DOI: 10.15825/1995-1191-2025-2-179-188

ASSOCIATION OF PLASMA TGF-β1 LEVELS WITH POLYMORPHIC LOCI AND *TGFB1* HAPLOTYPES RS1800469 AND RS1800470 IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS

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Objective: to investigate the association between plasma TGF-β1 levels in pediatric liver transplant (LT) recipients, both pre- and post-transplantation, and the polymorphic alleles and haplotypes at rs1800469 and rs1800470 loci of the *TGFB1* gene. **Materials and methods.** The study cohort comprised 135 pediatric LT recipients, aged 3 to 98.4 months (mean age 8.2 years, median 8 months). The control group consisted of 77 healthy individuals, aged 30.3 ± 5.2 years. Plasma TGF-β1 levels were quantified using ELISA. Genomic DNA from participants was analyzed for the polymorphic loci rs1800469 and rs1800470 of the *TGFB1* gene using real-time polymerase chain reaction PCR with TaqMan probes. **Results.** Blood TGF-β1 level in pediatric LT recipients pre-transplant was 4.6 (1.1–9.5) ng/mL. One month post-transplant, cytokine level increased to 6.3 (1.7–15.0) ng/mL (p = 0.008), and after one year, it rose further to 7.0 (1.9–13.5) ng/mL (p = 0.0001). Healthy adults had significantly higher TGF-β1 levels, with a median of 11.7 (6.4–16.9) ng/mL (p = 0.0000), compared to pediatric recipients. The distribution frequencies of the rs1800469 and rs1800470 polymorphic alleles in pediatric LT recipients did not significantly differ from those in healthy individuals. However, the occurrence of rare haplotypes (T-T and C-C) was significantly higher in pediatric recipients. Before transplantation and 1 month after the procedure, TGF-β1 levels in pediatric recipients were not associated with the carriage of the studied alleles or haplotypes. However, at 1-year post-transplant, higher TGF-β1 levels in pediatric recipients were significantly associated with the major alleles (C/C + C/T) of rs1800469 and the rs1800470 T/T genotype, as well as with the T-T haplotype. In healthy individuals, TGF-β1 levels were not influenced by the rs1800469 and rs1800470 alleles individually, but high cytokine levels were associated with the C-C haplotype. **Conclusion.** In pediatric LT recipients, elevated TGF-β1 levels at 1-year post-transplant are associated with the presence of the major alleles C (rs1800469) and T (rs1800470), as well as the T-T haplotype of the *TGFB1* gene. This suggests that these polymorphic loci may influence the development of post-transplant complications and could potentially serve as biomarkers for predicting clinical outcomes in LT.

Keywords: single nucleotide polymorphism, profibrogenic cytokine, congenital liver diseases, biliary atresia and hypoplasia/

INTRODUCTION

Liver transplantation is generally considered the only effective treatment for young children with end-stage liver failure, whether caused by congenital or acquired liver diseases. Overall posttransplant survival currently exceeds 85% at 5 years [1, 2]. To further improve treatment outcomes, it is crucial to enhance the prediction and diagnosis of post-transplant complications, which can be achieved through the use of minimally invasive molecular genetic markers.

One promising candidate is transforming growth factor β1 (TGF-β1), a multifunctional cytokine with immunosuppressive and profibrogenic properties. The level of TGF-β1 has been associated with graft dysfunction, infectious complications, and variations in the immunosuppression regimen in pediatric liver transplant (LT) recipients, making it a potential biomarker for monitoring the clinical status of these patients [3–5].

TGF-β1 is synthesized in almost all tissues, and its effects vary depending on its concentration and the cell type involved. Low levels of TGF-β1 are believed to promote inflammation, whereas high levels can lead to tissue fibrosis [6, 7]. Regulation of this cytokine is influenced by multiple factors and interactions, including the cellular TGF-β signaling pathway, which comprises a family of ligands and their transmembrane receptors and interacts with other cellular pathways such as SMAD

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and Notch [8, 9]. Additionally, TGF-β1 secretion may be influenced by polymorphisms in the *TGFB1* gene. To date, eight single nucleotide polymorphisms (SNPs) have been identified that may affect TGF-β1 production and have been associated with various diseases, including liver diseases [10, 11].

