

GENOMICS.
TRANSCRIPTOMICS. PROTEOMICS

UDC 575.113:616-002.5

Association of the Ile50Val Polymorphism of the Interleukin-4 Receptor Gene *IL4RA* with Chronic Viral Hepatitis

I. A. Goncharova¹, M. B. Friedin¹, L. E. Dunaeva²,
E. V. Beloborodova³, E. I. Beloborodova², and V. P. Puzyrev¹

¹*Institute of Medical Genetics, Tomsk Research Center, Siberian Division,
Russian Academy of Sciences, Tomsk, 634050 Russia
e-mail: irgon@img.tsu.ru, irgon67@mail.ru*

²*Department of Therapy, Siberian State Medical University,
Tomsk, 634050 Russia*

³*Department of Hospital Therapy, Siberian State Medical University,
Tomsk, 634050 Russia*

Received September 20, 2004

Abstract—The Ile50Val polymorphism of the *IL4RA* gene was tested for association with chronic viral hepatitis and the character of its progression (the stage of hepatic fibrosis). In total, 61 patients were examined. The control group was a random sample of Tomsk residents ($N = 128$). Genotyping was based on RFLP analysis. The allele and genotype frequencies of the Ile50Val polymorphism did not significantly differ between the patients and the controls. However, a significant difference in genotype frequency distribution was observed for the patients with different stages of hepatic fibrosis. The frequency of heterozygotes Ile/Val in patients without signs of fibrosis was lower than in the control group (7.1% vs. 51.6%, $P = 0.002$), while the frequency of the homozygous genotypes was higher. In addition, this subgroup significantly differed in genotype frequency distribution from subgroups of patients with early or severe fibrosis ($P = 0.035$ and $P = 0.004$, respectively).

Key words: chronic viral hepatitis, genetic polymorphism, Ile50Val polymorphism, interleukin-4 receptor gene (*IL4RA*)

INTRODUCTION

Studies of the association between genetic factors and predisposition to various diseases have identified the genetic status of the macroorganism as an important factor in individual response to viral infection. Polymorphic variants of some genes have been associated with predisposition to AIDS (*CCR5*), tuberculosis (*HLA-DR*, *NRAMP1*, and *VDR*), leprosy (*HLA-DR* and *TNF*), malaria (*DARC*, *G6PD*, and *iNOS*), leishmaniasis (*TNF*), and pneumococcal infection (the C-reactive protein gene) [1–7]. One of the most pressing problems is identification of the genetic determinants that affect susceptibility, individual features of disease progression, and the outcome in viral hepatitis. This problem is of immense importance because hepatitis is widespread, has a progressive course, and leads to severe complications such as hepatic cirrhosis and hepatocellular carcinoma [8]. According to the WHO, the cohort infected with the hepatitis C virus increased to 3% of the global population (200 million people) in the 15 years that elapsed after the virus was first described; the incidence of this disease is still increasing. In Russia, more than 1.1 million people were diagnosed for the first time as carriers of the hep-

atitis B and hepatitis C viruses between 2000 and 2003 [9].

In view of the above, attention now focuses on the cytokine genes, which play a major role in the development and regulation of defense mechanisms. Infection with the hepatitis virus activates both Th1 and Th2 cells; their ratio observed at the early stage of the disease affects the outcome [10]. The cytokine-secreting activity of Th1 cells dominates in patients recovering from acute hepatitis. When the virus persists and infection becomes chronic, predominance is observed for Th2 cells (IL-4, IL-5, and IL-10), which have an anti-inflammatory activity and are involved in regeneration and fibrogenesis [11].

Various, including viral, diseases of the liver are accompanied by an increase in serum IL-4 [12]. IL-4 acts as a T-cell growth factor, provides the major signal for differentiation of CD4⁺ T cells into Th2 cells, affects antibody production, restricts generalization and intensity of inflammation, and stimulates fibroblasts [12]. As a result, IL-4 is potentially capable of regulating the fibrogenic activity.

