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Interleukin-1β gene promoter polymorphism is associated with higher liver fibrosis progression rate in Croatian patients with biochemically active chronic hepatitis C

Povezanost polimorfizma genskog promotora interleukina 1β s ubrzanom pojavom fibroze jetre kod hrvatskih pacijenata s biokemijski aktivnim kroničnim hepatitisom C

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Summary

Background and aims: Genetic polymorphisms of immune mediators have been associated with differences in the natural course of chronic hepatitis C (CHC). The aim of this study was to analyze the association of IL-1β gene polymorphism with the stage of liver fibrosis (LF), grade of necroinflammatory activity (NIA) and fibrosis progression rate (FPR) in CHC patients.

Patients and methods: The study included 50 treatment-naive Croatian CHC patients (36 male and 14 female; age median 37.5 years) with elevated ALT. Diallele polymorphism (C/T) at locus -31 in the IL-1β gene promoter region was determined by restriction fragment length polymorphism (RFLP). Stage of LF and NIA were assessed from liver biopsy sample according to Ishak classification.

Results: There was no difference in the stage of LF and NIA level between particular patient genotypes. However, patients with at least 1 C allele at locus -31showed significantly faster FPR than those with no C allele (0.4 vs. 0.258 Ishak's units/year; p = 0.043). Higher stages of fibrosis were observed in older patients (p = 0.001) and those infected at an older age (p = 0.017).

Conclusion: Our study demonstrated that the carriage of at least 1 C allele at -31 locus of IL-1β gene led to faster progression of LF in CHC patients with a biochemically active disease, but did not determine the final stage of fibrosis development. Combined with other risk factors, this finding may serve as a genetic marker to identify patients that require earlier introduction of therapy, since delay could hamper therapeutic success due to rapid disease progression.

Key words: Hepatitis C, interleukin -1beta, gene polymorphism

Sažetak

Uvod: Polimorfizmi gena različitih medijatora imunološkog odgovora mogu se dovesti u vezu s različitostima u prirodnom tijeku i ishodu kroničnog hepatitisa C (KHC). Cilj ovoga istraživanja bio je analizirati odnos dialelnog polimorfizma gena za IL-1β sa stadijem jetrene fibroze, stupnjem nekroinflamatorne aktivnosti (NIA) i brzinom napredovanja fibroze kod bolesnika s KHC.

* *Errata: Editorial board apologizes to prof. Adriana Vince, MD Phd and Sanja Kozić Dokmanović, MD Phd for the error in quoting their full names. Instead of Sanja Kozić it should have been written Sanja Kozić Dokmanović, and instead of Adriana Vince Dokmanović it should have been Adriana Vince. We included this correction in PDF version on Hrcak Portal of scientific journals in Croatia.*

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Ispitanici i metode: U studiju je uključeno 50 neliječenih bolesnika (36 muškaraca i 14 žena; medijan dobi 37,5 g (19-65)) s KHC, koji su imali povišene vrijednosti ALT-a. Svim bolesnicima određen je dialelni polimorfizam (C/T) na lokusu -31 u promotorskoj regiji gena za IL-1β metodom PCR-RFLP. Iz nalaza biopsije jetre određeni su stadij fibroze i stupanj upalne aktivnosti prema Ishakovoj klasifikaciji.

Rezultati: Nije bilo razlike u stadiju fibroze niti stupnju NIA između pojedinih genotipova bolesnika. Međutim, značajno brže napredovanje fibroze uočeno je kod bolesnika s barem jednim C alelom na lokusu -31 u odnosu na bolesnike bez C alela (0.4 vs. 0.258 Ishakovih jedinica/godinu; p = 0.043). Viši stadiji fibroze zabilježeni su kod starijih bolesnika (p = 0,001) i kod onih zaraženih kasnije (p = 0,017).

Zaključak: Rezultati ovoga istraživanja ukazuju da se jetrena fibroza brže razvija kod bolesnika s biokemijski aktivnim KHC koji imaju barem 1 C alel na lokusu -31 gena za IL-1β, no istovremeno ovaj genski polimorfizam ne određuje krajnji stadij do kojega će se razviti fibroza. Uz ostale čimbenike rizika, nosilaštvo C alela na ovom lokusu može predstavljati genski marker koji bi ukazivao na bolesnike kod kojih treba što prije započeti s terapijom, jer se progresijom bolesti umanjuju šanse za uspješno liječenje.