A review of the literature suggests that two polymorphic loci, rs1800469 and rs1800470, are of particular significance in liver diseases [12–16]. The rs1800469 polymorphism involves a cytosine-to-thymine substitution (–509C>T) in the promoter region of the *TGFB1* gene, while the rs1800470 variant results from a thymine-to-cytosine substitution (+869T>C), leading to an amino acid change from leucine to proline in the TGF-β1 protein [17, 18].

To date, there is no definitive consensus regarding the effect of these loci on TGF-β1 production. It is suggested that the –509C>T substitution may either increase TGF-β1 levels or have no significant effect, while the +869T>C substitution is generally believed to enhance its secretion [19, 20]. *In vitro* experiments using HeLa cell cultures infected with various vectors showed increased TGF-β1 production when cells were infected with a construct carrying the minor C allele of rs1800470 [20]. However, it is known that regulation of TGF-β1 secretion in cancer cells differs substantially from that in normal cells [6–9].

Studies investigating the association between *TGFB1* genetic polymorphisms and plasma TGF-β1 levels in patients with liver disease have predominantly focused on adults with cirrhosis secondary to chronic hepatitis B or C infections and hepatic steatosis [21–24]. Plasma TGF-β1 concentrations in patients with liver disease are generally higher than in healthy individuals; however, findings regarding the relationship between TGF-β1 levels and specific polymorphic alleles have been inconsistent. For example, Mohy et al. [22], de Brito et al. [23], and Felicidade et al. [24] reported that elevated TGF-β1 levels were associated with the minor homozygous TT genotype of rs1800469, whereas Wang et al. [21] found that higher TGF-β1 levels correlated with the major alleles C (rs1800469) and T (rs1800470).

In young children with terminal liver failure caused by congenital or acquired liver diseases, TGF-β1 levels differ from those observed in healthy children and may correlate with the severity of liver fibrosis [3–5, 25]. However, no data are currently available regarding the extent to which blood cytokine levels are influenced by *TGFB1* gene polymorphisms. Our previous studies showed that distribution of the polymorphic alleles rs1800469, rs1800470, and rs1800471 of the *TGFB1* gene in young children with liver diseases did not differ significantly from that in healthy controls. However, the frequency of rare haplotypes at these loci was significantly higher in the group of pediatric patients listed for LT [26].

The aim of the present study was to assess the association between plasma TGF-β1 levels in pediatric LT candidates, both before and after transplantation, and the carriage of polymorphic alleles and haplotypes at the rs1800469 and rs1800470 loci of the *TGFB1* gene.

MATERIALS AND METHODS

The study included 135 children (59 boys and 76 girls) who underwent LT, aged between 3 and 98.4 months (mean age: 8.2 months; median: 8 months).

To evaluate the association between blood TGF-β1 levels and TGFB1 gene polymorphisms in healthy individuals, a comparison group consisting of 77 healthy adults (35 boys and 42 girls) with a mean age of 30.3 ± 5.2 years was used. Although cytokine levels are generally considered to be independent of age, the limited number of studies addressing this relationship report ambiguous results [3–5].

The indication for LT in the children was the end stage of liver disease resulting from various conditions, including biliary atresia (n = 74), biliary hypoplasia (n = 10), Alagille syndrome (n = 9), Caroli's disease (n = 10), and Byler's disease (n = 6). Additionally, 26 children had other rare liver disorders such as Crigler–Najjar syndrome, von Gierke disease, alpha-1 antitrypsin deficiency, tyrosinemia, fulminant hepatitis, autoimmune hepatitis, cryptogenic cirrhosis, and others.

Following transplantation, recipients received immunosuppressive therapy consisting of two or three agents: tacrolimus, mycophenolate, and corticosteroids. Examination and treatment protocols adhered to the clinical guidelines of the Russian Transplant Society and the protocols established by the Shumakov National Medical Research Center of Transplantology and Artificial Organs.

TGF-β1 levels in blood plasma was measured by quantitative enzyme-linked immunosorbent assay (ELISA) with a reagent kit from Bender MedSystems (Austria), following the manufacturer's instructions. Optical density was measured in microplate wells using a Zenyth 340r spectrophotometer (Biochrom Anthos, UK) at a wavelength of 450 nm. Cytokine levels were assessed at three time points: before transplantation, one month after transplantation, and one year after transplantation.