The key role in the function of IL-4 is played by its receptor (IL-4R), which is present on IL-4 target cells

Table 1. Characterization of the patients examined

Parameter	Patients with chronic viral hepatitis		
	males (N = 42)		females (N = 19)
Mean age, years	30.8 ± 1.39		
Virus	HCV (N = 42)		HBV (N = 13) HBV + HCV (N = 6)
Virus genotype	1b (n = 11)	non-1b (N = 8)	– –
Histological activity, score	2–18		2–9 6–14
Stage of hepatic fibrosis	0–3		0–2 0–2

Note: HCV, hepatitis C virus; HBV, hepatitis B virus; 1b, HCV genotype 1b; non-1b, other HCV genotypes.

and consists of two subunits, α and γ . Activating intracellular messengers, IL-4R induces expression of the genes that are sensitive to the IL-4 signal. Analysis of the competence of the IL-4 and IL-4R genes has associated their variants with atopy [13], bronchial asthma [14, 15], type 1 diabetes mellitus [16], and infections such as AIDS [17] and respiratory syncytial virus disease [18].

The above data make it possible to assume that the IL-4R α subunit gene, *IL4RA*, affects predisposition to viral hepatitis, virus persistence, and development of the chronic process in the liver. In this work, we tested the Ile50Val polymorphism of *IL4RA* for association with chronic viral hepatitis and the character of its course, depending on the stage of hepatic fibrosis.

EXPERIMENTAL

We examined 61 chronic viral hepatitis patients aged 17–56 years (Table 1). The diagnosis was based on the clinical data and the results of laboratory and instrumental tests, including biopsy of the liver. To verify the etiological diagnosis, blood serum was tested for hepatitis B virus (HBV) DNA and hepatitis C virus (HCV) RNA by PCR and for serological markers by ELISA.

HCV was detected in 42 patients. The HCV genotype was assayed in 20 patients. Of these, 11 showed HCV genotype 1b and 9 presented with other variants, which were not identified in this work and were collectively designated as the non-1b genotype (Table 1).

HBV was found in 13 patients. Combined infection with HCV and HBV was detected in six patients. In addition, we verified the diagnosis morphologically and estimated the histological activity [19] and the stage of fibrosis [20].

To study the association of the Ile50Val polymorphism of *IL4RA* with disease progression, the patients were divided into three subgroups differing in fibrosis stage. Group 0 included patients without signs of fibrosis; group I, patients with early (portal and periportal) fibrosis; and group II, patients with moderate (stage 2, porto-portal septa) or severe (stage 3, porto-central septa) fibrosis.

The control group was a random sample of 128 Tomsk residents.

DNA was isolated from blood lymphocytes by the standard nonenzymatic method [21]. Genotyping with respect to the Ile50Val (A148G) polymorphism of *IL4RA* was performed by RFLP analysis of PCR products according to a published protocol [22, 23].

The genotype frequency distribution was checked for the Hardy–Weinberg equilibrium by the χ^2 test. To compare the main groups with respect to allele and genotype frequencies, we used the χ^2 test with Yates correction for continuity and unity taken as the number of degrees of freedom. To compare genotype frequencies for the subgroups differing in fibrosis stage, contingency tables (3 × 2) were analyzed using Fisher's exact test. One-way analysis of variance was used to compare the subgroups with respect to mean values of independent variables such as fibrosis stage and disease record [24]. Computations were performed using the programs STATISTICA for Windows, Microsoft Excel and Graph PAD Instat (Graph PAD Software ver. 1.12a).

RESULTS AND DISCUSSION

The genotype frequency distribution of the Ile50Val polymorphism in the Tomsk residents obeyed the Hardy–Weinberg equilibrium. The frequency of allele Ile50 was about 59%, much the same as reported for Caucasoids [25]. The allele diversity estimated from the observed heterozygosity was similar to its maximal value possible for a dinucleotide polymorphism ($P > 0.05$).