Ključne riječi: hepatitis C, interleukin 1 beta, polimorfizam gena.

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Introduction

Chronic hepatitis C (CHC) is characterized by great differences in the natural course of the disease, ranging from mild disease with normal liver aminotransferases, minimal liver fibrosis and slow progression through rapidly progressing disease leading to the development of cirrhosis and its complications such as decompensation and hepatocellular carcinoma (HCC). Factors associated to the observed variations can be related to the virus, the host, and to extrinsic effects.[1] Considering host factors, the disease has been found to assume a more unfavorable course in men, black race, elderly individuals, and those acquiring infections at an older age.[1] Host genetic factors, especially single nucleotide polymorphisms (SNP) of the mediators of inflammatory response and fibrogenesis, have also been associated with a progressive course of the disease (HLA B54, DR3, DRB1*0405, DQB1*0401, DQB1*0502), interleukins (-1082A for IL-10, IL-1 RA (intron2VNTR)*2) and other biological compounds (matrix-metalloproteinase (MMP)-1 genotype 2G, MMP-3 genotype 5A and MMP-9 genotype C; mutation Factor V Leiden (Arg560Gln); TGF-β and angiotensin II gene polymorphism), whereas more extensive genomic analysis identified 7 gene signature that can predict risk for cirrhosis development in the Caucasian population.[2]

Interleukin-1β (IL-1β) is a pleiotropic cytokine that stimulates secretion of other cytokines, growth factors and adhesion molecules, thus leading to inflammatory response enhancement and even tumorigenesis.[3-5] Upon IL-1β gene sequencing Chen et al. identified 20 SNP, of which SNP at loci -511, -31 and +3954 have been most frequently analyzed and their association to different diseases has been established in several studies.[6] SNP at loci -511 and -31 are diallele polymorphisms based on the nucleotide base C and T exchange and showing nearly complete linkage disequilibrium according to previous studies.[6] The -31C/-511T haplotype has been associated to the highest IL-1β synthesis.[4-6] In our study, we analyzed SNP at the locus -31 because of its functional role as it is located within the region containing TATA box necessary to initiate gene transcription. In the CHC context, IL-1β has been found to interfere with intracellular HCV replication, to reduce IFN action, and to stimulate synthesis of the tissue inhibitor of matrix metalloproteinases (TIMMP-1).[7-9] The IL-1β gene polymorphism (-31CC/-511TT) is associated with the development of HCC while -31T homozygotes have poorer clinical prognosis.[10,11] No association has been observed between different IL1B gene SNPs with spontaneous or therapy induced viral clearance.[12] Two studies failed to find association for polymorphisms at loci -511 and +3954 with the risk of cirrhosis.[12,13] The association of these polymorphisms with the rate of fibrosis progression (FPR) has not been analyzed. The aim of this study was to analyze the possible influence of IL-1 β SNP at -31 locus on the stage of liver fibrosis, grade of necroinflammatory activity (NIA) and FPR in a cohort of Croatian CHC patients with biochemically active disease (elevated ALT).

Patients and methods

Patients

This prospective study consecutively included a cohort of 50 treatment-naive patients with biochemically active chronic hepatitis C that have been hospitalized because of liver biopsy as a part of pre-treatment evaluation at the Department for Viral Hepatitis at the University Hospital for Infectious

Diseases "Dr. Fran Mihaljević", Zagreb, Croatia in the period from February 2006 till February 2007. They were all anti-HCV and HCV RNA positive. The liver biopsy and elevated ALT levels were prerequisite for reimbursed therapy with pegylated interferon alfa-2a or 2b with ribavirin as defined by guidelines of the Croatian Institute for Health Insurance. Exclusion criteria comprised additional causes of liver diseases, particularly HBV or HIV co-infection, alcohol consumption of more than 30g/day, metabolic syndrome (abdominal obesity, increased body mass index, glucose intolerance or diabetes mellitus type 2, decreased HDL, increased triglycerides, and arterial hypertension) as well as autoimmune hepatitis.

The duration of infection was assessed on the basis of history data (surgery, blood transfusion, i.v. drugs, use, multiple sexual partners, or accidental exposure). The fibrosis progression rate (FPR) was calculated as a ratio between the observed histological stage of liver fibrosis and duration of infection (FPR = stage/ duration of infection) as described previously.[1]

The study protocol was approved by the Ethics Committee of "Dr. Fran Mihaljevic" University Hospital for Infectious Diseases, Zagreb and informed consent regarding the study and liver biopsy procedure was obtained from all the patients.

