, the indicator of acid steatocrit and the rate of APRI) and ultrasonography of the liver and bile ducts.

The authors gained the *consent of the Bioethics Committee* at the Medical University of Silesia in Katowice.

RESULTS

The age of the patients analysed ranged from 6 months to 18 years. The largest group was formed by the youngest patients, up to 6 years old (70%) with 12/30 (40%) being infants. No correlation between the age and the degree of manifestations of lesions to the liver and bile ducts in patients with diagnosed cystic fibrosis was concluded. The clinical picture was dominated by the symptoms of exocrine pancreatic insufficiency, symptoms from the respiratory tract and insufficient body weight. The results in the Swachmann-Kulczycki scale were from 30 to 100 points, an average of 78.6 points, the lowest was in the oldest patients (Table II).

The characteristics of damaged liver were noticed in 9 out of 30 patients (30%) with diagnosed cystic fibrosis. Elevated levels of the activity of aminotransferases (mainly AlAT) were observed in 9 out of 30 (30%) patients with cystic fibrosis and of gamma glutamyl transpeptidase in 6 out of 30 patients (20%). In 4 out of 30 patients with CF, ultrasonography revealed an enlarged liver of elevated echogenicity. The APRI rate (AspAT/number of platelets rate) ranged from 0.05 to 0.9 (average of 0.32). The AAR rate (AspAt/AlaT) was from 0.48-1.3 (the average of 0.8) (Table III).

Mutations within the *SERPINA1 gene* were observed in one out of 30 patients (3.3%) with diagnosed cystic fibrosis. Currently the patient is 7 years old and the values of the activity of aminotransferases, gamma glutamyl transpeptidase and alkaline phosphatase were reported as normal, however, a significant intensification

Table I. Analysis of the mutation of the *CFTR gene* in the patients analysed. The analysis was conducted by NZOZ GENOMED and MEDGEN.

Tabela I. Analiza mutacji genu CFTR u analizowanych pacjentów. Badania wykonane NZOZ GENOMED oraz MEDGEN.

Number of patients ***Liczba pacjentów***	**Type of mutation of the CFTR gene** ***Rodzaj mutacji genu CFTR***		
	F508del/F508del ***F508del/F508del***	**F508del/other** ***F508del/inne***	**Other** ***Inne***
	16/30 (53.3%)	10/30 (33.3%)	4/30 (13.3%)
Mutation of *SERPINA1 gene* *Mutacja genu SERPINA1*	1/30 (3.3%)	0/0 (0%)	0.0 (0%)

Table II. Clinical picture of the analysed group of patients with diagnosed cystic fibrosis.
Tabela II. Obraz kliniczny analizowanej grupy pacjentów z rozpoznaną mukowiscydozą.

Clinical picture ***Obraz kliniczny***	**Number of patients** ***Liczba pacjentów***	**Percentage of patients** ***Odsetek pacjentów***
Pancreatic insufficiency *Niewydolność trzustki*	30/30	100%
Insufficient body mass *Niedobór masy ciała*	6/30	20%
Symptoms from the respiratory tract *Objawy ze strony dróg oddechowych*	20/30	66.6%
History of meconium ileus *Niedrożność smółkowa w wywiadzie*	1/30	3.3%
Electrolyte abnormalities (hyponatremia, hypokaliemia) *Zaburzenia elektrolitowe (hyponatremia, hypokaliemia)*	3/30	10%
The Schwachman-Kulczycki score (average) *Skala Schwachmana-Kulczyckiego (średnie)*	78,6 points	

Table III. Assessment of the liver function in the patients analysed.
Tabela III. Ocena funkcja wątroby u analizowanych pacjentów.

Parameter ***Parametr***	**Number of patients** ***Liczba pacjentów***	**Percentage of analysed patients** ***Odsetek analizowanych pacjentów***
Increased activity of aminotransferases *Podwyższona aktywność aminotransferaz*	9/30	30%
Increased activity of GGTP *Podwyższona aktywność GGTP*	6/30	20%
Elevated concentration of bile acids *Podwyższone stężenie kwasów żółćiowych*	8/30	26.7%
Abnormal abdominal ultrasonography *Nieprawidłowości w usg jamy brzusznej*		
– fatty liver *stłuszczenie watroby*	5/30	16.7%
– enlarged liver *powiększenie wątroby*	4/30	13.3%
– portal hypertension *nadciśnienie wrotne*	1/30	3.3%
– cirrhosis of the *liver marskość* wątroby	1/30	3.3%

of the symptoms from the respiratory tract have been observed.

