

# Genetic characteristics of the course of chronic hepatitis

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**Abstract.** The aim of the study was a comparative analysis of genotypic variants of TNF- $\alpha$  and CTLA-4 genes in patients with chronic hepatitis C and liver cirrhosis and their effect on the course of the disease. The analysis of the effect of TNF- $\alpha$  gene genotypes on the course of the disease indicates an increased risk of CVHC development with both moderate and highly active course of the disease in carriers of the G / A + A / A genotype combination. The data obtained allow us to conclude that the carriage of the “G” allele and the combination of genotypes A / G + G / G of the –A49G polymorphism of the CTLA-4 gene are associated not only with a decrease in the risk of CVHC development, but also with a lower intensity of inflammation and fibro-formation in the liver and a high probability of favorable course of the disease.

## 1 Introduction

The problem of the spread and treatment of chronic viral hepatitis C continues to be one of the significant problems of internal medicine. The urgency of the problem of hepatitis C is determined by the high epidemiological and socio-economic significance of this disease, as well as the widespread occurrence, severity of the course and the frequency of development of chronic forms [1-4]. It is HCV infection that is the main reason for the formation of the entire group of chronic liver diseases - chronic hepatitis, cirrhosis, hepatocarcinoma [5-7]. At the same time, the study of this issue is not only medical, but also socio-economic in nature. It is believed that chronic hepatitis C is always potentially dangerous, but the pathogenesis of

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this disease is not fully understood. This will require a search for new approaches and new fundamental research [8-10]. An active study of the genetic basis of predisposition to various infectious diseases has collected a sufficient amount of information indicating the importance of dysfunction of anti-inflammatory cytokines (TNF- $\alpha$ ) and cytotoxic T-lymphocytes (CTLA-4) in the genesis of viral liver diseases [11-13]. The influence of genetic factors determines the immune status, in this regard, it is the immunogenetic aspects that determine the features of the development and progression of viral hepatitis [14, 15].

## **2 Aim of the research**

The aim of the study was a comparative analysis of genotypic variants of TNF- $\alpha$  and CTLA-4 genes in patients with chronic hepatitis C and liver cirrhosis and their effect on the course of the disease.

## **3 Materials and methods**

The main group of the study included 107 patients with chronic viral hepatitis C (CVHC). To assess the association of polymorphic markers of TNF-4 and CTLA-4 genes, patients with CVHC were divided into three subgroups. The first subgroup includes patients with moderate activity, chronic hepatitis C (CHC) (n = 33). The second subgroup consisted of patients with a high degree of CHC activity (n = 37). The third subgroup included patients with liver cirrhosis (n = 37). The criteria for inclusion in the study were clinical, biochemical and instrumental verification of the diagnosis with the definition and stage and severity of the disease, as well as the detection of hepatitis C virus RNA by the AmpliSens® HCV-FRT test system detected by polymerase chain reaction (PCR) on a RotorGene 6000 device.

As a comparison group, a population control was used, which was represented by DNA samples (n = 81) of apparently healthy donors without chronic liver infection.

The material for the molecular genetic study was the peripheral blood of the examined persons. DNA isolation was carried out according to the standard procedure with some modifications and using the reagents of the “Interlabservice” company (Russia). Identification of alleles of gene polymorphism was carried out using polymerase chain reaction.

Molecular genetic examination of biomaterials (DNA) was performed in the period 2015-2018 on the basis of the Department of Molecular Medicine and Cellular Technologies of the Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan.

## 4 Results and discussion

Comparative analysis of genotypic variants of the TNF- $\alpha$  marker -G308A revealed the accumulation of A / G and A / A genotypes in the group of patients with chronic hepatitis C and recorded differences in the distribution of these genotypes between patients and individuals of the control group. The total frequency of genotypes G / A + A / A in the study and control groups was 24.3% and 9.9%, respectively (tab.1). The calculated total contribution of these genotypes to the development of CVHC turned out to be highly significant, while the odds ratio was OR = 3.0 ( $X^2 = 6.4$ ;  $P = 0.01$ ; OR = 3.0; 95% CI 1.248-6.875), which indicates a 3-fold increase in the risk of developing CVHC in carriers of this combination of genotypes.