Methods

- HCV RNA detection and genotyping

Detection of HCV-RNA in patient sera was performed by polymerase chain reaction (PCR) using commercially available Cobas® Amplicor HCV kit, version 2.0 (Roche Diagnostics, USA), and quantification (IU/mL) by use of Cobas® Amplicor HCV Monitor test, version 2.0 of the same manufacturer. Viral genotype was determined by reverse hybridization (INNO LiPA HCV II test; Innogenetics, Gent, Belgium).

- Determination of the IL-1β gene promoter polymorphism

The IL-1β gene promoter polymorphism was determined by the PCR-RFLP method, as previously described[4]. Briefly, DNA was isolated from 200 mL whole blood by use of the commercial kit (QIAmp DNA Blood Mini Kit, QIAGEN GmbH, Hilden, Germany) and diluted in 100 mL buffer, according to the manufacturer's instructions. Then the IL-1β gene promoter region fragment containing restriction site -31 was amplified by the use of a pair of primers of the following nucleotide sequence: sense 5'-AGAAGCTTCCACCAATACTC-3'; antisense 5'-ACTAACCTTTAGGGTGTCAG-3'.[17] The fragment had a length of 448 bp. It was followed by region digestion using 5 units of Alu I restriction enzyme (endonuclease) (New England Biolabs Inc., USA), which resulted in the amplified region fragments of variable length depending on the restriction site allele. These fragments were separated by 3% agarose gel electrophoresis. Fragments of 344, 79, 20 and 5 bp were obtained in patients with CC genotype; fragments of 247, 97, 79, 20 and 5 bp in patients with TT genotype; and fragments of 344, 247, 97, 79, 20 and 5 bp in patients with CT genotype.

- Liver biopsy and histopathology

Liver biopsies were performed using Menghini's technique with a 1.6 mm diameter needle. The obtained cylinder of liver tissue was paraffin embedded, stained with H&E, Masson trichrome and PAS diastase, and analyzed by an experienced hepatopathologist. Only the ≥ 2cm long specimens with at least 11 portal areas were considered adequate for inclusion in the study. The stage of fibrosis and grade of necroinflammatory activity (NIA) were assessed according to the scoring system of Ishak et al. classifying fibrosis into 6 stages and NIA into 18 grades.[14]

Statistical analysis

Between-group differences in qualitative variables were analyzed by χ^2-test. Differences in quantitative variables among several groups were analyzed by the Kruskal-Wallis test (KW χ^2), and those between two groups by the Mann-Whitney U test (MW-U). The correlation of two variables was analyzed by Pearson and Spearman's test of correlation. Statistical significance was set at $p < 0.05$.

Results

Demographic, epidemiological, clinical and histological data of CHC patients are shown in Table 1. The patient group showed a male predominance (2.57:1). Female patients were older than male patients; the difference was not statistically significant ($p = 0.552$). The frequency of genotype 1 was significantly higher, but there was no difference in viremia between genotypes 1 and 3 ($p = 0.655$). Transmission by intravenous drug use and blood transfusion were the most common routes of infection.

Table 1 Demographic, epidemiologic and clinical characteristics of patients with chronic hepatitis C
Tablica 1. Demografske, epidemiološke i kliničke karakteristike pacijenata s kroničnim hepatitisom C