As in the control group, *IL4RA* genotype frequencies corresponded to the Hardy–Weinberg proportions in the patients (Table 2). However, in the patients, the observed heterozygosity tended to be lower than the expected heterozygosity. The observed and expected heterozygosities did not significantly differ within either group (Table 2) or between the patients and controls ($P > 0.05$).

Genetic markers can affect general predisposition to a disease or be associated with particular, pathogenetically important traits. In view of this, we studied

Table 2. Genotype and allele frequencies of the *IL4RA* Ile50Val polymorphism in patients with chronic viral hepatitis and healthy residents of Tomsk

Group	n	Genotypes			χ^2*	P*	Ile	χ^2***	P**	H_o	H_e	D	χ^2****	P****
		Ile/Ile	Ile/Val	Val/Val										
Controls	128	42 (32.8%)	66 (51.6%)	20 (15.6%)	1.924	0.378	0.59 ± 0.03	0.171	0.678	0.52 ± 0.04	0.48 ± 0.01	0.062	0.266	0.90
Patients	61	21 (34.4%)	26 (42.6%)	14 (23%)			0.56 ± 0.04			0.43 ± 0.06	0.49 ± 0.01	-0.136	0.539	0.80

Note: The χ^2 test was used to estimate the significance of differences in (χ^2*) genotype or (χ^2**) allele frequencies and (χ^2****) to test the genotype frequency distribution for correspondence to the Hardy–Weinberg equilibrium. The significance is characterized for the differences in (P*) allele or (P**) genotype frequencies and (P****) correspondence to the Hardy–Weinberg equilibrium. H_o and H_e , observed and expected heterozygosities, respectively; D, relative departure of the observed heterozygosity from the expected value.

Table 3. Stage of hepatic fibrosis in patients with chronic viral hepatitis as dependent on the type of hepatitis and the HCV genotype

Subgroup of patients		Fibrosis stage			P*
		0	I	II	
Virus	HCV	7 (16.7%)	15 (35.7%)	20 (47.6%)	0.083
	HBV	7 (53.8%)	3 (23.1%)	3 (23.1%)	
	HBV + HCV	1 (16.7%)	3 (50%)	2 (33.7%)	
HCV genotype	1b	0	1 (9%)	10 (91%)	0.347
	non 1b	0	2 (25%)	6 (75%)	

Notes: * The significance was evaluated using the χ^2 test.

Table 4. Stage of hepatic fibrosis in patients with chronic viral hepatitis as dependent on disease record (mean ± S.E.)

Subgroup by fibrosis stage	Disease record, years	P*
0	7.1 ± 1.5	0.403
I	5.9 ± 0.64	
II	7.5 ± 0.78	

Notes: * The significance was evaluated using one-way analysis of variance.

the effect of the Ile50Val polymorphism on the character of disease progression (fibrosis stage), although the allele and genotype frequencies of this polymorphism did not significantly differ between the patients and controls (Table 2). The fibrosis stage depends on several factors, such as the type of hepatitis, the genotype of the virus, and the disease record. However, we did not detect any association with these factors (Tables 3, 4).

The *IL4RA* allele frequencies did not significantly differ between the subgroups differing in fibrosis stage (Table 5).

Both alleles, which often affect the prognosis, and genotypes can be of functional importance for genetic predisposition to a particular disease.

It is individual genotypes that are subject to selection in natural populations, and the fitness of a population depends on the contributions of various genotypes. If a disease is a selective factor affecting the fitness in carriers of a particular genotype, then differences in genotype frequencies between patients and healthy people are of considerable interest. Concerning changes in genotype frequencies of the Ile50Val polymorphism, published data are discrepant. A decrease and an increase in the frequency of heterozygotes have been variously reported for asthma patients from the Japanese population [22, 26, 27]. In Tomsk, heterozygotes Ile/Val tend to accumulate from grandparents to patients with bronchial asthma in affected families: their frequencies are 37.7% in grandparents, 52.2% in parents, and 55.4% in probands [28].