Polymorphic loci rs1800469 and rs1800470 of the TGFB1 gene were identified in genomic DNA using real-time polymerase chain reaction (PCR) with TaqMan probes (Applied Biosystems, USA) on a CFX96™ real-time PCR system (Bio-Rad, USA), following the manufacturer's instructions. The TaqMan probes used

for genotyping were C_8708473_10 for rs1800469 and C_22272997_10 for rs1800470. Genomic DNA was extracted from venous blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) and the QIAcube™ automated analyzer (Qiagen, Germany), in accordance with the manufacturers' protocols.

Data were collected and initially processed using Microsoft Excel. Statistical analyses were performed using the STATISTICA software package (StatSoft Inc., USA). Quantitative variables are presented as mean ± standard deviation for parametric data, or as median and interquartile range (Q1–Q3) for nonparametric variables (with range excluding outliers shown in the graphs). Comparisons between two dependent groups were conducted using the Wilcoxon signed-rank test, while comparisons between two independent groups employed the Mann–Whitney U test. For comparisons among multiple independent groups, the Kruskal–Wallis test was used. Genotype and haplotype frequencies were analyzed using Fisher's exact test (p-value) via the SNPstats software. Differences were considered statistically significant at a p-value <0.05.

The study was approved by the Local Ethics Committee, Shumakov National Medical Research Center of Transplantology and Artificial Organs. Informed consent was obtained from all participants or their legal guardians and documented in the patients' medical records.

RESULTS

The median plasma TGF-β1 levels in pediatric LT recipients prior to transplantation was 4.6 ng/mL (interquartile range [IQR]: 1.1–9.5 ng/mL). One month after transplantation, the median TGF-β1 level significantly increased to 6.3 ng/mL (IQR: 1.7–15.0 ng/mL; p = 0.008). One year after transplantation, plasma TGF-β1 levels remained significantly higher compared to pre-transplant levels, reaching 7.0 ng/mL (IQR: 1.9–13.5 ng/mL; p = 0.0001) (Fig. 1).

The results of DNA genotyping for polymorphic alleles rs1800469 and rs1800470 of the TGFB1 gene in pediatric LT recipients are presented in Fig. 2, showing the distribution frequencies of different genotypes.

The frequency of occurrence of these SNP alleles in pediatric recipients did not differ significantly from that observed in healthy controls. In healthy adults, the genotype frequencies were as follows: for rs1800469 – 40% C/C, 44% C/T, and 16% T/T; for rs1800470 – 43% T/T, 40% T/C, and 17% C/C. Notably, plasma TGF-β1 level in healthy adults was 11.7 ng/mL (IQR: 6.4–16.9 ng/mL), which was significantly higher than the levels observed in pediatric liver recipients both before and after transplantation (p = 0.0000).

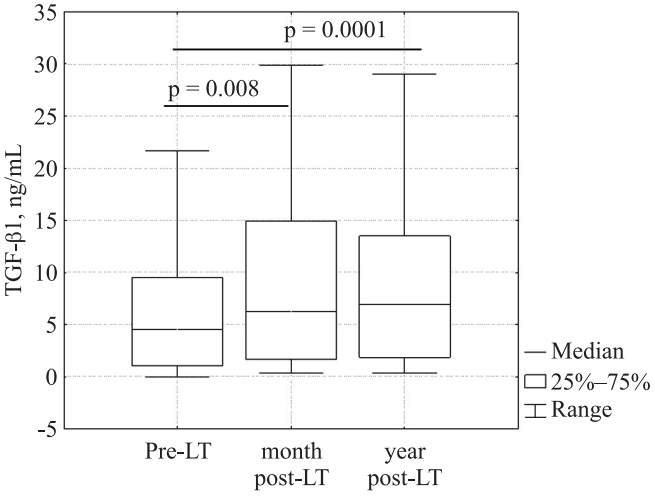

Fig. 1. Plasma TGF-β1 levels in pediatric liver transplant recipients measured before transplantation, one month after, and one year after transplantation