Sex (n (%))/*spol*	
Male/*muško*	36 (72%)
Female/*žensko*	14 (28%)
Age (yrs) (median (range)) *(starost- prosjek (opseg))*	
Total/*sveukupno*	37.5 (19-65)
Male/*muško*	36 (19-65)
Female */žensko*	45 (22-58)
Age at infection (yrs) (median (range)) *Starost kod infekcije (prosjek (opseg))*	24.5 (11-57)
Route of infection (n (%)) / *Tijek infekcije*	
Transfusion/Op/*Transfuzija*	20 (40%)
IVDU	21 (42%)
Sexual/*seksualno*	6 (12%)
Prick/*ubod*	3 (6%)
Stage of fibrosis (median (range)) *Stadij fibroze (prosjek (opseg))* (Ishak 0-6)	4 (2-6)
0	0 (0%)
1	0 (0%)
2	13 (26%)
3	10 (20%)
4	16 (32%)
5	10 (20%)
6	1 (2%)
HAI (Ishak 0-18; median (range)) *(prosjek (opseg))*	8 (3-15)
FPR (median (range))/ *(prosjek (opseg))* (unit/year)/*(jedinica/godina)*	0.333 (0.08-1.5)
HCV genotype (n (%))	
1	35 (70%)
3	15 (30%)
Viremia (IU/mL) (median(range))/*(prosjek (opseg))*	
All genotypes/(*svi genotipovi*)	551.500 (1.600-7.241.000)
HCV genotype 1	695.400 (1.600-7.241.000)
HCV genotype 3	429.000 (251.000-979.000)
Laboratory findings (median (range)) / *(prosjek (opseg))*	
AST (IU/mL)	59.5 (13-294)
ALT (IU/mL)	121.5 (17-647)
γGT (IU/mL)	52 (13-343)
Bil (μmol/L)	13.8 (5.1-85)
PT (%)	87 (61-129)
TP (g/L)	76 (65-94)
Alb (g/L)	46 (35-53)
Plt ($Nx10^9$)	195 (94-476)
L ($Nx10^9$)	6.9 (4.3-26)
Hb (g/L)	146.5 (112-165)

IVDU = intravenous drug use/*intravenozno korištenje lijeka;*; HAI = histology activity index/*indeks histološke aktivnosti;* FPR = fibrosis progression rate/*stopa razvoja fibroze;* AST = aspartate aminotransferase/*aspartat aminotransferaza*; ALT = alanine aminotransferase/*alanin aminotransferaza*; γGT = gamma glutamyl aminotransferase/*gama glutamil aminotransferaze*; PT = prothrombin time/*protrombinsko vrijeme*; TP = total proteins */sveukupni proteini*; Alb = albumins/*albumini*; Plt = platelets/*trombociti*; L = leukocytes/*leukocitis*; Hb = hemoglobin/*hemoglobin*.

Fibrosis was observed in all specimens; median stage of liver fibrosis was 4, liver cirrhosis (stage 6) was found in only one patient, whereas lower (stages 2 and 3) and higher (stages 4 and 5) stages of liver fibrosis were quite equally distributed in the rest of the study patients. Laboratory findings revealed a biochemically active disease with preserved synthetic function of the liver. Genetic analysis showed that CT and TT genotypes were more common and quite uniformly distributed (Table 2), while CC genotype was rare. There was no sex difference in the rate of C allele carrier state (Pearson $\chi^2 = 0.149$; $p = 0.7$).

Table 2 Genotype frequency at IL-1 β gene promoter region locus -31
Tablica 2. Učestalost genotipa kod IL-1β gena promotora na lokusu-31 regije

IL-1β promoter genotype at locus -31 *IL -1β promotor genotipa kod locus-31 regije*	No. of patients (%) *Broj pacijenata*
CC	7 (14%)
CT	23 (46%)
TT	20 (40%)

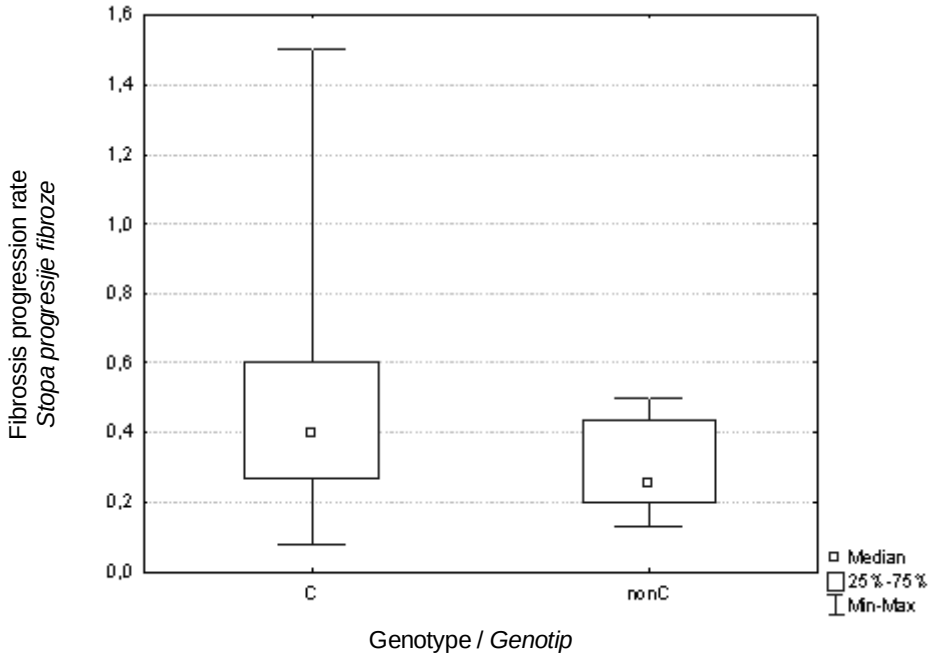
IL-1β gene polymorphism and stage of liver fibrosis, level of necroinflammatory activity and fibrosis progression rate