In our sample, the allele frequencies were much the same in the subgroups of patients, but the genotype frequencies differed (Table 5). A deviation from the Hardy–Weinberg proportions was observed for the genotype frequency distribution of the patients showing no signs of fibrosis ($\chi^2 = 10.2681$, d.f. = 1, $P = 0.0013$). The deficiency of heterozygotes Ile/Val was about 86% ($D = -0.8564$). Compared with the control group, this subgroup again showed a significantly lower frequency of heterozygotes (7.1% vs. 51.6% in the controls, $P = 0.002$) and differed in genotype fre-

Table 5. Genotype and allele frequency distributions of *IL4RA* in patients with chronic viral hepatitis and different stages of hepatic fibrosis

Subgroup by fibrosis stage		Frequency				
		genotype			allele	
		Ile/Ile	Ile/Val	Val/Val	Ile	Val
0 (<i>n</i> = 14)		7 (50%)	1 (7.1%)	6 (42.9%)	15 (53.6%)	13 (46.4%)
I (<i>n</i> = 21)		7 (16.7%)	10 (47.6%)	4 (19%)	24 (57.1%)	18 (42.9%)
II (<i>n</i> = 26)		7 (26.9%)	15 (57.7%)	4 (15.4%)	29 (55.8%)	23 (44.2%)
<i>P</i>	0 and I	0.035*			0.768**	
	0 and II	0.004*			0.851**	
	I and II	0.787*			0.893**	

Note: Subgroup 0, patients without signs of fibrosis; subgroup I, patients with early fibrosis; and subgroup II, patients with the second or third stage of fibrosis. The significance was evaluated using (*) Fisher's exact test or (**) the χ^2 test.

quencies from the subgroups with early or severe fibrosis (Table 5). In the subgroups of fibrosis patients, the genotype frequency distributions obeyed the Hardy–Weinberg equilibrium. The frequencies of heterozygotes were, respectively, 47.6 and 57.7%, much the same as in the control groups and higher than in the patients without signs of fibrosis (Table 5).

Notwithstanding the lack of differences in heterozygote frequencies between the control subjects and the fibrosis patients, the above findings make it possible to assume that genotype Ile/Val is prognostically valuable, predisposing to fibrosis in chronic viral hepatitis. There are several arguments for this assumption. First, it is known that some alleles and genotypes are widespread in human populations, regardless of their association with various pathologies. For instance, pathological allele 667T of the methylene tetrahydrofolate reductase gene (*MTHFR*) contributes to various diseases (thrombophilia, cardiovascular disorders, and pregnancy complications), but its frequency in Caucasoid populations varies from 15% in the United Kingdom to 55% in Spain [29]. The frequency of the null genotype of the glutathione S-transferase μ gene (*SGTM1*) in Caucasoids greatly varies, reaching 73% in some populations [30]. Yet this genotype, which determines the complete absence of an enzyme involved in the first phase of xenobiotic detoxification, plays a role in pathogenesis of various forms of cancer and is associated with a higher risk of various high-incidence diseases [31]. Population frequencies of pathology-associated alleles or genotypes are maintained at a high level probably because their carriers have certain advantages. Recent studies have shown, for instance, that carriers of the null genotype of *SGTM1* have a lower risk of myocardial infarction [32]. Second, only one full decade elapsed from the discovery of HCV and the wide spreading of hepatitis C: this period is too short for the disease to exert a considerable effect on the genotype frequency distribution.

Thus, alleles and genotypes associated with pathologies can occur at high frequencies in human populations. Our results suggest that carriers of the common genotype Ile/Val have a higher risk of hepatic fibrosis in viral hepatitis.

The prognostic value of heterozygous genotype Ile/Val of *IL4RA* cannot be fully explained in terms of allele-specific features of relevant biochemical processes. High frequencies are characteristic of the homozygous genotypes, although one determines a high and the other, a low level of IgE: compared with Val50, variant Ile50 is associated with a threefold higher activity of IL-4 and, consequently, a higher level of IgE. A medium level of IgE might be expected for heterozygotes. It is known, however, that the Ile50Val polymorphism is in the extracellular domain of the IL-4R α -chain, and the presence of two different amino acid residues in receptors may alter the binding and activation of IL-4 and the subsequent development of the inflammatory response.

