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ANGIOTENSIN CONVERTING ENZYME (ACE) POLYMORPHISM AND ITS RELATION TO PROGRESSION LIVER CIRRHOSIS CAUSED BY CHRONIC HEPATITIS C IN THE KAZAKH POPULATION

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Abstract

To study the inheritance value of the polymorphism of the gene angiotensin single nucleotide polymorphism located in (AT-6) promoter-6 region in the progression of cirrhosis as a result of viral hepatitis C. Material and methods: This prospective study was performed on 120 patients having chronic hepatitis C, 53 of them are women and 67 are men, and on healthy donors 70 people. Patients were divided into groups: 1 group - patients diagnosed with chronic viral hepatitis without cirrhosis (stage of fibrosis F1-F3 (Metavir) - 40 people, 2 group - patients diagnosed with cirrhosis in the outcome of viral hepatitis C Class A (Child-Pugh) -35 people and group 3 with diagnosis of cirrhosis Class B and C - 45 people. Results: thymosin-thymosin is more often found in the homozygous genotype of the inheritance of the angiotensinogen AT-6 gene (P <0.05) than in patients with chronic viral hepatitis. The inheritance of the homozygous genotype C/C of the AT-6 angiotensinogen gene is associated with an easier flow of chronic HCV infection (P <0.005) in the Kazakh population. The inheritance of the T allele in the group with compensated and decompensated cirrhosis is also statistically significant in comparison with the group of patients with chronic viral hepatitis C (P <0.05, P <0.005, respectively). Conclusion: An association was identified between of the gene angiotensin single nucleotide polymorphism located in (AT-6) promoter region -6 and fibrosis in chronic HCV infection.

Keywords: *angiotensin converting enzyme angiotensin converting enzyme, liver cirrhosis*

Introduction

Renin-angiotensin system (RAS) is an important hormonal regulatory mechanism of the blood pressure and body fluid homeostasis. Several studies have shown upregulation of RAS activity during liver fibrosis [*H.Yoshiji et al, 2007*]. The key RAS protein, angiotensin II (Ang II), is produced in the liver from its precursor angiotensin I by the proteolytic cleavage by angiotensin I converting enzyme (ACE) [*H.Yoshiji et al, 2007*]. Ang II exerts its diverse biological effects by binding with one of its multiple receptors, particularly Ang II type 1 receptor (AT1-R), overexpressed in activated HSCs [*M.Y.Kim et al, 2008*]. Ang II induces HSC activation, proliferation, and contraction [*R.Bataller, et al 2000*], as well as increased TGFβ, TIMP1 expression, and collagen deposition [*H.Yoshiji et al, 2007; M.G.Ghany, et al 2004*]. Finally, Ang II also contributes to the oxidative stress in the fibrotic liver.

Given the influence of RAS on the formation of fibrosis and participation in the pathogenesis of severe complications of liver cirrhosis, the study of polymorphisms of the AT6 gene in viral hepatitis C will allow to determine the genetic predictors of

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the progression of viral hepatitis C.Since polymorphism of RAS genes is associated with increased angiotensin II production, it can be associated with progressive liver cirrhosis

The aim of the present study was To study the inheritance value of the polymorphism of the gene angiotensin single nucleotide polymorphism located in -6 (AT-6) promoter region in the progression of cirrhosis as a result of viral hepatitis C.

Subjects and Methods

This prospective study was performed on 120 patients having chronic hepatitis C, 53 of them are women and 67 are men, and on healthy donors 70 people. Patients were divided into groups: 1 group - patients diagnosed with chronic viral hepatitis without cirrhosis (stage of fibrosis F1-F3 (Metavir) - 40 people, 2 group - patients diagnosed with cirrhosis in the outcome of viral hepatitis C Class A (Child-Pugh) -35 people and group 3 with diagnosis of cirrhosis Class B and C - 45 people. The diagnosis of chronic viral hepatitis C was established based on the detection of a positive result on anti-HCV by enzyme immunoassay and qualitative PCR analysis for the detection of RNA HCV. All patients included in the study were of Kazakh nationality and informed about the study. The patients were attending the Outpatient Clinic of National Scientific Centre of Surgery named Syzganov (Almaty, Kazakhstan). Informed oral consents were taken from all participants and the study was approved by the local ethical committee.

