https://doi.org/10.22416/1382-4376-2025-35-2-95-104
UDC 616.36-006.6-07:577.21

The Risk of Developing Liver Fibrosis is Associated with Polymorphism and Rare Haplotypes of the *TGFB1* Gene in Children with End Stage Liver Disease

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Aim: to determine the distribution of the three most significant single nucleotide polymorphisms (SNPs) of the *TGFB1* gene (rs1800469, rs1800470, rs1800471) and their haplotypes in children with liver fibrosis.
Materials and methods. The study included 107 pediatric liver recipients (45 boys, 62 girls) aged from 3 to 73 months (median — 8 months). The control group consisted of 199 healthy individuals (78 men, 121 women) aged 32.7 ± 9.6 years. During histological examination of the liver removed before transplantation, fibrosis of different severity grades was diagnosed in all children in accordance with the criteria of the METAVIR scale: 5 cases — Grade F1, 9 cases — Grade F2, 14 cases — Grade F3 and 79 cases — Grade F4. The indication for liver transplantation was end-stage liver disease: biliary atresia ($n = 61$) and hypoplasia ($n = 8$), Alagille syndrome ($n = 8$), Caroli disease ($n = 8$), Byler disease ($n = 6$) and other liver diseases ($n = 16$). SNPs were determined by real-time polymerase chain reaction using TaqMan probes in genomic DNA, isolated from peripheral blood.
Results. In children with liver fibrosis of different severity grades, the frequency distribution of the studied *TGFB1* gene SNPs was: for rs1800469 — 38 % GG homozygotes, 42 % AG heterozygotes and 20 % AA homozygotes; for rs1800470 — 50 % AA, 29 % AG, 21 % GG; for rs1800471 — 93 % CC, 7 % GC, 0 % GG. The distribution of SNPs rs1800469 and rs1800471 corresponded to the Hardy — Weinberg equilibrium and did not differ from that in healthy individuals. The distribution of rs1800470 in children with fibrosis, in contrast to healthy controls, did not correspond to the Hardy — Weinberg law ($p = 0.00026$). For the studied SNPs, linkage disequilibrium was shown; five main combinations were observed: three haplotypes, including two most common ones, were distributed, in total, in about 55 % of children with fibrosis and 91 % of healthy individuals; these frequencies were not statistically different in the group of patients and healthy individuals. Significant differences were detected in the distribution of two rarer haplotypes — A-A-C and G-G-C (respectively rs1800469, rs1800470, rs1800471), which were observed more often in patients with liver fibrosis than in healthy individuals: respectively, in 6.03 (95% CI: 3.06–11.89; $p < 0.0001$) and 3.71 (95% CI: 1.94–7.08; $p = 0.0001$) times.
Conclusions. In children with liver fibrosis, the distribution of single nucleotide polymorphism rs1800470 and two rare haplotypes rs1800469, rs1800470, rs1800471 of the TGFB1 gene differs significantly from that in healthy individuals. Polymorphism of rs1800470, as well as haplotypes A-A-C or G-G-C at positions rs1800469, rs1800470, rs1800471, may predispose to the development of liver fibrosis in children with liver failure.
Keywords: congenital and hereditary liver diseases, biliary atresia and hypoplasia, pediatric liver recipients, rs1800469, rs1800470, rs1800471
Conflict of interest: the authors declare no conflict of interest.

For citation: Kurabekova R.M., Gichkun O.Ye., Tsiroulnikova O.M., Pashkova I.Ye., Shevchenko O.P., Gautier S.V. The Risk of Developing Liver Fibrosis is Associated with Polymorphism and Rare Haplotypes of the *TGFB1* Gene in Children with End Stage Liver Disease. Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2025;35(2):95–104. https://doi.org/10.22416/1382-4376-2025-35-2-95-104

Риск развития фиброза печени ассоциирован с полиморфизмом и редкими гаплотипами гена *TGFB1* у детей с печеночной недостаточностью