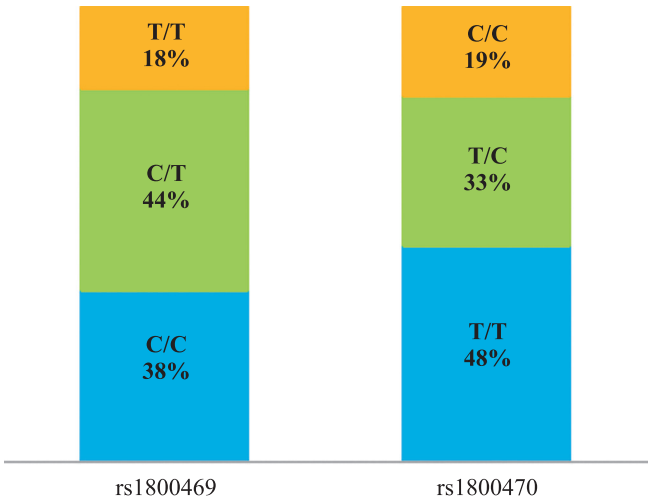

Fig. 2. Frequencies of polymorphic variants rs1800469 and rs1800470 of the TGFB1 gene in pediatric liver recipients

A comparative analysis of plasma TGF-β1 levels was conducted in pediatric recipients and healthy individuals across different genotypes of the rs1800469 polymorphism, considering the main genetic models of allelic interaction: codominant, dominant, recessive, and over dominant models. In pediatric recipients, TGF-β1 levels were assessed at three time points: before liver transplantation, one month post-transplant, and one year post-transplant. The results are illustrated in Fig. 3, using box plots showing the median, interquartile ranges (2nd–3rd quartiles), and the data range excluding outliers.

The results showed that plasma TGF-β1 levels in pediatric liver transplant recipients (PLTRs) with different genotypes of the rs1800469 polymorphic locus of the TGFB1 gene did not differ significantly either before or one month after LT. However, one year after transplantation, under the recessive model of allelic interaction,

carriers of the homozygous minor allele (T/T genotype) exhibited significantly lower TGF-β1 levels compared to carriers of the C/T and C/C genotypes (p = 0.045). In healthy individuals, TGF-β1 levels were found to be independent of the rs1800469 genotype (p > 0.05 in all comparisons).

Similarly, the TGF-β1 content in PLTRs with different genotypes of another TGFB1 polymorphism, rs1800470, was analyzed based on models of allelic gene interaction. The results are presented in Fig. 4.

Fig. 4 shows that TGF-β1 levels in the blood of PLTRs with different rs1800470 genotypes did not differ significantly before or one month after LT, similar to the findings for rs1800469. However, one year after transplantation, under the dominant model of allelic interaction, carriers of the homozygous major allele (T/T genotype) exhibited significantly higher TGF-β1 levels compared to carriers of the T/C and C/C genotypes (p = 0.039). In healthy individuals, TGF-β1 levels were independent of the rs1800470 genotype (p > 0.05 in all comparisons).

Fig. 5 presents the frequencies of haplotypes formed by the rs1800469 and rs1800470 polymorphic variants in PLTRs and healthy adult subjects

In both PLTRs and healthy adults, four haplotype variants were identified, with the most common being the C-T haplotype, which consists of the major alleles of both polymorphic loci. The second most frequent haplotype in healthy individuals was T-C, containing two minor alleles. The frequencies of the most common haplotypes did not differ significantly between recipients and healthy individuals. However, the occurrence of the T-T and C-C haplotypes was significantly higher in PLTRs compared to healthy adults (p = 0.007 and p = 0.021, respectively).

The results of the comparative analysis of TGF-β1 levels based on carriage of different haplotypes of the rs1800469 and rs1800470 polymorphic alleles in pediatric recipients and healthy adults are presented in Fig. 6.

