DOI: 10.15825/1995-1191-2024-4-166-170

NATIVE LIVER FIBROSIS IN PEDIATRIC LIVER RECIPIENTS: ASSOCIATION WITH GENETIC POLYMORPHISM IN THE *TGFB1* GENE

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Objective: to examine the relationship between native liver fibrosis and *TGFB1* gene polymorphism in pediatric liver recipients. **Materials and methods.** Fibrosis of varying severity was diagnosed (METAVIR scale) based on histological analysis of the native liver of children (45 boys and 62 girls aged 3 to 73 months). Genomic DNA was genotyped by real-time polymerase chain reaction using TaqMan probes. **Results.** The prevalence of the *TGFB1* single nucleotide polymorphisms (SNPs) rs1800469, rs1800470, and rs1800471 was examined in both children with liver fibrosis of varying severity and in healthy individuals. The distribution of rs1800470 in children with fibrosis was 50% homozygotes of major allele, 29% heterozygotes and 21% homozygotes of minor allele. This distribution was not consistent with the Hardy–Weinberg principle ($p = 0.00026$). Conclusion. Liver fibrosis in pediatric liver recipients is linked to the rs1800470 polymorphism of the *TGFB1* gene. Carriage of the heterozygous rs1800470 genotype may be a protective factor against liver fibrosis in children with liver failure.

Keywords: liver fibrosis, biliary atresia and hypoplasia, pediatric liver recipients, rs1800469, rs1800470, rs1800471.

INTRODUCTION

In recent years, substantial progress has been achieved in developing a highly effective treatment system for children with congenital hepatobiliary disorders. Advanced surgical techniques for LT have been introduced, many of which are recognized as globally pioneering – including procedures involving ABO-incompatible donors. The number of LTs has notably increased, even among very young pediatric patients. Arguably, the most remarkable accomplishment in this field has been the complete fulfillment of the national demand for pediatric LT, offering a full recovery to patients who were once considered untreatable [1, 2].

Key areas of current research include identifying genetic predisposition patterns, improving methods for predicting disease progression, and developing strategies to prevent post-LT complications. One promising direction for integration into clinical pediatric practice is the investigation of gene polymorphisms that influence the expression of key factors regulating the formation, development, and function of the hepatobiliary system in children before and after birth.

Previous studies have demonstrated that children with liver failure of various etiologies exhibit a distinct distribution of rare haplotypes of the *TGF-β1* (transforming growth factor beta 1) gene, which regulates the expression of the key profibrogenic cytokine *TGF-β1*, compared to healthy individuals. The clinical relevance of three single nucleotide polymorphisms (SNPs) in the TGFB1 gene – rs1800469, rs1800470, and rs1800471 – has been established, particularly in relation to post-transplant complications such as graft rejection and infections [3, 4].

However, the broad spectrum of liver diseases among the studied patients – including congenital cholestatic and metabolic disorders, as well as acquired cirrhosis and hepatitis – limits the ability to isolate the role of TGFB1 gene polymorphisms. This necessitates further investigation in more homogeneous patient groups. Cirrhosis, a terminal stage of liver fibrosis, is characterized by excessive production and accumulation of extracellular matrix, leading to partial or complete impairment of hepatic function. The fibrotic process involves hepatocytes, lymphocytes, and a cascade of proinflammatory and profibrogenic cytokines [5, 6].

The aim of the present study was to analyze the association between native liver fibrosis and SNPs in the *TGF-β1* gene – rs1800469, rs1800470, and rs1800471 – in pediatric LT recipients during the early post-transplant period. The findings of this research are expected to clarify the role of *TGF-β1* gene polymorphisms in the pathogenesis of liver fibrosis and to evaluate their potential

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clinical value in predicting fibrosis risk in pediatric liver recipients.

MATERIALS AND METHODS

The study included 107 pediatric LT recipients (45 boys and 62 girls) aged 3 to 73 months (median age: 8 months), and a control group of 199 healthy individuals (79 males and 120 females) with a mean age of 32.7 ± 9.6 years.