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Цель исследования: определить распределение трех наиболее значимых однонуклеотидных полиморфизмов гена *TGFB1* (rs1800469, rs1800470, rs1800471) и их гаплотипов у детей с фиброзом печени.
Материалы и методы. В исследование включено 107 детей-реципиентов печени (45 мальчиков, 62 девочки) в возрасте от 3 до 73 мес. (медиана — 8 мес.). Контрольная группа состояла из 199 здоровых лиц (78 мужчин, 121 женщина) в возрасте 32,7 ± 9,6 года. При гистологическом исследовании удаленной перед трансплантацией печени у всех детей был диагностирован фиброз различной степени тяжести в соответствии с критериями шкалы METAVIR: 5 случаев — степень F1, 9 случаев — степень F2, 14 случаев — степень F3 и 79 случаев — степень F4. Показанием к трансплантации была терминальная стадия болезней печени в исходе: атрезии (n = 61) и гипоплазии желчевыводящих путей (n = 8), синдрома Алажилля (n = 8), болезни Кароли (n = 8), болезни Байлера (n = 6) и других заболеваний печени (n = 16). Указанные однонуклеотидные полиморфизмы определяли в выделенной из периферической крови геномной ДНК методом полимеразной цепной реакции в режиме реального времени с помощью зондов TaqMan.
Результаты. У детей с фиброзом печени различной степени тяжести распределение частот изученных однонуклеотидных полиморфизмов гена *TGFB1* составляло: для rs1800469 — 38 % гомозигот GG, 42 % гетерозигот AG и 20 % гомозигот AA; для rs1800470 — 50 % AA, 29 % AG, 21 % GG; для rs1800471 — 93 % CC, 7 % GC, 0 % GG. Встречаемость однонуклеотидных полиморфизмов rs1800469 и rs1800471 соответствовала равновесию Харди — Вайнберга и не отличалась от таковой у здоровых лиц. Распределение rs1800470 у детей с фиброзом, в отличие от здоровых лиц, не соответствовало закону Харди — Вайнберга (p = 0,00026). Анализ показал сцепленное наследование изученных однонуклеотидных полиморфизмов. Наблюдалось пять основных сочетаний: три гаплотипа, в том числе два наиболее частых, суммарно имели около 55 % детей с фиброзом и 91 % здоровых лиц — эти показатели статистически не различались в группе пациентов и здоровых лиц. Достоверные различия выявлены в распределении двух более редких гаплотипов — A-A-C и G-G-C (соответственно rs1800469, rs1800470, rs1800471), которые у пациентов с фиброзом печени наблюдались чаще чем у здоровых лиц: соответственно, в 6,03 (95% ДИ: 3,06–11,89; $p < 0,0001$) и 3,71 (95% ДИ: 1,94–7,08; p = 0,0001) раза.
Выводы. У детей с фиброзом печени распределение однонуклеотидного полиморфизма rs1800470 и двух редких гаплотипов rs1800469, rs1800470, rs1800471 гена *TGFB1* значительно отличается от такового у здоровых лиц. Полиморфизм rs1800470, а также гаплотипы A-A-C или G-G-C в положении rs1800469, rs1800470, rs1800471 могут предрасполагать к развитию фиброза печени у детей с печеночной недостаточностью.
Ключевые слова: врожденные и наследственные болезни печени, атрезия и гипоплазия желчевыводящих путей, дети — реципиенты печени, rs1800469, rs1800470, rs1800471
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования: Курабекова Р.М., Гичкун О.Е., Цирульникова О.М., Пашкова И.Е., Шевченко О.П., Готье С.В. Риск развития фиброза печени ассоциирован с полиморфизмом и редкими гаплотипами гена *TGFB1* у детей с печеночной недостаточностью. Российский журнал гастроэнтерологии, гепатологии, колопроктологии. 2025;35(2):95–104. https://doi.org/10.22416/1382-4376-2025-35-2-95-104

Introduction

Cirrhosis, the end-stage liver fibrosis, is the cause of over one million deaths worldwide each year [1]. Fibrosis develops in response to the damaging effects of traumatic, toxic or infectious agents, leading to excessive secretion and deposition of extracellular matrix, resulting in partial or complete impairment of tissue function. The regulation of liver fibrosis involves various liver cells, B and T lymphocytes, proinflammatory and profibrogenic cytokines, one of which is transforming growth factor-β1 (TGF-β1) [2, 3].