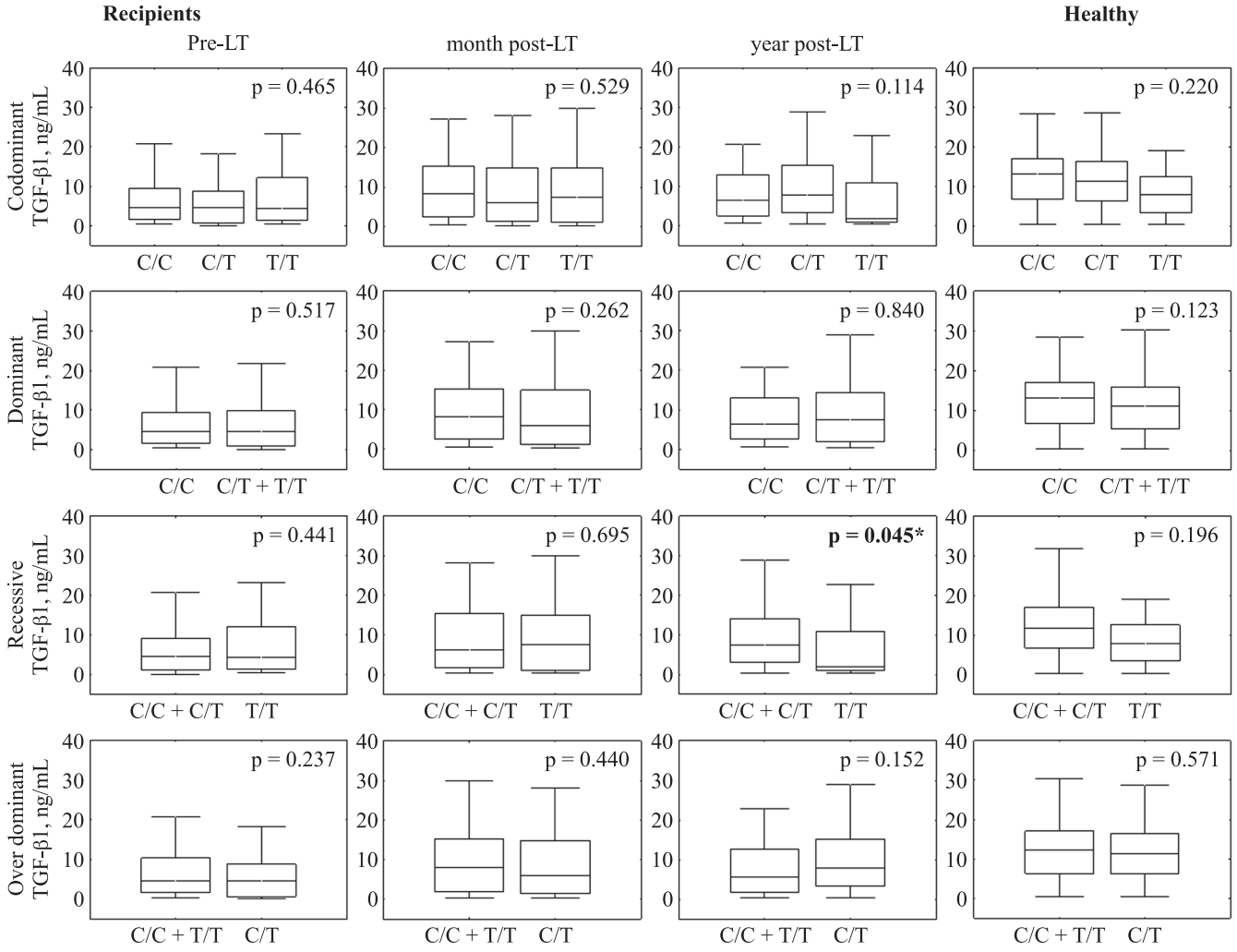

Fig. 3. Comparison of plasma TGF-β1 levels in pediatric liver transplant recipients measured before transplantation, one month after, and one year after transplantation, and in healthy adults, stratified by genotypes of the rs1800469 polymorphic allele of the TGFB1 gene, analyzed using allelic interaction models. p < 0.05

The results showed that TGF-β1 levels in the blood of PLTRs carrying different haplotypes did not differ significantly before LT or one month after LT. However, one year after LT, recipients carrying the haplotype consisting of two minor alleles (T-C) showed the lowest cytokine levels, which were significantly lower compared to recipients carrying the haplotype composed of a minor and a major allele (T-T) ($p = 0.019$).

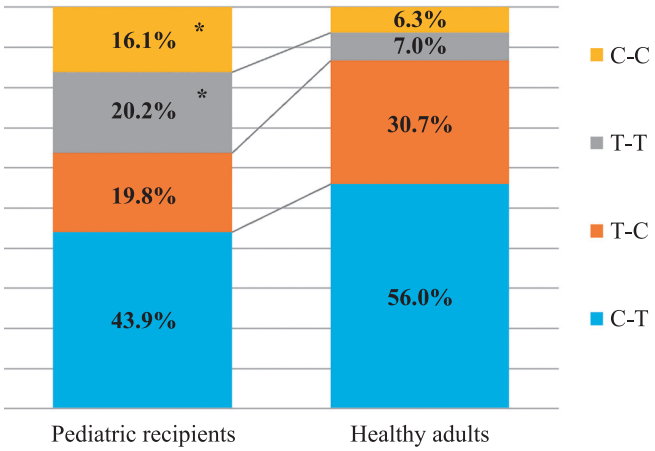

Fig. 5. Haplotype frequencies of the rs1800469 and rs1800470 polymorphic variants of the TGFB1 gene in pediatric liver transplant recipients and healthy adults. $p < 0.05$

In contrast, among healthy individuals, the lowest TGF-β1 levels were observed in carriers of the T-T haplotype, while the highest levels were found in carriers of the C-C haplotype ($p = 0.03$).