There was no difference in the stage of fibrosis or in the level of NIA among the three IL-1β genotypes (CC vs. CT vs. TT) at locus 31 (Table 3). Analysis of the stage of fibrosis according to the rate of C allele carrier state yielded no difference either, i.e. between patients with at least 1 C allele at locus -31 (CC and CT= "inflammatory genotype") and patients with no C allele at the locus (TT genotype) ($p = 0.601$). There was no difference in the level of NIA between patients with at least 1 C allele and those without C allele at locus -31 ($p = 0,420$). FPR was lowest in patients with TT genotype, intermediate in patients with CT genotype, and highest in patients with CC genotype (Table 3) which was close to be significant ($p = 0.092$). However, the comparison of FPR between patients with at least 1 C allele at locus -31 and patients without C allele at this locus yielded higher FPR in the former; the difference was statistically significant ($p = 0.043$) (Picture 1).

Table 3 Stage of liver fibrosis, HAI and FPR in patients with different genotypes of IL-1β gene promoter polymorphism. Results are presented for the three possible genotypes at locus -31 (CC, CT and TT) and patients carrying at least 1 C allele at locus -31 (inflammatory genotype C *vs.* non-C) ($p < 0.05$). (*) = significant result**.**
Tablica 3 Stadij fibroze jetre, HAI te FPR kod pacijenata s različitim genotipovima IL-1β gena promotora polimorfizma. Rezultati su prikazani kod tri mogućih genotipa u locus-31 (CC, CT i TT) te kod pacijenata nositelja najmanje 1 C alele na lokusu – 31 (upalni genotip C protiv non-C) (p < 0,05). (*) = značajan rezultat

	CC (n = 7)	CT (n = 23)	TT (n = 20)	p (K-W)	C (CC+CT) (n = 30)	Non-Cn (TT) (n = 20)	p (M-W)
Stage of fibrosis *Stadij fibroze* Median (range) *Prosjek (opseg)*	4 (2-5)	3 (2-6)	3.5 (2-5)	0.263	4 (2-6)	3.5 (2-5)	0.601
HAI median (range) *Prosjek (opseg)*	8 (6-12)	8 (4-15)	8.5 (3-15)	0.689	8 (4-15)	8.5 (3-15)	0.420
FPR (Ishak's units/year) *jedinica /god.* median (range) *Prosjek (opseg)*	0.417 (0.125-1)	0.333 (0.08-1.5)	0.258 (0.133-0.5)	0.092	0.4 (0.08-1.5)	0.258 (0.133-0.5)	0.043*

HAI = histology activity index/*indeks histološke aktivnosti*; FPR = fibrosis development rate/*omjer razvoja fibroze*; p-value/*p-vrijednost*; K-W = Kruskal-Wallis test; M-W = Mann-Whitney U test



Picture 1 Difference in the fibrosis progression rates (FPR) between the patients with or without at least 1 C allele at locus -31 of the IL-1β gene promoter region (FPR (C) = 0.4 (0.08-1,5) vs. FPR (non-C) = 0.258 (0.133-0.5) Ishak's units/year; p = 0.043).

Slika 1. Razlika kod stope progresije fibroze (FPR) između pacijenata sa ili bez najmanje 1 C alele na lokusu-31 IL-1β gena promotora regije (FPR (C) = 0,4 (0,08-1,5) vs. FPR (non-C) = 0,258 (0,133-0,5) Ishak jedinice/godina; p = 0,043).