The content of TGF-β1 in the blood and tissues in liver fibrosis may depend on many factors, including genetic predisposition. The role of various variants of the *TGFB1* gene in the development of liver fibrosis is not fully understood, and in children with liver failure it has not been practically studied [4–6].

The *TGFB1* gene is characterized by significant single nucleotide polymorphism (SNP), which may play a certain role in the development of various pathologies [5, 7]. The most significant are considered to be three SNPs: rs1800469, rs1800470 and rs1800471. Polymorphism rs1800469 — C(–509)T, replacement of cytosine with thymine in the promoter region leads to a change in binding to transcription factors; rs1800470 — T(+869)C, substitution of thymine for cytosine in codon 10 leads to substitution of leucine for proline in the protein molecule; rs1800471 — C(+915)G, substitution of cytosine for guanine in codon 25 leads to substitution of arginine for proline in the protein product. These SNPs can lead to different levels of expression and activity of the TGF-β1 protein in tissues [8, 9].

In our previous work, it was shown that in children with liver failure, the distribution of haplotypes of the three specified SNPs of the *TGFB1* gene differs significantly from that in healthy individuals [10]. The etiology of liver diseases in the studied patients included both congenital cholestatic or metabolic diseases and acquired cirrhosis and various hepatitis. The variety of causes of liver failure

in the studied group did not allow us to assess the contribution of *TGFB1* genetic polymorphism to the pathogenesis of liver diseases.

The aim of this study: to determine the distribution of the three most significant single nucleotide polymorphisms of the *TGFB1* gene (rs1800469, rs1800470, rs1800471) and their haplotypes in children with liver failure with morphologically confirmed liver fibrosis.

Understanding the role of *TGFB1* genetic polymorphism in the development of tissue fibrosis can be of both scientific and practical importance. The obtained data can be used to create diagnostic panels to assess the risk of developing fibrosis and to search for new targets for fibrosis therapy.

Materials and methods

The study included 107 pediatric liver recipients (45 boys, 62 girls) aged 3 to 73 months (median — 8 months). The control group consisted of 199 healthy individuals (78 men, 121 women) aged 32.7 ± 9.6 years.

The stage of liver fibrosis was determined in a morphological study, including a macroscopic description and histological analysis of samples of the liver removed before transplantation, in accordance with the METAVIR scale criteria: stage F0 — no fibrosis; stage F1 — stellate dilation of the portal tracts without septa; stage F2 — dilation of the portal tracts with single portoportal septa; stage F3 — multiple portocentral septa without cirrhosis; stage F4 — cirrhosis. All children were diagnosed with fibrosis of varying severity: 5 cases — F1, 9 cases — F2, 14 cases — F3 and 79 cases — F4.

The indication for transplantation was the terminal stage of liver diseases in the outcome: biliary atresia and hypoplasia, Alagille syndrome, Caroli disease, Byler disease and other rare liver diseases, including Crigler — Najjar syndrome, Gierke syndrome, alpha 1 antitrypsin deficiency, tyrosinemia, fulminant and autoimmune hepatitis, cryptogenic cirrhosis. The demographic and clinical characteristics of the pediatric liver recipients included in the study are presented in Table 1.

The children included in the study underwent transplantation of a liver fragment from a living related donor. The recipients received 2- or 3-component immunosuppressive therapy, which included tacrolimus, corticosteroids and mycophenolates. Routine examination and treatment of patients was carried out in accordance with the clinical recommendations of the Russian Transplant Society and the protocols of the V.I. Shumakov Center for Transplantology and Artificial Organs.

Genomic DNA was isolated from peripheral blood using QIAamp DNA Blood Mini Kit by QIAcube™ automated analyzer (Qiagen, Germany) according to the manufacturers' protocols. Polymorphic variants rs1800469, rs1800470, rs1800471 of the *TGFB1* gene were tested by real-time polymerase chain reaction using TaqMan probes (Applied Biosystems, USA) by CFX96™ amplifier (Bio-Rad, USA) in accordance with the manufacturer's instructions.