On the other hand, it is necessary to consider the general properties of cytokines. These substances mediate communication between different systems of the organism, organize an integrated defense network, are interchangeable, and exert pleiotropic biological effects. Hence, it is possible to assume that some gene complexes and interacting sets of protein isoforms, which are determined by different variants of genes, contribute to the clinical phenotype along with the Ile50Val polymorphism. Moreover, the association of a genetic polymorphism with a disease can be explained by functional significance of the polymorphism itself or by its linkage disequilibrium with another locus, identification of which may implicate new genes in the given disease.

REFERENCES

1. O'Brien S.J., Nelson G. W. 2004. Human genes that limit AIDS. *Nature Genetics*. **36**, 565–574.
2. Kwiatkowski D. 2000. Susceptibility to infection. *Br. Med. J.* **321** (28), 1061–1065.
3. Gonzalez E., Dhanda R., Bamshard M., *et al.* 2001. Global survey of genetic variation in CCR5, RANTES and MIP-1: Impact on the epidemiology of the HIV-1 pandemic. *Proc. Natl. Acad. Sci. USA*. **98**, 5199–5204.
4. Dessein A.J., Chevillard C., Marquet S., *et al.* 2001. Genetics of parasitic infections. Drug metabolism disposition. **29**, 484–488.
5. Lipsitch M., Sousa A. 2002. Historical intensity of natural selection for resistance to tuberculosis. *Genetics*. **161**, 1599–1607.
6. Roy S., Hill A. 2002. Association of common genetic variant with susceptibility to invasive pneumococcal disease. *Br. Med. J.* **324**, 1369.
7. Plebanski M., Proudfoot O., Pouniotis D., *et al.* 2002. Immunogenetics and the design of *Plasmodium falciparum* vaccines for use in malaria-endemic populations. *J. Clin. Invest.* **110**, 295–301.
8. Yagoda A.V., Geivandova N.I., Khubiev Sh.Kh., *et al.* 2000. *Immunologiya*. **2**, 36–38.
9. Ivashkin V.T., Bueverov A.O., Gryazin A.E. 2004. Mechanisms of hepatitis C virus resistance to antiviral drugs. *Mol. Meditsina*. **2**, 18–23.
10. Sennikov S.V., Kuramshin D.Kh., Tolokonskaya N.P., Kozlov V.A. 2003. Gene expression and production of main immunoregulatory cytokines in viral hepatitis C. *Tsitokiny i Vospalenie*. **4**, 1–4.
11. Ivashkin V.T., Mamaev S.N., Lukina E.A., *et al.* 2001. The cytokine system in patients with chronic diffuse liver diseases. *Immunologiya*. **1**, 46–48.
12. Tsaregorodtseva T.M., Serova T.I. 2003. *Tsitokiny v gastroenterologii* (Cytokines in Gastroenterology). Moscow: Anakharsis.
13. Mitsuyasu H., Yanagihara Y., Mao X.-Q. *et al.* 1999. Cutting edge: Dominant effect of Ile50Val variant of the human IL-4 receptor alpha-chain IgE synthesis. *J. Immunol.* **162**, 1227–1231.
14. Howard T.D., Wiesch D.G., Postma D.S., *et al.* 1998. Linkage and association study of the IL4 receptor (IL4R) gene on chromosome 16 in asthma and allergic phenotype. *Am. J. Hum. Genet.* **63** (Suppl), 293.
15. Puzyrev V.P., Freidin M.B. 1999. Role of interleukin genes and receptors in predisposition to bronchial asthma. *Byull. Eksp. Biol. Med.* **127** (Suppl. 1), 3–6.
16. Mirel D.B., Valdes A.M., Lazzeroni L.C. *et al.* 2002. Association of IL4R haplotypes with type 1 diabetes. *Diabetes*. **51**, 3336–3341.