Indications for LT in the pediatric cohort were end-stage liver disease resulting from the following conditions: biliary atresia (n = 61), biliary tract hypoplasia (n = 8), Alagille syndrome (n = 8), Caroli's disease (n = 8), Byler's disease (n = 6), and other rare hepatic disorders (n = 16), including Crigler–Najjar syndrome, glycogen storage disease type I (Von Gierke disease), alpha-1 antitrypsin deficiency, tyrosinemia, fulminant hepatitis, autoimmune hepatitis, and cryptogenic cirrhosis.

Liver fibrosis of varying severity was diagnosed in the recipients based on macroscopic and histologic examination of the native liver explants obtained during transplantation, evaluated according to the METAVIR scoring system: F1 (stellate enlargement of portal tracts without septa) in 5 cases, F2 (portal expansion with isolated porto-portal septa) in 9 cases, F3 (numerous septa without cirrhosis) in 14 cases, and F4 (cirrhosis) in 79 cases.

All patients received treatment and underwent comprehensive clinical, laboratory, and instrumental evaluation in accordance with the protocols at Shumakov National Medical Research Center of Transplantology and Artificial Organs. LT was performed using grafts from living-related donors. Post-transplant, recipients were maintained on double- or triple-drug immunosuppressive therapy regimens.

The detailed methodology and statistical analysis employed in this study have been previously described in our publication, "Association between the Tgfb1 Gene Haplotype and Liver Diseases in Children" [7].

RESULTS AND DISCUSSION

Fig. 1 illustrates the distribution frequencies of three polymorphic variants of the *TGF-β1* gene – rs1800469, rs1800470, and rs1800471 – among pediatric liver recipients with fibrosis.

Comparative analysis of genotype frequencies of the studied SNPs between children with liver fibrosis and healthy controls revealed no statistically significant differences for rs1800469 and rs1800471. However, a significant difference was observed in the distribution of rs1800470 genotypes between the two groups (Fig. 2).

As shown in Fig. 2, a statistically significant difference was observed in the distribution of the heterozygous genotype of rs1800470. In children with liver fibrosis, the AG genotype was 1.6 times less frequent than in healthy individuals ($\chi^2 = 9.4778$, $p = 0.0236$).

In healthy individuals, all three SNPs conformed to Hardy–Weinberg equilibrium. Among children with liver fibrosis, rs1800469 and rs1800471 also demonstrated equilibrium ($\chi^2 = 1.7648$, $p = 0.23$; $\chi^2 = 0.1236$, $p = 0.99$, respectively). However, the distribution of rs1800470 significantly deviated from Hardy–Weinberg expectations ($\chi^2 = 13.7673$, $p = 0.00026$).

These findings suggest that among the three studied SNPs in the TGF-β1 gene, rs1800470 displays a notable deviation from Hardy–Weinberg equilibrium in the group of children with liver fibrosis. This deviation may reflect a potential medical significance of this locus under study.

A comparative analysis of genotype frequencies in children with liver fibrosis and healthy individuals was conducted using different allelic interaction models, including codominant, dominant, recessive, and over dominant (Table).

Table shows significant differences in the distribution of rs1800470 SNP genotypes in codominant (OR = 0.49, CI 0.29–0.84, p = 0.0088) and over dominant (OR = 0.47, CI 0.28–0.77, p = 0.0024) models. The findings suggest that, in both models, the heterozygous AG genotype is significantly less frequent in children with liver fibrosis, potentially serving as a protective factor against

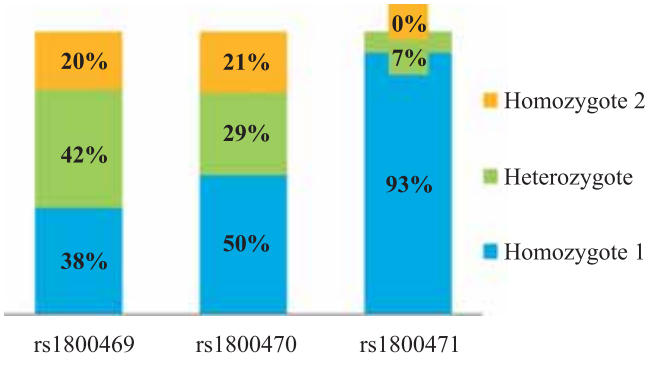

Fig. 1. Distribution of genotypes rs1800469, rs1800470 and rs1800471 of the TGFB1 gene in pediatric liver recipients with liver fibrosis