In patients with elevated parameters of damaged liver cells, cholestasis and/or abnormal ultrasonography of the abdomen the mutation of *SERPINA1 gene* was not observed.

No correlation between the presence of the *SERPINA1 gene* mutation and clinical symptoms, the type of mutation of the CFTR protein, the results of the laboratory tests of the function and damage to the liver and abdominal ultrasonography was concluded.

OVERVIEW AND DISCUSSION

In the majority of cystic fibrosis patients, the course of liver-related complications in the initial stage of the disease is symptomless. The first symptom is usually incidentally diagnosed hepatomegaly and/or hypertransaminasemia. In infants, liver fattiness may be detected incidentally during a routine abdominal ultrasonography. The most common lesions within the liver and bile ducts in patients with cystic fibrosis are focal cirrhosis of the liver, focal biliary cirrhosis, fattiness of the liver and portal hypertension [16, 17].

The factors contributing to the development of liver diseases in cystic fibrosis are as follows:
– male gender – in women oestrogens probably serve as a protector,
– coexisting meconium ileus,
– significant malnutrition,
– pancreatic insufficiency,
– „heavy" genotype (especially F508del) [18, 19, 20].

As far as our patients are concerned, boys accounted for 56.6% (17/30) of the total of the children analysed and the symptoms of damaged liver and cholestasis were equally observed in both sexes. The history of meconium ileus was reported in one out of 30 children. Damage to the liver in that child manifested itself by elevated activity of aminotransferases and GGTP. The abdominal ultrasonography revealed characteristics of a fatty liver. An additional factor in that boy which contributed to the occurrence of lesions to the liver was chronic parenteral nutrition, insufficient body weight and F508del/F508del mutation, while no mutation of the *SERPINA1 gene* was observed. Wilschanski and Siano did not conclude a correlation between lesions to the liver and lesions to the lungs, respiratory failure and the level of malnutrition [21, 22]. Our observations would confirm that. Rowland observed a statistically more frequent occurrence of lesions to the liver in children with cystic fibrosis and concomitant diabetes [2]. Diabetes is a statistically important factor in the development of lesions to the liver. None of the patients with cystic fibrosis have yet been diagnosed with diabetes, despite the fact that in patients over the age of 10 this diagnosis is the most frequent. According to Rowland, the number of days of hospitalization, the amount of medications administered orally and intravenously was comparable in the subgroups of cystic fibrosis patients with and without lesions to the liver. No differences were reported with relation to the frequency of liver damage in cystic fibrosis depending on ethnicity [2].

Aldamiz-Echevarria proved that children diagnosed with cystic fibrosis and concomitant liver damage weigh less, are shorter, have smaller arm circumference and lower BMI. Patients with cystic fibrosis have a significantly lower level of the following acids: linoleic (LA), docosahexaenoic (DHA) and docosapentaenoic (DPA). Additionally, he reported high levels of long-chain fatty acids (arachidic acid) and low levels of omega-6 acids. The more ravaged the patient's body, the lower the concentration of adiponectin [23]. As regards our patients, insufficient body mass was observed in 20% and it did not correlate with the features of damaged liver cell and with cholestasis. The connection between the duration of parenteral nutrition in patients with cystic fibrosis with concomitant meconium ileus and the characteristics of damaged liver and/or its cirrhosis is known. Furthermore, it has been proven that antioxidants play a significant role in protecting hepatocytes from oxidative stress, which through free radicals contributes to revealing lesions to the liver in cystic fibrosis [16, 17].

Recurrent bacterial, viral and/or fungal infections of the respiratory tract and right ventricular heart insufficiency during respiratory failure also play a role in revealing lesions to the liver. If we consider the above factors to indirectly indicate the existence of liver diseases, why is the maximum level of detecting liver diseases in children with CF between 10 and 20 years of age and it does not increase later? The symptoms of pulmonary insufficiency and right ventricle heart insufficiency intensify with age, and so does the number of infections and administered medications [5]. In our patients the elevated levels of activity of aminotransferases and gamma glutamyl transpeptidase occurred most frequently in the first year of life.