Statistical calculations were performed using Microsoft Excel (Microsoft Corp., USA). Analysis of the genotype distribution frequencies of the studied SNPs, their haplotypes, and assessment of linkage disequilibrium (LD) were performed using the SNPstats program [11]. To confirm the independent distribution of the alleles of the studied polymorphisms, their compliance with the Hardy — Weinberg law was assessed. To compare the frequencies of genotypes or individual alleles in different groups, the Pearson χ^2 criterion was used. To quantitatively represent the strength of the influence of a possible genotype on a trait, odds ratios (OR) and their 95 % confidence intervals (95 % CI) were calculated. To assess linkage disequilibrium, D statistics and the correlation coefficient r were calculated. The critical value of the significance level was taken to be 0.05.

The protocol of this study was approved by the local ethics committee of the V.I. Shumakov Center for Transplantology and Artificial Organs. To participate in the study, patients or their guardians signed

Table 1. Characteristics of patients included in the study
Таблица 1. Характеристика пациентов, включенных в исследование

Parameter / *Параметр*	Value / *Значение*
Number of patients, *n* / *Количество пациентов, n*	107
Age, months, *Me* (Q_{25}; Q_{75}) / *Возраст, мес., Me* (Q_{25}; Q_{75})	8 (3–73)
Gender, male/female, *n* (%) / *Пол, мужской/женский, n (%)*	45 (42 %) / 62 (58 %)
Pathologies, *n* (%) / *Заболевания, n (%)*	
Biliary atresia / *Атрезия желчевыводящих путей*	61 (57 %)
Hypoplasia of the biliary tract / *Гипоплазия желчевыводящих путей*	8 (7.5 %)
Caroli syndrome / *Синдром Кароли*	8 (7.5 %)
Alagille syndrome / *Синдром Алажилля*	8 (7.5 %)
Byler disease / *Болезнь Байлера*	6 (5.5 %)
Other / *Другие*	16 (15 %)

a written informed consent, which is stored in their medical record.

Results

The results of DNA genotyping of children with liver fibrosis and healthy individuals included in the study for the presence of three polymorphic variants of the *TGFB1* gene: rs1800469, rs1800470 and rs1800471, are presented as the frequency of occurrence of various genotypes and alleles in Figure 1.

Significant differences were found in the distribution of rs1800470 genotypes in children with liver fibrosis and healthy individuals (Fig. 1B): $\chi^2 = 9.4778$; $p = 0.0236$. In children with liver fibrosis, the heterozygous genotype AG was 1.6 times less common than in healthy individuals ($p = 0.0024$). Comparative analysis of the frequencies of genotypes and alleles of two other SNPs — rs1800469 (Fig. 1A) and rs1800471 (Fig. 1C), did not show significant differences in the studied groups.

The analysis of the equilibrium distribution of the frequencies of the studied genotypes in accordance with the Hardy — Weinberg law is presented in Table 2.

In children with liver fibrosis, the distribution of SNP rs1800470 does not correspond to the Hardy — Weinberg equilibrium: $\chi^2 = 13.7673$; $p = 0.00026$. In all other cases, in children with fibrosis and in healthy individuals, the distribution of the studied SNPs corresponded to the Hardy — Weinberg law.

A comparative analysis of the distribution of genotype and allele frequencies in children with liver fibrosis and healthy individuals for three SNPs was carried out in various models of interaction between allelic genes: codominant, dominant, recessive, and superdominant. For each model, the odds ratio and the error value for encountering a particular genotype in the group of children with liver fibrosis compared to healthy individuals were calculated (Table 3).

Significant differences were found in the distribution of genotypes of SNP rs1800470 in the codominant (OR = 0.49; 95 % CI: 0.29–0.84; $p = 0.0088$) and superdominant (OR = 0.47; 95 % CI: 0.28–0.77; $p = 0.0024$) models. The presented result shows that in both models the heterozygous genotype AG is significantly less common in liver fibrosis and can be a protective factor. In all other cases, no significant differences in the distribution of frequencies of genetic variants in children with fibrosis and healthy individuals depending on the model of interaction of allelic genes were found.

The studied polymorphisms are located in one gene and on one chromosome, which increases the likelihood of linked inheritance of nearby loci. The results of the analysis of linkage disequilibrium (linked inheritance of loci and formation of haplotypes) are presented in Table 4.

The obtained result, as expected, revealed statistically significant linkage between all studied variants. The highest linkage is characteristic of the pair rs1800469 – rs1800470.