DISCUSSION

In PLTRs, TGF-β1 levels can vary significantly depending on the underlying disease etiology, degree of liver fibrosis, presence of post-transplant complications, immunosuppressive therapy regimen, and other factors [3–5]. However, the causal mechanisms underlying this variability remain poorly understood. In this study, we evaluated the extent to which plasma TGF-β1 levels may be associated with carriage of polymorphic loci and hap-

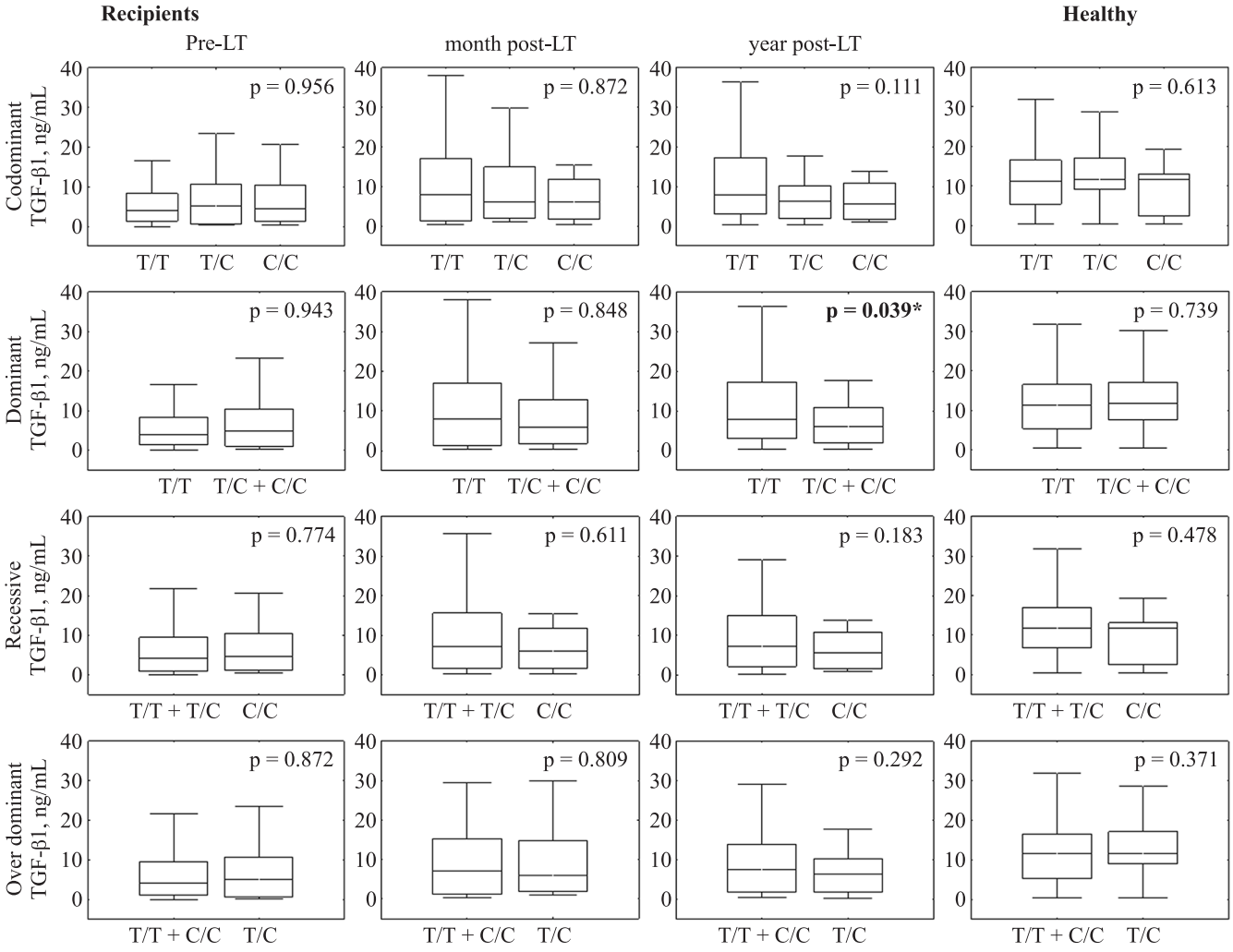

Fig. 4. Comparison of plasma TGF-β1 levels in pediatric liver transplant (LT) recipients measured before transplantation, one month after, and one year after transplantation, and in healthy adults, stratified by genotypes of the rs1800470 polymorphic allele of the TGFB1 gene, analyzed using allelic interaction models. $p < 0.05$

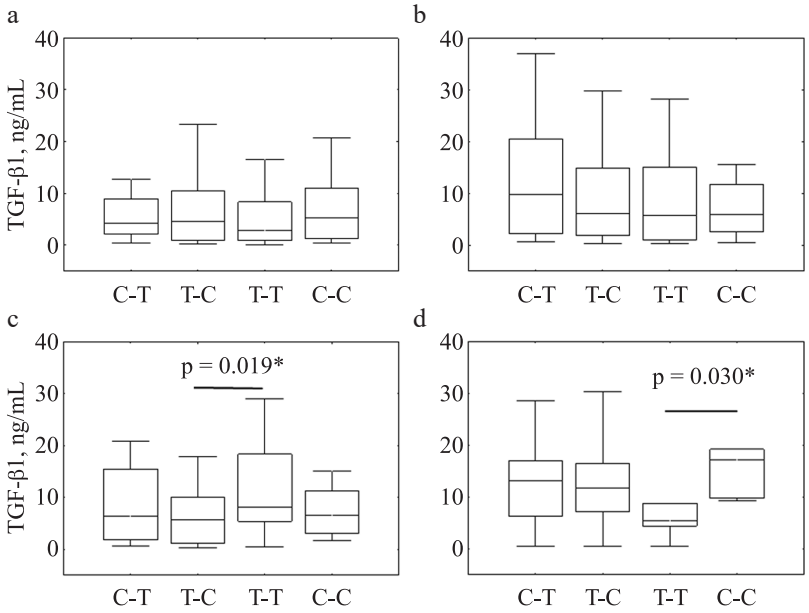

Fig. 6. TGF-β1 levels in pediatric liver recipients measured before (a), one month (b), and one year (c) after liver transplantation, as well as in healthy adults (d), stratified by haplotypes of the rs1800469–rs1800470 polymorphic variants of the TGFB1 gene. $p < 0.05$

lotypes (rs1800469 and rs1800470) of the TGFB1 gene in PLTRs.

LT was associated with a significant increase in TGF-β1 levels in pediatric recipients; however, cytokine concentrations did not reach those observed in healthy adults or, as shown previously, in healthy children of the same age group [5]. The distribution of the rs1800469 and rs1800470 polymorphic loci and their most common haplotypes (C-T and T-C) did not differ significantly