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## Genetic Factors Predisposing to a Chronic Course of Virus Hepatitis and Liver Fibrosis

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**Abstract**—The *IL4* C(–590)T, *IL4RA* Ile50Val, and *TNF* G(–308)A polymorphisms were tested for association with the chronic development of virus hepatitis, the extent of which was inferred from the liver fibrosis stage. The frequency of allele A of the *TNF* G(–208)A polymorphism in patients with mild fibrosis was higher (24.5%) than in patients with moderate or severe fibrosis (13.4%) or cirrhosis (8.7%). The frequency of heterozygous genotype CT of the *IL4* C(–590)T polymorphism significantly differed between cirrhosis (68.2%) and moderate or severe fibrosis (39.1%).

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### INTRODUCTION

Liver fibrosis is an excessive accumulation of collagen in the liver and typically arises in response to chronic liver damage, which is caused by various exogenous (persistent virus or helminth infection and alcohol intoxication) and endogenous (hereditary defects in metal metabolism and other genetic factors determining the response to various agents) factors [1, 2].

The specifics of the genetic regulation of the production and degradation of collagen in carriers of certain genotypes may underlie genetic predisposition to active fibrogenesis and rapid disease progression upon exposure to damaging agents. Some mutations and polymorphic variants of genes have been associated with the fibrogenesis rate in chronic virus hepatitis. For instance, rapid disease progression is associated with *HFE* (hemochromatosis) and/or *TFR1* (transferin receptor 1) mutations leading to an excessive accumulation of iron in liver tissue [3]. Genetic  $\alpha$ 1-antitrypsin deficiency, caused by a mutation of the proteinase inhibitor (Pi) gene, also facilitates the rapid progression of fibrosis. A higher frequency of heterozygous genotype MZ of the Pi gene have been observed in patients with severe liver diseases [4].

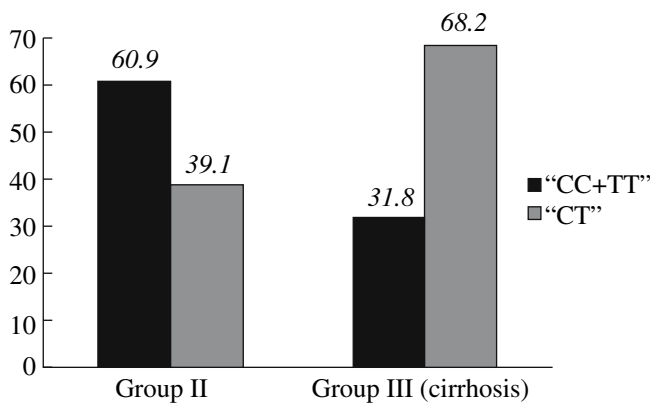
Polymorphic variants of immune response genes have been extensively studied in the recent years. Ample data associating the polymorphic markers of various immune system genes with chronic virus hepatitis (CVH), features of its course, and complications

demonstrate that an adequate immune response, which depends on the genetic characteristics of the host organism, play an important role in the development of infection [5]. Interleukins (ILs) are not only involved in the immune response, but they also modulate the activity and metabolism of the enzymes that determine collagen production in the liver. For instance, synthesis of the proteolytic enzyme collagenase is regulated by IL1, IL6, IL10,  $\gamma$ - and  $\beta$ -interferons, transforming growth factor  $\alpha$ , tumor necrosis factor  $\alpha$  (*TNF- $\alpha$* ), while its suppression depends on IL4, IL11, and IL13 [6, 7].

The objective of this work was to test the polymorphisms of some immune system genes involved in controlling collagen synthesis (*IL4* C(–590)T, *IL4RA* Ile50Val, and *TNF* G(–308)A) for association with the extent of fibrosis in CVH. This work was performed with additional genetic polymorphisms and a larger sample as compared with our previous analysis of the association of *IL4RA* Ile50Val with CVH [8].

### EXPERIMENTAL

The sample included 130 CVH patients. The inclusion criteria were type B and/or C CVH serological markers detected in the serum by ELISA (with an ELISA kit) and hepatitis B virus DNA or hepatitis C virus RNA detected by the polymerase chain reaction (PCR). The diagnosis was morphologically verified in all patients, with the histological activity score esti-



Frequencies of genotypes CC + TT and CT of the *IL4* C(-590)T polymorphism in CVH patients with moderate or severe fibrosis (II) or cirrhosis (III).

mated according to Knodell et al. [9] and the fibrosis stage assessed according to Desmet et al. [10]. To test the polymorphisms for association with the fibrosis stage, the CVH patients were stratified into three groups. Group I included 44 patients with mild fibrosis (stage I). Group II included 64 patients with moderate or severe fibrosis (stages II and III, respectively). Group III included 22 patients with liver cirrhosis (fibrosis stage IV).

The association with CVH and fibrosis stage was studied for the three polymorphisms of the immune system genes involved in controlling collagen synthesis, i.e., C(-590)T of *IL4*, Ile50Val of *IL4RA*, and G(-308)A of *TNF*.

DNA of peripheral blood lymphocytes was isolated by the standard nonenzymatic method [11]. Genotyping was performed by restriction fragment length polymorphism (RFLP) analysis of PCR products [12, 13].