Relation of liver fibrosis stage, necroinflammatory activity and fibrosis progression rate with demographic and clinical characteristics of CHC patients

The comparison of demographic, clinical and histopathologic variables with the stage of liver fibrosis (Table 4) showed that higher stages were mostly present in older patients as well as in those who acquired infection later in life. Patients with higher stages of liver fibrosis had a longer duration of infection than those with lower stages of fibrosis (borderline significance p = 0.055). The grade of NIA was higher in patients with higher stages of liver fibrosis. There was no difference in the stage of liver fibrosis according to viral genotype, viremia or ALT level. There was no difference in the stage of liver fibrosis according to sex (p = 0.780).

Grade of NIA correlated with patient age (Spearman's correlation coefficient r_s = 0.397; p = 0.004) and ALT level (r_s = 0.548; p < 0.001) while there was no significant correlation with the age at infection (r_s = 0.258; p = 0.071), duration of infection (r_s = 0.268) and viremia (r_s = 0.066; p = 0.709). There was no difference in the grade of NIA between patients with viral genotypes 1 and 3 (p = 0.328).

FPR increased with age at infection but the difference did not reach statistical significance (p = 0.489). There was no correlation of FPR with ALT (Spearman's correlation coefficient r_s = 0.069) and viremia (r_s = 0.220; p = 0.212). There was no difference in FPR according to sex (p = 0.319) and viral genotype (p = 0.538).

Discussion

The results of this study have shown that carriers of at least one C allele at locus -31 of IL-1β gene promoter region have significantly higher fibrosis progression rate than those without C allele. The association between this particular gene polymorphism and FPR in CHC was not documented previously. No difference was found in the stage of liver fibrosis and grade of inflammatory activity between patients with different alleles at locus -31 of the IL-1β gene promoter region. This finding suggests that IL-1 β -31 SNP is not a risk factor for developing a particular stage of fibrosis but may influence the rate by which fibrosis progresses to that stage. The association between IL-1 β -31 SNP and fibrosis progression rate in CHC is an important finding

Table 4 Stage of liver fibrosis according to age, age at infection, duration of infection, HAI, ALT and viremia ($p < 0.05$)
Tablica 4. Stadij fibroze jetre prema godinama starosti, dobi kod infekcije, trajanju infekcije, HAI, ALT te viremije

	Stage 2 *2. stadij* (n = 13)	Stage 3 *3. stadij* (n = 10)	Stage 4 *4. stadij* (n = 16)	Stage 5 *5. stadij* (n = 10)	P (K-W)
Age (yrs) / *Dob (god.)* median (range) *prosjek (opseg)*	29 (23-56)	31 (19-38)	45.5 (26-60)	45.5 (33-65)	p = 0.001
Age at infection *Dob kod infekcije* (yrs) median (range) *god. prosjek (opseg)*	22 (11-49)	23.5 (17-28)	31 (17-48)	32.5 (22-57)	p = 0.017
Duration of infection *Trajanje infekcije* (yrs) median (range) *god. prosjek (opseg)*	7 (4-25)	8 (2-15)	13.5 (6-30)	12 (5-20)	p = 0.055
HAI median (range) *prosjek (opseg)*	5 (3-15)	7 (6-15)	10 (6-14)	10 (8-15)	p = 0.002
ALT (IU/mL) median (range) *prosjek (opseg)*	80 (17-220)	139.5 (27-335)	128.5 (37-647)	140.5 (44-310)	p = 0.324
Viremia (IU/mL) median (range) *prosjek (opseg)*	312 500 (1 600 – 3 185 000)	289 000 (29 240- 7 241 000)	447 000 (84 800 - 3 468 000)	1 425 000 (416 000 – 4 335 320)	p = 0.243

HAI = histology activity index/*indeks histološke aktivnosti*; ALT = alanine aminotransferase/*alanin aminotransferaza*; p - p-value/*p-vrijednost*; K-W = Kruskal-Wallis test;