17. Vasilescu A., Heath S.C., Ivanova R., *et al.* 2003. Genomic analysis of *Th1–Th2* cytokine genes in an AIDS cohort: Identification of *IL4* and *IL10* haplotypes associated with the disease progression. *Genes Immun.* **4**, 441–449.
18. Choi E.H., Lee H.J., Yoo T., Chanock S.J. 2002. A common haplotype of interleukin-4 gene *IL4* is associated with severe respiratory syncytial virus disease in Korean children. *J. Infect. Dis.* **186**, 1207–1211.
19. Knodell R.G., Ishak R.G., Black W.S., *et al.* 1981. Formulation and application of numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology*. **1**, 431–435.
20. Desmet V.J., Gerber M., Hoofnagle J.H., *et al.* 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. *Hepatology*. **19**, 1513–1520.
21. Lahiri D.K., Bye S., Nunberg J.I., *et al.* 1992. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods used. *J. Biochem. Biophys. Methods*. **25**, 193–205.
22. Mitsuyasu H., Izuhara K., Mao X.-Q., *et al.* 1998. Ile50Val variants or IL4R? upregulates IgE synthesis and associates with atopic asthma. *Nature Genet.* **19**, 119–120.
23. Noguchi E., Shibasaki M., Arinami T., *et al.* 1999. Lack of association of atopy/asthma and the interleukin-4 receptor α gene in Japanese. *Clin. Exp. Allergy*. **29**, 228–233.
24. Lakin G.F. 1990. *Biometriya* (Biometry), Moscow: Nauka.
25. He J.O., Connett J.E., Anthonisen N.R., Sandford A.J. 2003. Polymorphisms in the *IL13*, *IL13RA1*, and *IL4RA* genes and rate to decline in lung function in smokers. *Am. J. Respir. Cell Mol. Biol.* **28**, 379–385.
26. Ober C., Leavitt S.A., Tsalenko A., *et al.* 2000. Variation in the interleukin 4-receptor gene confers susceptibility to asthma and atopy in ethnically diverse population. *Am. J. Hum. Genet.* **66**, 517–526.
27. Noguchi E., Shibasaki M., Arinami T. *et al.* 1999. No association between atopy/asthma and the Ile50Val polymorphism of IL-4 receptor. *Am. Respir. Crit. Care Med.* **160**, 342–345.
28. Puzyrev V.P., Freidin M.B., Ogorodova L.M., Kobaykova O.V. 2002. Interrelation of polymorphic variants of interleukine genes and receptors with atopic bronchial asthma. *Med. Genet.* **1**, 86–92.
29. Spiridonova M.G., Stepanov V.A., Puzyrev V.P. 2001. On the role of polymorphic variants of the 5,10-methylene tetrahydrofolate reductase gene (*MTHFR*) in pathogenesis of cardiovascular diseases. *Klin. Med.* **2**, 10–16.
30. Freidin M.B., Bragina E.Yu., Ogorodova L.M., Puzyrev V.P. 2002. Polymorphism of glutathione transferase 1 and 1 genes (*GSTT1* and *GSTM1*) in patients with atopic bronchial asthma in the Western Siberian Region. *Mol. Biol.* **36**, 1–5.
31. Ivashchenko, T.E., Strelakov D.L., Solov'eva D.V., *et al.* 2004. Testing for genetic predisposition to multifactor diseases and the genetic passport. In: *Molekulyarno-biologicheskie tekhnologii v meditsinskoj praktike* (Molecular-Biological Technologies in Practical Medicine), Novosibirsk: Al'ta Vista, pp. 9–28.
32. Wilson M.H., Grant P.J., Hardie L.J., Wild C.P. 2000. Glutathione S-transferase M1 null genotype is associated with a decreased risk of myocardial infarction. *FASEB Lett.* **14**, 791–796.