between PLTRs and healthy individuals. In contrast, the rare haplotypes T-T and C-C were significantly more frequent among PLTRs than among healthy controls, corroborating previous findings [26] and suggesting a potential association between these rare haplotypes and the development of liver disease in PLTRs.

Comparison of TGF-β1 blood levels in PLTRs carrying different genotypes of the studied polymorphic loci and their haplotypes revealed no significant differences before or one month after LT. However, one year after LT, significant associations were observed: for rs1800469, carriage of the major allele (C/C + C/T) was associated with higher TGF-β1 levels, whereas carriers of the homozygous minor genotype (T/T) exhibited lower cytokine levels. Similarly, for rs1800470, the homozygous major genotype (T/T) was associated with higher, and carriage of the minor allele (T/C + C/C) with lower TGF-β1 levels.

Among pediatric recipients, carriers of the T-T haplotype (combining the minor allele of rs1800469 and the major allele of rs1800470) had higher TGF-β1 levels, while carriers of the T-C haplotype (both minor alleles) exhibited the lowest cytokine levels.

In healthy individuals, TGF-β1 levels did not differ significantly based on the individual carriage of rs1800469 or rs1800470 genotypes. However, differences emerged when rare haplotypes were analyzed: the C-C haplotype (major allele of rs1800469 and minor allele of rs1800470) was associated with higher, and the T-T haplotype with lower TGF-β1 levels – the opposite pattern compared to pediatric recipients.