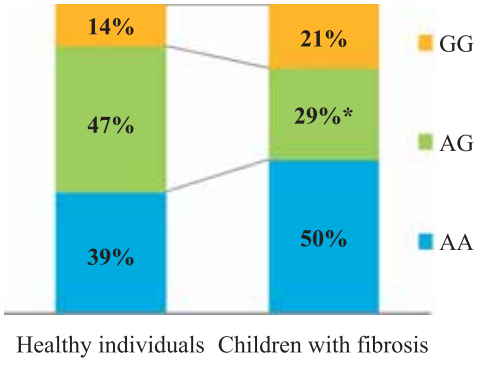

Fig. 2. Distribution of rs1800470 genotypes of the TGFB1 gene in healthy individuals and in pediatric liver recipients with liver fibrosis. * $p < 0.05$ *vs.* healthy individuals

Table

Distribution of the TGFB1 polymorphism rs1800470 in children with liver fibrosis and in healthy individuals in different m odels

SNPs/Model	Genotype	Frequency, % children with fibrosis	Frequency, % healthy individuals	OR (95% CI)	P value
Codominant	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	AA	50.0	39.4	1.00	0.076
	AG-GG	50.0	60.6	0.65 (0.40–1.05)	
Recessive	AA-AG	79.2	86.4	1.00	0.11
	GG	20.8	13.6	1.66 (0.89–3.09)	
Over dominant	AA-GG	70.8	53.0	1.00	0.0024*
	AG	29.2	47.0	0.47 (0.28–0.77)	

* – $p < 0.05$.

its development. No significant differences in genotype distribution were observed in the other models. It is important to note that in our previous study, the distribution of the rs1800470 polymorphism in 225 children with end-stage liver failure did not show significant differences compared to healthy individuals. This discrepancy can likely be attributed to the absence of liver fibrosis in some recipients, where the indication for transplantation was based on conditions such as hepatitis and metabolic liver diseases, rather than liver fibrosis [7].

Our data show significant differences in the frequency of *TGF-β1* gene polymorphisms between children with liver fibrosis and healthy individuals, suggesting a potential association between these genetic variants and susceptibility to liver fibrosis.

Several studies have investigated the role of *TGF-β1* gene polymorphisms in liver fibrosis in adult patients, but their results have been inconsistent. The authors suggest that these discrepancies may be attributed to the ethnic origin of the populations studied [8–10]. While some studies in European populations show a link between liver fibrosis and *TGF-β1* gene polymorphisms, this association has not been consistently observed in some Asian populations. Furthermore, research has indicated that *TGF-β1* gene polymorphisms could also play a role in the development of myocardial fibrosis and myocardial infarction [11–13].

The findings of our study may hold both scientific and practical significance. They contribute to a deeper understanding of the role of *TGF-β1* gene polymorphisms in the development of tissue fibrosis and may be useful in assessing individual risk for fibrosis or identifying new therapeutic targets. Moreover, the presence of genotypes associated with an increased risk of fibrosis could be valuable in predicting post-transplant complications or individual responses to immunosuppressive therapy – areas that warrant further investigation.

CONCLUSION

Liver fibrosis remains a significant clinical issue, with its causes and underlying mechanisms still under active investigation. In the present study, liver fibrosis was linked to TGF-β1 gene polymorphisms. Specifically, among pediatric liver recipients with verified native liver fibrosis, the heterozygous genotype at the rs1800470 locus of the TGF-β1 gene was found to be 1.6 times less frequent compared to healthy individuals. This finding suggests a potential protective role of the heterozygous rs1800470 variant against the development of liver fibrosis. Continued investigation into the TGF-β1 gene polymorphisms may enable personalized prediction of post-transplant complications.

The authors declare no conflict of interest.

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The article was submitted to the journal on 22.10.2024