All patients were subjected to full history taking, complete clinical examination, abdominal ultrasound, while laboratory investigations were done to all subjects

Isolation and genotyping of DNA

For the isolation of DNA was used Wizard Genomic DNA Purification kit(Promega, USA) according to the manufacturer's protocols with modification of the Genetics Laboratory of the Biotechnology Centre of the Ministry of Education and Science of the Republic of Kazakhstan (Figure 1).

Genotyping of polymorphisms was carried out by direct sequencing in both directions. For the sequence of reactions, BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) was used. DNA sequencing was performed on a 3730XL DNA analyzer device, Applied Biosystems, Foster city, CA, USA (Figure 2). The analysis of the obtained nucleotide sequences was carried out using the Applied Biosystems software package (Sequence Analysis 5.3.1, SeqScape v.2.6, etc.), Finch TV v1.3.1. And using international bases of nucleotide sequences (Blast, ENSEMBL, GeneBank, etc.)

Results

Comparison of the frequency distribution of genotypes of T / C genotypes and AT-6 angiotensinogen gene alleles in patients with chronic viral hepatitis C and healthy individuals of Kazakh nationality showed that the homozygous inheritance of the T / T genotype and the heterozygous inheritance of the C / T genotype are not significantly different from the baseline and control groups (P> 0.05). (Table 1). In the group of patients with chronic HCV infection, a significantly lower frequency of homozygous inheritance of the C / C genotype (P <0.05) is observed. The distribution of

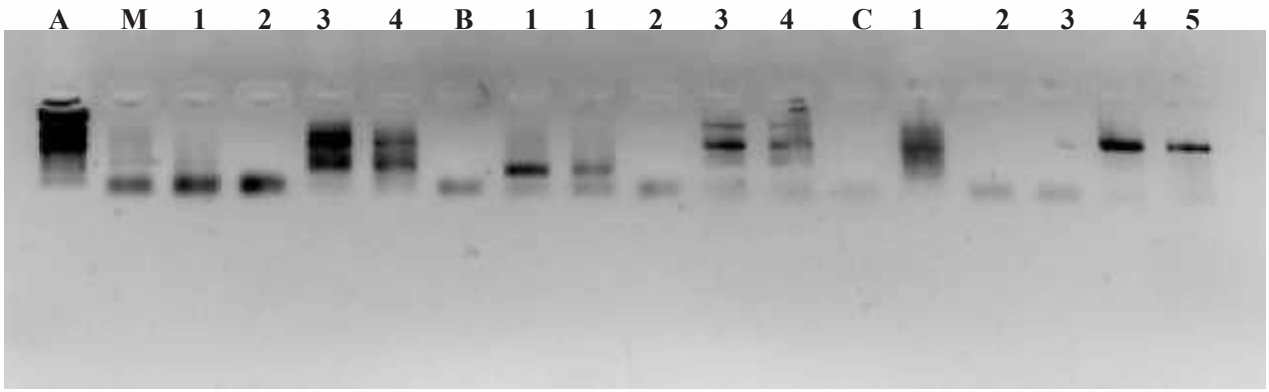

Figure 1. *Electrophoresis in 1% agarose gel after staining with ethidium bromide showing PCR-RFLP analysis of the TGF. PCR with 4 samples of isolated DNA. 5 modes of PCR mixtures with different concentrations of MgCl2 (A = 1.5 mM, B = 2.0 mM, C = 2.5 mM and with the addition of betaine to D, E mixture). M = DNA marker.*

Table 1

Frequency distribution of T/C genotypes and alleles of the angiotensinogen AT-6 gene in patients with chronic viral hepatitis C and healthy individuals of Kazakh nationality

Genotypes and alleles	Frequency of alleles and genotypes		P
	Patients (n=120)	Health donors (n=70)	
Genotype T/T	82 (68,3%)	46(65,7%)	>0,05
Genotype C/T	9 (7,5%)	5 (7,1 %)	>0,05
Genotype C/C	29 (24,2%)	19(27,1 %)	<0,05
allele T	91 (75,8 %)	51(72,9%)	>0,05
allele C	38 (31,70%)	24 (34,3%)	>0,05

the alleles C and T of the angiotensinogen AT-6 gene did not differ significantly in both groups (P> 0.05) and showed that in both groups the highest percentage was found in the T allele (75.8% and 72.9% in patients and Of healthy donors, respectively) than to allele C (31.7% and 34.3% in patients and healthy donors, respectively).