The change of the profile of bile acids to hydrophobic is related to the stimulation of enterohepatic circulation. Bile secretion of the fraction of bilirubin conjugated with monoglucuronides, bile salts, phospholipids and cholesterol are significantly higher in patients with cystic fibrosis. Liver fibrosis is a result of haemodynamic disorders of the balance between fibrogenesis and fibrolysis of the constituents of connective tissue which lead to excessive and modified accumulation of the elements of the extracellular matrix (ECM) in the organ. Mutation within the *SERPINA1 gene* may also have an impact on those processes. Stellate cells of the liver are the main source of fibrotic tissues – collagen I, III, IV, glycoproteins (laminin, elastine, hyaluronate, vesicant). It has not been satisfactorily explained why that balance becomes lost in the course of cystic fibrosis [24, 25, 26, 27].

Among our patients, mutation of the *SERPINA1 gene* was observed in only 1 child (3.3%). As far as the patient is concerned , currently the activities of aminotransferases, gamma glutamyl transpeptidase are normal and so is the concentration of bile acids in the blood serum. Also, abdominal ultrasonography did not reveal any lesions. But, there has been an increase in the intensity of symptoms from the respiratory tract.

Patients with cystic fibrosis should undergo regular laboratory tests (the activity of aminotransferases, gamma glutamyl transpeptidase, the concentration of bilirubin and bile acids, and the APRI rate). It should also be remembered that an increase in 2 parameters of the liver above the norm within 3 months indicates progressive lesions to the liver [16, 19]. However, laboratory tests have low sensitivity. Most patients with multifocal liver cirrhosis have normal results of the laboratory tests. Isolated increase in the activity of aminotransferases with normal activities of gamma glutamyl transpeptidase may suggest fatty liver. In our patients, elevated activities of aminotransferases in blood serum were observed in 9 out of 30 patients (30%) and of glutamyl amino transpeptidase in 6 out of 30 (20%). In none of the patients was mutation within the *SERPINA1 gene* observed. Relevant European recommendations suggest performing Doppler abdominal ultrasonography every 12 months (changes to the echogenicity of the liver as factors in the assessment of the organ's fattiness, symptoms of portal hypertension, cirrhotic transformation of the liver). The examination is inexpensive and non-invasive. However, a normal picture of the liver does not exclude an ongoing process of fibrosis. As far as our patients are concerned, in 5 out of 30 of them (16.7%) their ultrasonography revealed characteristics of fatty liver and in 4 out of 30 (13.3%) the liver was enlarged. Liver cirrhosis with the symptoms of portal hypertension was observed in one female patient. Despite the advancement of the lesions to the liver, no mutation of the *SERPINA1 gene* was concluded.

Further studies on the causes of lesions to the liver in the course of cystic fibrosis seem of paramount importance, which may contribute to a decrease in the mortality rate, extending the survival rate and an improvement in the patients' quality of life.

CONCLUSIONS

We did not find more often occurrence of the *SERPINA1 gene* mutation in children with cystic fibrosis and coexisting features of damaged liver and cholestasis. The results obtained suggest the contribution of other than *SERPINA1 gene* mutations factors responsible for the development of changes in the liver in patients diagnosed with cystic fibrosis. The studies on the subject should be extended and performed on a larger group of patients.