In the examined groups of children with fibrosis and healthy individuals, five main (frequency of occurrence — more than 1 %) combinations of the studied SNPs were observed. Table 5 presents the observed haplotypes in order of decreasing frequency of occurrence, the frequencies themselves for different groups, OR between patients and healthy individuals, as well as the error value for the calculated OR.

Statistically significant differences ($p < 0.05$) were found for haplotypes No. 3 and 4, which were observed more often in children with fibrosis than in healthy individuals (Table 5). Haplotype No. 3 (A-A-C) was found in 23.7 % of children with fibrosis, which is 6.03 times more often than in healthy individuals ($p < 0.0001$). Haplotype No. 4 (G-G-C) was found in 18.5 % of children with fibrosis, 3.71 times more often than in the group of healthy individuals ($p = 0.0001$). In total, haplotypes No. 3 and 4 were found in almost half (42.2 %) of

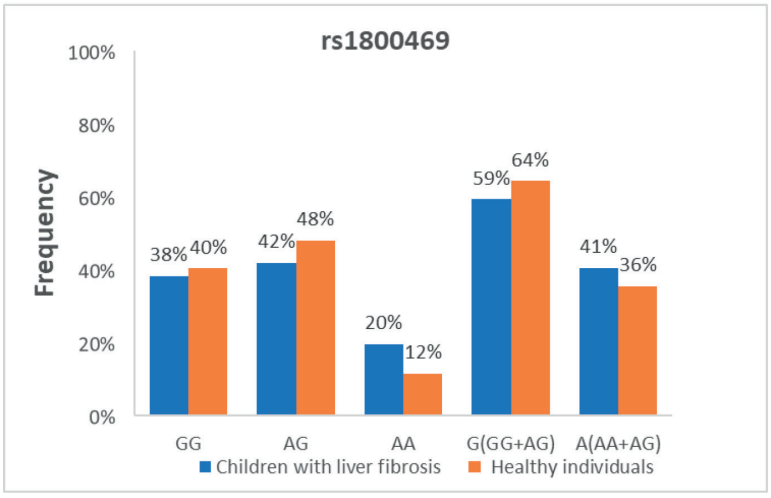
Table 2. Compliance with the Hardy — Weinberg law of the distribution of *TGFB1* polymorphism in children with liver fibrosis and healthy individuals

Таблица 2. Соответствие закону Харди — Вайнберга распределения полиморфизма *TGFB1* у детей с фиброзом печени и здоровых лиц

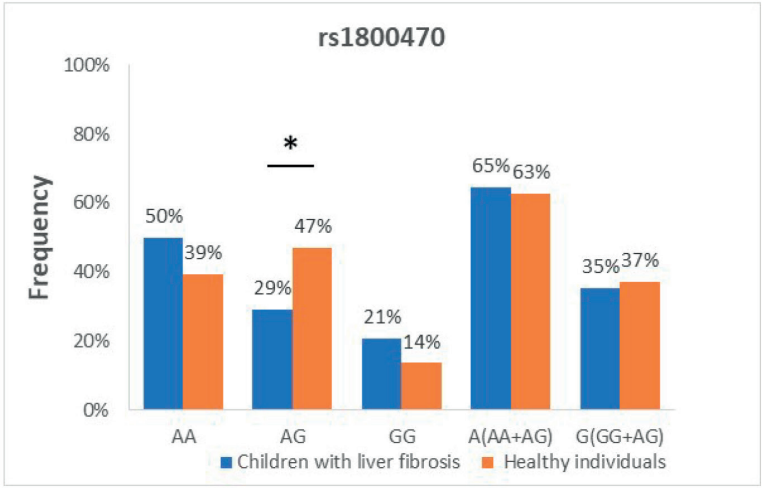
Groups *Группы*	Single nucleotide polymorphism / *Однонуклеотидный полиморфизм*					
	rs1800469		rs1800470		rs1800471	
	χ^2	*p*	χ^2	*p*	χ^2	*p*
Children with fibrosis *Дети с фиброзом*	1.7648	0.23	13.7673	0.00026*	0.1236	1
Healthy individuals *Здоровые лица*	0.4246	0.64	0.0075	1	0.0837	1