These findings suggest that the T allele of rs1800469 may reduce, while the C allele of rs1800470 may enhance TGF-β1 production. The opposing effects of these polymorphisms may explain the relatively higher frequency of the T-C haplotype among healthy individuals, where the influence of the two variants appears to compensate each other, resulting in normal cytokine levels comparable to those associated with the major alleles.

The absence of differences in TGF-β1 levels in recipients before or one month after surgery can be attributed to the disruption of normal regulatory processes during these periods, which may be influenced by disease-related complications or drug therapies. It is also possible that, one year post-surgery, the patients' condition stabilizes, leading to partial normalization of TGF-β1 production regulation, despite the ongoing influence of immunosuppressive therapy. In healthy adults, cytokine regulation may differ significantly from that in pediatric recipients. For example, under normal conditions, TGF-β1 is produced by liver stellate cells at a basal le-

vel, with production increasing in response to activating factors [28].

Thus, our findings suggest that blood TGF-β1 levels in PLTRs may be influenced by the carriage of specific loci and haplotypes of the *TGFB1* gene, namely rs1800469 and rs1800470.

As mentioned in the introduction, no other studies have specifically examined the association between TGF-β1 levels and TGFB1 genetic polymorphism in PLTRs. When compared to data from adults with liver disease, our findings align with those of Chinese researchers [21] but differ from results observed in Brazilian and Egyptian populations [22–24]. Additionally, there are studies that did not find a significant association between cytokine levels and its gene polymorphism [12].

Interestingly, studies investigating the relationship between TGF-β1 levels and TGFB1 genetic polymorphism in other diseases also present mixed results. These studies can be divided into two groups: one group associates higher cytokine levels with the major alleles (C rs1800469 or T rs1800470) in diseases such as human papillomavirus infection [31], systemic lupus erythematosus [32], gastric adenocarcinoma [33], and rheumatoid arthritis [34], while the other group finds higher levels associated with the minor alleles (T rs1800469 and C rs1800470) in conditions like breast cancer [20, 29] and coronary artery ectasia [30].

The contradictory findings in these studies can be attributed to the pleiotropic nature of TGF-β1 cytokine, its complex regulation, variations in experimental designs, or differences in the ethnic backgrounds of the study populations. However, given the significant number of studies that have found an association between protein levels and gene polymorphisms, and the consistent division of results into two opposing trends (with high TGF-β1 levels being associated either with major or minor alleles), it is reasonable to assume that such an association exists. Yet, depending on various factors, this association could be either direct or inverse.

One such factor could be the stage of disease progression when TGF-β1 levels are measured. For example, cytokine levels may fluctuate significantly depending on the severity of liver fibrosis [5, 23]. An initially low or high cytokine production, which may contribute to the disease pathogenesis, could either increase or decrease over time, potentially reflecting the progression of the disease rather than causing it. Therefore, while the studies reviewed suggest an association between TGF-β1 levels and its gene polymorphism, they do not conclusively answer the question of causal relationships.

The retrospective nature of this study, its reliance on the case-control method, and the genetic heterogeneity of the sample may present certain limitations to the conclusions drawn. Most phenotypic traits are influenced by numerous genetic loci, making it challenging to isolate the contribution of a single locus. Additionally, the haplotype occurrence analysis performed in this study is approximate, as precise haplotype determination for heterozygous variants requires sequencing. Further research is necessary to confirm the findings presented here.

Despite these limitations, the results of this study suggest a potential association between blood TGF-β1 levels and carriage of polymorphic loci and haplotypes rs1800469 and rs1800470 of the TGFB1 gene. As a critical regulator of fibrosis and immune response, TGF-β1 may play a significant role in the regulation of protein levels in PLTRs. These findings open up new avenues for understanding protein regulation and may position the studied variants as potential prognostic markers for complications in PLTRs.

CONCLUSION

Elevated TGF-β1 levels in the blood of PLTRs one year after LT are associated with carriage of the major alleles – C rs1800469 and T rs1800470, as well as the T-T haplotype of the TGFB1 gene. This finding suggests that these polymorphic loci may influence the development of post-transplant complications, highlighting their potential use as predictive markers for transplant outcomes.

The authors declare no conflict of interest.

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The article was submitted to the journal on 17.03.2025