REFERENCES

1. Rudnick D. Cystic fibrosis – associated Liver Disease: when will the future be now? J Pediatr Gastroenterol Nutr. 2012;3:312--317.
2. Rowland M, Gallagher C, O`Laoide R *et al.* Outcome in cystic fibrosis liver disease. Am J Gastroenterol. 2011;106:104--109.
3. Lamireau T, Monnerau S, Martin S *et al.* Epidemiology of liver disease in cystic fibrosis: a longitudinal study. J Hepatol. 2004;41:920-925.
4. Freudenberg F, Broderick A, Yu B *et al.* Pathophysiological basis of liver disease in cystic fibrosis employing a DeltaF508 mouse model. Am J Gastrointest Liver Physiol. 2008;294:1411--1420.
5. Guilbault C, Saeed Z, Downey G *et al.* Cystic fibrosis mouse model. Am J Respir Cell Mol Biol. 2007;36:1-7.
6. Herrmann U, Dockter G, Lammert F. Cystic fibrosis-associated liver disease. Best Practise& Research. Clin Gastroenterol. 2010;24:585-592.
7. Ala A, Schilsky M. Genetic modifiers of liver injury in hereditary liver disease. Semin. Liver Dis. 2011;31(2):208-214.
8. Barker S, Bale S, Booker J, Buller A *et al.* Development and characterization of reference materials for *MTHFR, SERPINA1, RET, BRCA1, and BRCA2* genetic testing. J Mol Diag. 2009;11(6):553-561.
9. Bartlett J, Friedmann K, Ling S *et al.* Genetic modifiers of liver disease in cystic fibrosis. JAMA 2009;9:1076-1083.
10. Abbound R, Nelson T, Mattman A. Alfa1-antitrypsyn deficiency: a clinical-genetic overview. Appl Clin Genet. 2011;4:55-65.
11. Cooley J, Sontaq M, Accurso F, Remold O`Donnell. SerpinB1 in cystic fibrosis airway fluids: quantity, molecular form and mechanism of elastase inhibition. Eur Res J. 2011;37(5):1083-1090.
12. Courtney J, Plant B, Morqan K, Rendall J. Association of improved pulmonary phenotype in Irish cystic fibrosis patients with a 3' enhancer polymorphism in alpha-1-antitrypsin. Pediatr Pulmonol. 2006;41(6):584-591.
13. Fisher H, Ortiz-Pallardo M, Ko Y, Esch C. Chronic liver disease in heterozygous alfa1-antitrypsin deficiency P i Z. J Hepatol. 2000;33(6):883-892.
14. Ramos M, Trujillano D, Olivar R, Sotillo F *et al.* Extensive sequence analysis of CFTR, SCNN1A, SCNN1A, SCNN1B, SCNN1G and SERPINA1 suggests an oligenic basis for cystic fibrosis-like phenotypes. Clin Genet. 2014;86(1):91-95.
15. Wei Z, Yan Y, Carrell R, Zhou A. Crystal structure of protein Z–dependent inhibitor complex shows how protein Z functions as a cofactor in the membrane inhibition of factor X. Blood 2009;114(17):3662-3667.
16. Colombo C, Battezzati P, Crosignani A *et al.* Liver disease in cystic fibrosis: a prospective study on incidence, risk factors and outcome. Hepatology. 2002;36:1374-1382.
17. Colombo C, Russo M, Zazzeron L *et al.* Liver disease in cystic fibrosis. J Pediatr Gastroenterol Nutr. 2006;43:49-55.
18. De Haan W. A marker associated with increased risk for severe liver disease in cystic fibrosis. Clin Genet. 2010;77:434-437.
19. Debray D, Kelly D, Houwen R *et al.* Best practice guidance for the diagnosis and management of cystic fibrosis- associated liver disease. J Cyst Fibros. 2011; 10 suppl. 2:529-536.
20. Kearns G. Hepatic drug metabolism in cystic fibrosis: recent developments and future directions. Ann Pharmacother. 1993;27:74-79.
21. Wilschanski M, Rivlin J, Cohen S *et al.* Clinical and genetic risk factors for cystic fibrosis – related liver disease. Pediatrics. 1999;103:52-57.
22. Siano M, De Gregorio F, Boggia B *et al.* Ursodeoxycholic acid treatment in patients with cystic fibrosis at risk for liver disease. Dig Liver Dis. 2010;42:428-431.
23. Aldamiz-Echevarria L, Prieto J, Andrade F *et al.* Persistence of essential fatty acid deficiency in cystic fibrosis despite nutritional therapy. Pediatr Res. 2009;66:585-589.
24. Pereira T, Lewindon P, Greer R *et al.* Transcriptional basis for hepatic fibrosis in cystic fibrosis- associated liver disease. J Pediatr Gastroenterol Nutr. 2012;3:328-335.
25. Kok K, te Morsche R, van Oijen M, Drenth J. Prevalence of genetic polymorphisms in the promoter region of the alpha-1 antitrypsin (SERPINA1) gene in chronic liver disease: a case control study. BMC Gastroenterol. 2010 Feb 20;10:22. doi: 10.1186/1471-230X-10-22.
26. Saunders D, Tindall E, Shearer R *et al.* A novel *SERPINA1* mutation causing serum alfa(1) – antitripsin deficiency. PLoS One 2012;7(12):e51762. doi: 10.1371
27. Topic A, Alempijevic T, Kovacevic N. Alfa 1-antitrypsin phenotypes in adult liver disease patients. J Med Sci. 2009;114(4):228-234.

Conflicts of interest/Konflikt interesu
The Authors declare no conflict of interest.
Autorzy pracy nie zgłaszają konfliktu interesów.

Received/Nadesłano: 29.09.2014 r.
Accepted/Zaakceptowano: 30.12.2014 r.

Published online/Dostępne online

Address for correspondence:
Sabina Więcek
Department of Paediatrics, Medical University of Silesia
16 Medykow Street, 40-752 Katowice, Poland
e-mail: sabinawk@wp.pl