Note: ** — $p < 0.05$ (does not comply with the Hardy — Weinberg law).*
Примечание: ** — $p < 0,05$ (не соответствует закону Харди — Вайнберга).*

A



B



C

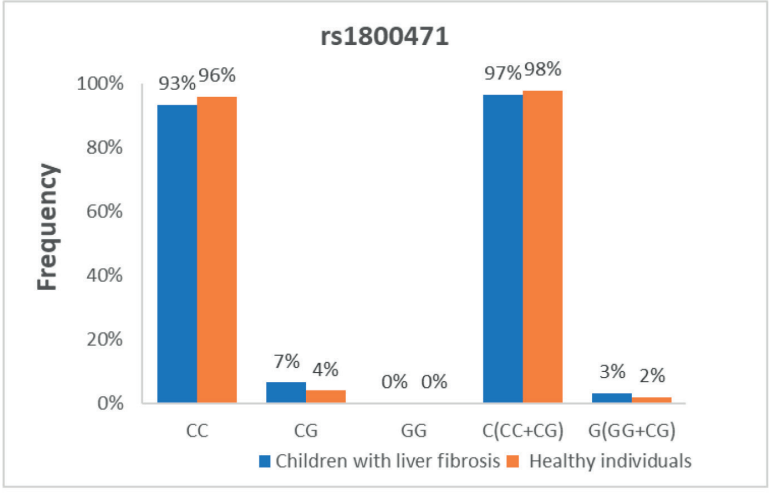


Figure 1. Frequency of occurrence of genotypes and alleles of the *TGFB1* gene SNPs — rs1800469 (A), rs1800470 (B) and rs1800471 (C) — in children with liver fibrosis and healthy individuals (* — $p < 0.05$)

Рисунок 1. Частоты встречаемости генотипов и аллелей ОНП гена *TGFB1* — rs1800469 (A), rs1800470 (B) и rs1800471 (C) — у детей с фиброзом печени и здоровых лиц (* — $p < 0,05$)

Table 3. Distribution of *TGFB1* polymorphism in children with liver fibrosis and healthy individuals in different models

Таблица 3. Распределение полиморфизма *TGFB1* у детей с фиброзом печени и здоровых лиц в разных моделях

SNP / Model ***ОНП / Модель***	**Genotype** ***Генотип***	**Frequency, %** ***Частота, %***		**OR (95% CI)** ***ОШ (95% ДИ)***	***p***
rs1800469		**Children with fibrosis** ***Дети с фиброзом***	**Healthy individuals** ***Здоровые лица***		
codominant *кодоминантная*	GG	38.3	40.4	1.00	0.17
	AG	42.1	48	0.92 (0.55–1.55)	
	AA	19.6	11.6	1.78 (0.88–3.59)	
dominant *доминантная*	GG	38.3	40.4	1.00	0.72
	AG-AA	61.7	59.6	1.09 (0.67–1.77)	
recessive *рецессивная*	GG-AG	80.4	88.4	1.00	0.062
	AA	19.6	11.6	1.86 (0.97–3.54)	
superdominant *сверхдоминантная*	GG-AA	57.9	52.0	1.00	0.32
	AG	42.1	48.0	0.79 (0.49–1.26)	
rs1800470					
codominant *кодоминантная*	AA	50.0	39.4	1.00	0.0088*
	AG	29.2	47.0	0.49 (0.29–0.84)	
	GG	20.8	13.6	1.20 (0.62–2.33)	
dominant *доминантная*	AA	50.0	39.4	1.00	0.076
	AG-GG	50.0	60.6	0.65 (0.40–1.05)	
recessive *рецессивная*	AA-AG	79.2	86.4	1.00	0.11
	GG	20.8	13.6	1.66 (0.89–3.09)	
superdominant *сверхдоминантная*	AA-GG	70.8	53.0	1.00	0.0024*
	AG	29.2	47.0	0.47 (0.28–0.77)	
rs1800471					
codominant *кодоминантная*	CC	93.4	96.0	1.00	0.33
	CG	6.6	4.0	1.69 (0.59–4.79)	