pointing to the possible genetic risk for faster fibrosis progression, which may prove useful in identifying the individuals in whom the therapy should not be postponed. The association of IL-1β gene polymorphism and risk of cirrhosis in CHC patients was analyzed in several studies, however, neither of them tackling the effect of this particular polymorphism on FPR.[12,13] Constantini et al. investigated IL-1β gene polymorphism at locus +3954 in the region of exon V and found no association with various stages of the disease or therapeutic response to IFN and ribavirin.[12] It is important to notice that SNP+3945 has no functional effects on IL-1B synthesis in vivo as opposed to different synthetic capacities resulting from the -31/-511 SNPs. Bahr et al. found no difference in the frequency of diallele polymorphisms at locus -31 in the promoter region of IL-1β gene between patients with cirrhosis and those with CHC without cirrhosis (Caucasians from Germany), while a difference was recorded in the case of IL-1RA gene polymorphism.[13] In a study of Fontanini et al., the risk for development of cirrhosis was increased in male HCV positive patients with high-inflammatory IL1β haplotype (-511/-31 TC/TC or TC/(CC or TT) and carrying at least one IL1RN A2 allele).[15] According to their results, inflammatory action of this IL1β haplotype appeared to be blunted in females, probably due to hormonal reasons. In a study published by Zhang et al. IL-1β was demonstrated to enhance TIMMP-1 expression, thus increasing intrahepatic fibrogenesis.[9] Tanaka et al. report on the CHC patients carrying T allele at locus -511 (implying C allele at locus -31 based on linked inheritance) to be at an increased risk of developing HCC; however, further analysis revealed no difference in the stage of liver fibrosis and different genotypes at the respective locus either.[10] According to these data, it seems that IL1β SNPs have important functional effects on the development and clinical behavior of HCC, while some discrepancies still exist regarding its impact on cirrhosis development in CHC patients. Otherwise, in patients with alcohol induced liver disease and primary biliary cirrhosis IL-1β gene polymorphism was found to be a significant risk

factor for the development of cirrhosis.[2] Obviously, this polymorphism exerts variable interaction with other factors in liver diseases of different etiologies. It should be noted that the frequency of C and T alleles at locus -31 greatly varies among different populations, i.e. races, and that such a genetic diversity may explain differences observed in the prevalence of particular diseases (e.g., HCC in Caucasian and Asian populations) as well as racial variability in the clinical picture and course of the same diseases.[2,6] The median stage 4 of liver fibrosis in our patients exceeded the values published in other studies on the natural course of CHC so far, which could be attributed to the study design including only the patients with elevated liver aminotransferases.[1,16] In the study by Poynard, as many as 46% of patients had stages 0 and 1 according to the METAVIR classification, corresponding to stages 0-2 by Ishak's classification (compared with stage 2 recorded in 26% of our patients and none with stages 0 and 1).[1] The median inflammatory activity in our patients also exceeded the levels reported in the cited studies which could be attributed to the fact that the Croatian patients had a biochemically active disease. This is probably also the reason why the rate of fibrosis progression shown in our study (0.333 Ishak's units/year) exceeded the results reported from large studies on the natural course of CHC. In the study by Poynard, median FPR was 0.13 METAVIR units/year (corresponding to 0.195 Ishak's units/year), whereas Wright reports on median FPR of 0.255 Ishak's units/year.[1,16] In our study, statistically significant higher stages of fibrosis were observed in older patients and those infected at older age. Although higher stages of fibrosis were associated with a longer duration of infection, this association was not statistically significant. Consistently with the results from large epidemiological studies, the stage of liver fibrosis was found to predominantly depend on the patient age and age at infection rather than the duration of infection, suggesting non-linear progression of fibrosis over time.[1,16,17] We found no association between the stage of fibrosis and gender, otherwise sex distribution in the study population was consistent with the sex distribution of hepatitis C recorded in Croatia[18]. There was no association of the stage of fibrosis with viral genotype, viremia, route of transmission and ALT levels, which is consistent with literature reports.[1,16]

We observed an increase in NIA in older age, as also reported elsewhere,[1,16] which was associated with a higher stage of fibrosis, the latter being variedly reported in literature.[17] As inflammatory mediators stimulate fibrogenesis in the liver, a higher inflammatory activity would be expected to imply stronger fibrogenous stimulation, thus resulting in a higher final stage of fibrosis.

In conclusion, our study demonstrated that genetic IL-1β polymorphism, i.e. carrier state of at least 1 C allele at locus -31 in the promoter region of this gene, led to faster progression of liver fibrosis in this cohort of Croatian CHC patients. The study has also shown that older patient age and older age at infection are the main risk factors for higher stages of liver fibrosis. Knowing the risk factors for the development of fibrosis and the rate of fibrosis progression can help in treatment and follow-up strategies of patients with CHC. Although small by numbers, this study contributes to the understanding of the natural progression of fibrosis in CHC in Caucasians.

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