Also, significant differences in the total frequency of genotypes containing the mutant allele "A" were found between the control group (9.9%) and patients of subgroups I and II (30.3% and 25.3%, respectively). At the same time, the risk of developing CVHC in a moderately active form in carriers of these genotypes was 4 times higher ( $X^2 = 7.3$ ;  $P = 0.007$ ; OR = 4.0; 95% CI 1.401-11.24), and highly active CVHC was 3 times higher ( $X^2 = 4.3$ ;  $P = 0.04$ ; OR = 3.0; 95% CI 1.029-8.358) compared with representatives of the general population sample that do not contain this combination of genotypes. The total share of G / A + A / A genotypes in the subgroup of patients with liver cirrhosis were also higher than in the control sample (18.9% versus 9.9%, respectively). However, despite the fact that the odds ratio, reflecting the degree of risk of developing cirrhosis for a given genotype, turned out to be higher than one (OR = 2.1), this difference did not reach the level of statistical significance ( $X^2 = 1.9$ ;  $P = 0.2$ ; OR = 2.1; 95 % CI 0.7089-6.395).

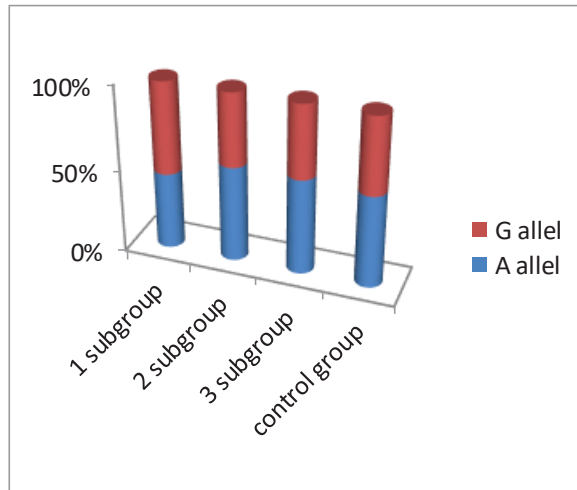
Our data indicate an increased risk of developing CVHC with both moderate and highly active course of the disease in carriers of a combination of genotypes G / A + A / A, but they do not confirm the relationship between the carriage of this combination of genotypes and the development of CVHC with an outcome in cirrhosis. To assess the effect of genotypes G / A + A / A on the severity of CVHC and the development of an unfavorable outcome, we also compared the frequency of this combination between different subgroups. It turned out that the frequency of the combination of genotypes G / A + A / A decreases linearly with an increase in the activity of the pathological process in the liver and the development of cirrhosis, however, the difference in the indicator between the studied subgroups turned out to be insignificant ( $p > 0.05$ ). The most likely reason for the observed phenomenon is that, as mentioned above, as a result of the expression of the TNF- $\alpha$  gene with such genotypes, a smaller amount of tumor necrosis factor with normal activity will be produced, and, therefore, its effect as an activator of the fibrogenesis process will be weakened. This was confirmed by the fact that in the subgroup of patients with a moderate degree of CVHC

activity, a significant predominance of the homozygous genotype A / A (12.1% versus 2.5%) was revealed in comparison with the control group. In subgroups of patients with highly active CVHC and liver cirrhosis, the difference in the frequency of occurrence of the A / A genotype with that in the healthy population was statistically insignificant ( $p > 0.05$ ).

**Table 1.** Allele frequency and genotype distribution of TNF- $\alpha$  gene polymorphism -G308A in patients with HCV, liver cirrhosis and healthy donors.

Name of group	N	Frequency distribution of genotypes TNF - $\alpha$							
		G/G		G/A **		A/A ***		G/A + A/A ****	
		n	%	N	%	N	%	N	%
Main Group	107	81	75.7	18	16.8	8	7.5	26	24.3
1- subgroup: moderately active HCV	33	23	69.7	6	18.2	4	12.1	10	30.3
2- subgroup: highly active HCV	37	28	75.7	7	18.9	2	5.4	9	25.3
3-subgroup: cirrhosis of the liver	37	30	81.1	5	13.5	2	5.4	7	18.9
Control group (healthy donors)	81	73	90.1	6	7.4	2	2.5	8	9.9

The excess of heterozygotes (negative value of D) in our study was recorded in almost all groups. An excess of heterozygotes in all clinical groups indicates a deviation from the equilibrium state – recessive alleles in the heterozygous state escape the action of selection directed against them. At the same time, the indicators in the main group and the control group were almost identical. However, there was a variation in the values of indicator D between the subgroups of patients with HCV. The highest deviation of expected and observed heterozygosity was found in the group of patients with moderately active HCV (-0.455) and the lowest in the group of patients with highly active HCV (0.213). Thus, the group of patients with cirrhosis of the liver demonstrated the least expected and observed heterozygosity, but the D index was the lowest in the group of patients with a moderately active form of HCV.