Note: *SNP — single nucleotide polymorphism; OR (95% CI) — odds ratio (95% confidence interval); * — $p < 0.05$.*
Примечание: *ОНП — однонуклеотидный полиморфизм; ОШ (95% ДИ) — отношение шансов (95%-ный доверительный интервал); * — $p < 0,05$.*

Таблица 4. Статистическая оценка неравновесия по сцеплению для пар полиморфных вариантов гена *TGFB1*

Table 4. Statistical assessment of linkage disequilibrium for pairs of polymorphic variants of the *TGFB1* gene

Single nucleotide polymorphism pairs ***Пары однонуклеотидных полиморфизмов***	**D**	***r***	***p***
rs1800469 – rs1800470	0.1448	0.6191	0*
rs1800469 – rs1800471	–0.0113	–0.1355	0.0001*
rs1800470 – rs1800471	0.0089	0.1066	0.002*

Note: ** — $p < 0.05$.*
Примечание: ** — $p < 0,05$.*

children with fibrosis and only in 7.4 % of healthy individuals.

The most frequent haplotypes — No. 1 and 2 (G-A-C and A-G-C) — were generally present in 57.8 % of children with fibrosis and 92.6 % of healthy individuals, while differences in the occurrence of each of the haplotypes No. 1, No. 2 or No. 5 separately did not reach statistical significance.

Discussion

Polymorphism of *TGFB1*, a gene encoding one of the key profibrogenic cytokines, can make a significant contribution to the development of fibrosis; however, its role in the outcome of congenital liver pathologies has not been studied in children with liver fibrosis. In this study, we showed that the frequency of polymorphic variants of the *TGFB1* gene in children with verified liver

Table 5. Frequency of occurrence of *TGFB1* gene haplotypes in children with liver fibrosis and healthy individuals

Таблица 5. Частоты встречаемости гаплотипов гена *TGFB1* у детей с фиброзом печени и здоровых лиц

No. №	Nucleotide in position *Нуклеотид в положении*			Frequency of occurrence *Частота встречаемости*			OR (95% CI) *ОШ (95% ДИ)*	*p*
	rs1800469	rs1800470	rs1800471	Total *Всего*	Children with fibrosis *Дети с фиброзом*	Healthy individuals *Здоровые лица*		
1	G	A	C	0.5268	0.3984	0.5885	1.00	
2	A	G	C	0.2724	0.146	0.3174	0.93 (0.60–1.46)	0.77
3	A	A	C	0.1006	0.2372	0.0372	6.03 (3.06–11.89)	< 0.0001*
4	G	G	C	0.0756	0.185	0.0369	3.71 (1.4–7.08)	0.0001*
5	G	G	G	0.0185	0.004	0.0175	2.14 (0.62–7.45)	0.23

Note: * — $p < 0.05$.
Примечание: * — $p < 0,05$.

fibrosis differs significantly from that in healthy individuals, suggesting an increased risk of liver fibrosis in individuals with this genotype.

The frequencies of SNPs rs1800469, rs1800470, and rs1800471 of the *TGFB1* gene in healthy individuals obtained in our study are consistent with the data of Russian authors and the NCBI database for the European population [12–14].

Analysis of the frequencies of the three most significant SNPs of the *TGFB1* gene in children with liver fibrosis showed that the distribution of rs1800470 differs from that in healthy individuals and does not correspond to the Hardy — Weinberg equilibrium. Nonequilibrium distribution of genotypes can be an important indicator of the medical significance of the locus under study. In children with liver fibrosis, the heterozygous genotype A/G was found 1.6 times less frequently than in the group of healthy individuals, which can serve as a protective factor against fibrosis in the codominant and superdominant models of gene interaction. It should be noted that in our previous work, analysis of the distribution of rs1800470 polymorphism in 225 children with terminal liver failure did not reveal significant differences from the healthy control, which can be explained by the absence of liver fibrosis in some recipients, for whom the indication for transplantation, among others, was various hepatitis and metabolic liver diseases [10].

TGF-β1 is a vital protein and is involved in the regulation of many key cellular processes, so significant impairments of its functions may be incompatible with life [15]. While single nucleotide substitutions may have little effect on protein function, a combination of several substitutions may already have a clinical manifestation. This is confirmed by the analysis of haplotypes of the studied SNPs, which revealed significantly greater differences in the distribution of haplotypes in children with fibrosis and healthy individuals than in the case of a single SNP rs1800470.