Frequency distribution of the genotypes of the angiotensinogen AT-6 gene depending on the progression of chronic viral hepatitis C (by groups). (Table 2).

The results of this study showed that in patients with cirrhosis of the liver with the outcome of viral hepatitis C, *thymosin-thymosin* is more often found in the homozygous genotype of the inheritance of the angiotensinogen AT-6 gene (P <0.05) than in patients with chronic viral hepatitis. The inheritance of the homozygous genotype C / C of the AT-6 angiotensinogen gene is associated with an easier flow of chronic HCV infection (P <0.005) in the Kazakh population. The inheritance of the T allele in the group with compensated and decompensated cirrhosis is also statistically significant in comparison with the group of patients with chronic viral hepatitis C (P <0.05, P <0.005, respectively).

Table 2.

Frequency of the distribution of C / T genotypes of the angiotensinogen AT-6 gene depending on the progression of chronic viral hepatitis C (by groups)

Genotypes and alleles	Frequency of alleles and genotypes		
	Group 1 chronic hepatitis C	Group2 HCVcirrhosis Class A	Group 3 HCV cirrhosis Class B и C
Genotype T/T (n=82)	20 (24,3%)	28 (40,0 %)	38 (46 %)
GenotypeC/T (n=9)	3(33,3%)	4 (44,4%)	2(22,2%)
GenotypeC/C (n=29)	17 (58,6%)	5 (17,24%)	5(17,24%)
Allele T (n=91)	23(25,3 %)	32 (35,2%)	42 (46,2%)
Alele C (n=38)	20(52,6 %)	9 (23,7%)	7 (18,4%)

Discussion

The natural history of the chronic liver disease caused by HCV remains controversial with varying rates of progression to cirrhosis [*Roskams T, et al, 2004*]. Although approximately 80% of patients who acquire hepatitis C virus (HCV) infection will develop a chronic low grade slowly progressive hepatitis, perhaps only 20–30% of infected patients will progress to clinically significant fibrotic disease after 20–30 years [*Roskams T, et al, 2004*].

In the process of fibrogenesis, the production of the key signal molecule of transforming growth factor-β1 can be enhanced by angiotensin II (A II), the main effector molecule of RAS (20). The primary effect of RAS is the regulation of vascular tone and the excretion of salts by the kidneys. However, recent studies have shown that, independently of these effects, AII increases the accumulation of the extracellular matrix (21). Functional polymorphism of RAS genes is described, including the nucleotide sequence of 6 bp from the transcriptional locus in the angiotensinogen (AT) promoter, the angiotensin I peptide precursor and the absence of a deletion with an intron 16, angiotensin-converting enzyme that converts AI to AII [*Bataller R, et al*, 2003; *Herath CB, et al 2007*]

Forrest et al. [*Forrest E, et al, 2005*] studied different four polymorphisms of RAS gene and could not identify any significant association between these four RAS polymorphisms and fibrosis in chronic HCV infection. In addition another study of Gu¨c¸lu¨ et al. [*Gu¨c¸lu¨ Mustafa et al. 2010*] suggested that ACE gene had no role in the development of fibrosis in non-alcoholic fatty liver disease. Serejo et al. [*Serejo F, et al, 2011*] recorded that TT genotype was significantly more prevalent among chronic hepatitis C (CHC) patients but in contrast to our results they declared

that ACE I allele may be a risk factor for liver fibrosis progression

Our study showed that inheritance of the T allele in the group with compensated and decompensated cirrhosis is also statistically significant in comparison with the group of patients with chronic viral hepatitis C ($P < 0.05$, $P < 0.005$, respectively

Conclusion An association was identified between of the gene angiotensin single nucleotide polymorphism located in (AT-6) promoter region -6 and fibrosis in chronic HCV infection In the Kazakh population.

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