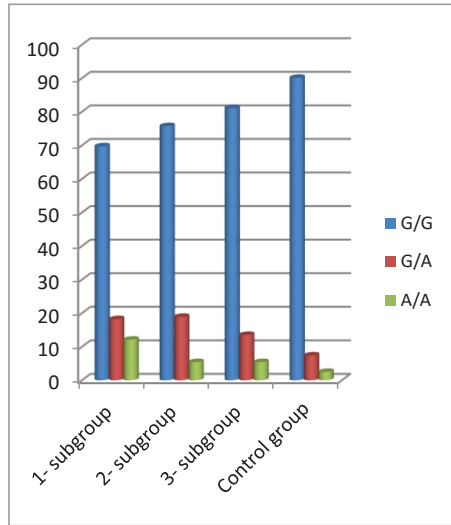
**Fig.1.** Allele frequency of CTLA-4 polymorphism -A49G in patients with HCV, liver cirrhosis and healthy donors.

The study also investigated the total frequency of the genotypes of the CTLA-4 gene containing the mutant allele (A / G + G / G) for a more complete representation of the role of this allele.

It was shown that in the main and control groups the total frequency of A / G + G / G was 74.8% and 67.9%, respectively (fig.2).

However, even the total contribution of genotypes carrying the mutant allele "G" to the development of CVHC turned out to be insignificant ( $X^2 = 1.1$ ;  $P = 0.3$ ; OR = 1.4; 95% CI 0.7396-2.653), which indicates that there is no increase in the risk of CVHC development in carriers of this combinations of genotypes.

Differences in the total frequency of genotypes containing the mutant allele "G" were also insignificant between the control group (67.9%) and patients of II and III subgroups (70.3% and 67.6%, respectively) (fig.1). And only in patients with moderately active CVHC, the difference of this indicator with the control (87.8% versus 67.9%) was statistically significant. It should be noted that a significant difference in the total frequency of genotypes containing the mutant allele was also noted between patients with moderately active CVHC and patients with liver cirrhosis. This fact is another confirmation of the connection between the accumulation of the mutant allele "G" of the -A49G polymorphism of the CTLA-4 gene with a low activity of the inflammatory process and fibrosis in the liver.



**Fig.2.** Genotype distribution of CTLA-4 polymorphism -A49G in patients with HCV, liver cirrhosis and healthy donors.

Analysis of the deviation of expected and observed heterozygosity in the study of polymorphism -A49G of the CTLA-4 gene in the control group of conditionally healthy individuals and patients with HCV showed that an excess of heterozygotes with a minimum negative value of D was recorded in the group of control subjects (-0.077) and in the group of patients with cirrhosis of the liver (-0.008). The excess of heterozygotes in these groups indicates a deviation from the equilibrium state. It is interesting to note that the D index in the main group of patients, as well as in the first and second subgroups, had a positive value, which indicates a lack of heterozygotes in these samples. At the same time, the highest value of D was observed in the group of patients with moderately active HCV (0.345), and the deficiency of heterozygotes was 3.3 times greater than in patients with progressive HCV and 7.6 times greater than in patients with irreversible liver changes. Apparently, in this case, the deficiency of heterozygotes in the sample is due to differential selection acting in favor of homozygotes.

## 5 Conclusion

The obtained results showed the existence of a difference in the frequency of occurrence of allelic variants of the studied polymorphisms in patients with favorable and unfavorable

course of HCV and varying degrees of activity in the liver of the pathological process caused by infection of viral hepatitis type C.

An analysis of the scientific literature on the effect of polymorphism - G308A of the TNF- $\alpha$  promoter region on the development of chronic hepatic pathology shows that most researchers believe that this polymorphism affects the development and course of C-viral hepatitis. The results of our study are consistent with the data of proponents of ideas about the significant pathogenetic role of the TNF- $\alpha$  gene in the course of hepatitis.

The data obtained allow us to conclude that the carriage of the “G” allele and the combination of genotypes A / G + G / G of the -A49G polymorphism of the CTLA-4 gene are associated not only with a decrease in the risk of CVHC development, but also with a lower intensity of inflammation and fibro-formation in the liver and a high probability of favorable course of the disease.

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