The studied polymorphisms are localized in one gene and, as expected, linkage disequilibrium was revealed between all pairs of SNPs rs1800469, rs1800470 and rs1800471.

In general, five main haplotype variants were observed in the studied group, the occurrence of two of which was significantly more frequent (from 3.7 to 6 times) in children with fibrosis than in healthy individuals. Rarer haplotypes A-A-C and G-G-C were found only in 7 % of healthy individuals and in more than 40 % of children with fibrosis, which may indicate a predisposition to the development of liver fibrosis in those with these haplotypes. Interestingly, both haplotypes carry one minor allele in the 1st or 2nd position and two major alleles. It can be assumed that the minor variant in position rs1800469 or rs1800470 in combination with two major ones can disrupt the function of the profibrogenic cytokine and predispose to the development of fibrosis. At the same time, the haplotype with two minor alleles (A-G-C) was the second most common, which did not differ significantly between children with fibrosis and healthy individuals.

It should be noted that the frequency of occurrence of the most common haplotype, containing all three major alleles (G-A-C), in healthy individuals in the studied sample was about 60 % and coincided with the data of other authors [12, 16, 17].

Thus, our data show reliable differences in the frequency of *TGFB1* gene polymorphism in children with fibrosis and healthy individuals, which indicates a possible association of *TGFB1* gene

polymorphic variants with the risk of developing liver fibrosis in children. Other studies of *TGFB1* gene genetic polymorphism in young children with liver fibrosis in the Russian or other populations have not been published to date.

In adult patients, the role of *TGFB1* gene polymorphism in the development of liver fibrosis has been researched in several studies, but the results are not always unambiguous [18], which, as the authors believe, may be due to the ethnic origin of the patients studied. In the European population, associations between liver fibrosis and *TGFB1* gene polymorphism have been identified, while in a number of Asian populations such a relationship was not found. In addition, it has been shown that *TGFB1* polymorphism can play a certain role in the development of myocardial fibrosis and myocardial infarction [13, 19].

Pathological conditions are often influenced by a large number of genetic factors/polymorphisms, which individually may contribute only a small share to the overall risk and their significance is difficult to assess when analyzing small groups. Independent studies with a significantly larger number of participants are needed to confirm the findings of this work.

The present study is observational, retrospective and hypothesis-provoking, its design is based on the case-control method. The limitations of the article's conclusions may be due to the fact that in case of a heterozygous genotype, it is impossible to unambiguously determine the haplotype by the genotype using the PCR method due to the diploid set of chromosomes. In such cases, an accurate determination of the haplotype is ensured only by sequencing.

Genetic analysis is non-invasive, is carried out once in a lifetime, regardless of age and physiological condition, can provide information on the individual characteristics of the patient and allow for personalized therapy. Predisposition to genetic diseases may depend on the ethnic origin of the individual, which necessitates conducting studies in genetically homogeneous groups. However, in our study, ethnicity was not determined, and the results obtained can be attributed to the open Russian population.

Conclusion

The development of liver fibrosis is regulated, among others, by the profibrogenic cytokine TGF-β1, the content of which in the tissue can be determined, among other things, by the genetic polymorphism of the *TGFB1* gene itself. The distribution of the three most significant polymorphisms of the *TGFB1* gene (rs1800469, rs1800470 and rs1800471) and its haplotypes in children with liver fibrosis was studied. It was shown that the occurrence of the heterozygous variant (AG) rs1800470 is 1.6 times less frequent, and the haplotypes A-A-C and G-G-C are 6 and 3.7 times more frequent in children with liver fibrosis than in healthy individuals. It is possible that the AG genotype of rs1800470 reduces, and the A-A-C and G-G-C haplotypes corresponding to rs1800469, rs1800470 and rs1800471 of the *TGFB1* gene increase the risk of developing liver fibrosis in children with liver failure.

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Submitted: 31.05.2024 Accepted: 25.09.2024 Published: 30.04.2025
Поступила: 31.05.2024 Принята: 25.09.2024 Опубликована: 30.04.2025