The genotype frequencies of the three polymorphisms were tested for correspondence to the Hardy-Weinberg equilibrium by the Pearson  $\chi^2$  test [14]. The allele and genotype frequencies of particular groups were compared by the  $\chi^2$  test with Yates correction for continuity at one degree of freedom and by Fisher's exact test.

## RESULTS AND DISCUSSION

The extent of liver fibrosis in CVH was associated with the *IL4* C(-590)T and *TNF* G(-308)A polymorphisms, located in the promoter regions. Group III with cirrhosis differed in genotype frequencies of the *IL4* C(-590)T polymorphism from group II with moderate or severe fibrosis ( $p = 0.047$ ): the frequency of heterozygous genotype CT was higher, while the frequencies of homozygous genotypes CC and TT were lower in cirrhosis patients ( $\chi^2 = 4.47$ ,  $p = 0.30$ ; figure).

A difference in *IL4* C(-590)T genotype frequencies has been observed for HIV-infected patients: genotype TT occurred at a frequency reaching 10% in the patients, but was not detected in healthy subjects [15]. In our sample of CVH patients from Tomsk, the frequency of genotype TT averaged 6.7% and varied from 0 (group III) to 9.1% (group I), depending on the fibrosis state (table).

Allele and genotype frequencies of the polymorphisms in CVH patients differing in fibrosis stage

Gene	Polymorphism	Genotype	Fibrosis stage						<i>p</i>		
			I		II		III		I and II	I and III	II and III
			n	%	n	%	n	%			
<i>IL4</i>	C-590T	CC	16	36.4	35	54.7	7	31.8	0.172*	0.279*	<b>0.047*</b>
		CT	24	54.5	25	39.1	15	68.2			
		TT	4	9.1	4	6.2	0	0			
		T	32	36.4	33	25.8	15	34.1			
<i>IL4RA</i>	Ile50Val	Ile/Ile	19	36.5	20	26.7	9	34.6	0.129*	0.603*	0.752*
		Ile/Val	29	55.8	40	53.3	13	50			
		Val/Val	4	7.7	15	20	4	15.4			
		Val	37	35.6	70	46.7	21	40.4			
<i>TNF</i>	G-308A	GG	29	59.2	54	76	19	82.6	0.134*	0.135*	0.870*
		GA	16	32.6	15	21.2	4	17.4			
		AA	4	8.2	2	2.8	0	0			
		A	24	24.5	19	13.4	4	8.7			

Note: *n* is the observed number of genotypes and alleles; *P* is the significance level obtained by the  $\chi^2$  and (\*) Fisher's exact tests.

Allele T, which is associated with an elevated production of IL4, predisposes to atopic dermatitis, asthma, and rheumatoid arthritis [16]. Acting as a key anti-inflammatory cytokine, IL4 limits the spreading and intensity of inflammation; inhibits the production of the proinflammatory cytokines IL6, IL8, IL12, and TNF- $\alpha$  in macrophages; and reduces the generation of highly reactive metabolites of oxygen and nitrogen. The absence of genotype TT from the group of cirrhosis patients can be explained by the fact that IL4 deficiency facilitates intense inflammatory reactions, activation of apoptosis in mononuclear cells and hepatocytes, and, consequently, disease progression. The higher frequency of heterozygous genotype CT in this group cannot be explained in terms of the biochemical processes associated with the different alleles. It seems better to consider the function of the promoter regions in this case. The structural polymorphism of the promoter region possibly affects the efficiency and specificity of transcription factor binding. In turn, this may affect the production of the ultimate gene product and, eventually, the specificity of cell reactions and the response of the body to various agents.

In addition, the CVH patients with different fibrosis stages differed in the frequency of allele A of the *TNF G(-308)A* polymorphism. The frequency of allele A decreased with fibrosis progression from mild (group I, 24.5%) to cirrhosis (group III, 8.7%; table).

Allele A of the *TNF G(-308)A* polymorphism is probably associated with a higher production of TNF- $\alpha$  both in vitro and in vivo [17]. TNF- $\alpha$  is a key proinflammatory cytokine that plays an important role in local and systemic pathological processes by triggering a cascade of inflammatory reactions and facilitates induction of necrosis and apoptosis in hepatocytes [18]. Based on these data, it is possible to assume that allele A of the *TNF G(-308)A* polymorphism is associated with a high rate of fibrogenesis and disease progression. However, our findings indicate that only mild liver fibrosis develops in virus hepatitis patients carrying allele A. The frequency of allele A was elevated in patients with fibrosis stage I, which must be accompanied by a high-level production of TNF- $\alpha$ . The lowest frequency of allele A was observed in cirrhosis patients. This finding needs further investigation and studies of the association between the *TNF G(-308)A* genotypes with TNF- $\alpha$  production in mononuclear cells.

It should be noted that the available data on the effect of *TNF* polymorphisms on the histological severity of the disease are discrepant. The *TNF* polymorphisms at -308 and -238 have not been associated with disease stage [19]. On the other hand, these polymorphisms are thought to affect the risk of cirrhosis in type C CVH patients [20]. For instance, the frequency of allele A in Caucasian patients with biliary cirrhosis is significantly lower than in healthy individuals [21].

To summarize, we showed that *IL4 C(-590)T* genotype CT, associated with cirrhosis, predisposes to a severe course of CVH, while *TNF G(-308)A* allele A, associated with mild fibrosis, determines a more favorable course.

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