The relationship between L-leucine-7-amido -4-methyl coumarin 1 gene polymorphism and susceptibility to the chronic hepatitis B virus infection in an Iranian population

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Background: Lamnin has important effects on human immunity system. The current study aimed to assess the role of L-leucine-7-amido-4-methyl coumarin 1 gene polymorphisms on hepatitis B virus (HBV) susceptibility. **Materials and Methods:** The *rs20558, rs20563, rs10911193, rs10911251,* and *rs1413390* polymorphisms were analyzed using polymerase chain reaction (PCR) and PCR-reaction–restriction fragment-length polymorphism and amplification-refractory mutation system-PCR using three different groups including chronic HBV-infected patients, HBV patients who were resolved their infection spontaneously and healthy volunteers. Laminin concentrations were also measured in the blood of these individuals. **Results:** People with *rs20558C, rs20563G,* and *rs10911193T* alleles have an increased risk of HBV infection. Moreover, we found that CGTAT haplotype was more frequent in chronically infected people who could affect the mechanism of disease. Furthermore, there was a significant relationship between laminin concentration and *rs20558, rs20563,* and *rs10911193* genotypes in patients. **Conclusion:** According to the statistical analysis, *rs20558, rs20563, rs10911193* polymorphisms probably, related to the chronic HBV infection. In addition, no association of the *rs10911251, rs1413390* single nucleotide polymorphisms with the disease was found.

Key words: Hepatitis B virus, laminin, single nucleotide polymorphism

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious public health problem worldwide, especially in Asia and Africa. The disease can develop into chronic hepatitis, cirrhosis, hepatocellular cancer, and finally death. Therefore, the incidence of the disease varies from the person-to-person,[1,2] but the mechanism of disease is not clearly found. Most likely, there is a close connection between T-cells and modes of infections.[3] Interleukin 10 (IL-10),[4] IL-28,[5,6] macrophage migration factor,[7] interferon lambda 4[8] are proteins that control the cascades of the human immune system in chronic infections such as HBV/hepatitis C virus (HCV) through the reduction of the viral replication. Furthermore, the genetic history of individuals has a significant impact on the incidence of disease.[9] On the other hand, the concentration of regulatory proteins in the blood can also affect the course of the disease.[10] Genetic changes such as single nucleotide polymorphisms (SNPs) in the sequences that affect the function of the gene can change the production of protein.[11]

The host immune responses are supported by the connective tissue (CT). The structure and normal function of CT is affected by the molecular composition of its extracellular matrix (ECM) which contains glycoproteins and structural proteins.[12] Abnormal

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synthesis or degradation of ECM contents such as laminin, collagen, and elastin can change the pathophysiology of infectious and some diseases including prolapse.[13] The results have revealed that the amount of collagen decreases in the CT of patients with female pelvic floor disorders.[14] Furthermore, an increase or no changes have reported in collagen expression in patients with stress urinary incontinence.[15] Moreover, ECM deficiency leads to fibrosis which is the result of failing in normal tissue contents and function. After a chronic infection, a wound-healing response induces in tissues. This phenomena causes a new ECM for maintaining the tissue homeostasis. If the infection is long-lasting, or the genetic mutations are existing in ECM pathways, may result in the fibrosis and disease severity.[16] The host immune responses against HBV infection involves signaling pathways and regulatory systems which can be disrupted. In this pilot study, we describe how the SNPs of L-leucine-7-amido-4-methyl coumarin 1 (LAMC1) contribute to susceptibility to chronic HBV infection. Laminin is a noncollagenous element of the basement membranes. It has been contributed to cell adhesion, differentiation, migration, signaling, and metastasis. Laminin has three nonidentical chains: Laminin alpha, beta and gamma. LAMC1 codes the laminin gamma1 chain, as a critical component of the ECM.[16] Previous studies indicated that the *rs10911193T* allele in the gene promoter was significantly prevalent among patients with prolapse.[17] The goals of this pilot study were to determine if polymorphisms in the LAMC1 gene (*rs20558, rs20563, rs10911193, rs10911251,* and *rs1413390*) were associated with the susceptibility to chronic HBV infection in a sample of Iranians.

METHODS

Study subjects

In this case–control, 100 chronic HBV patients showing positive hepatitis B virus surface antigen (HBsAg) and antibodies against anti-HBc and impaired liver function test (≥2 times the upper limit of normal) for at least 6 months were enrolled into the study (age: 29.03 ± 5.710, age range of 18–43). Serological tests (presence of HBsAg by enzyme-linked immunosorbent assay (ELISA) and HBV-DNA by reverse transcription polymerase chain reaction (PCR) and clinical findings were compatible with the chronic liver disease. 40 patients with negative HBsAg and anti-HCV and positive antibodies against anti-HBs and anti-HBc were also included as spontaneously recovered (SR) group (age: 29.72 ± 5.517, the age range of 18–42). The control group comprised 100 HBsAg, anti-HBs and anti-HBc negative, healthy volunteers with normal values for alanine transaminase, without any history of hepatitis B infection (age: 30.44 ± 4.539, the age range of 22–45). All participants were in the same geographical area.

All participants were unrelated Iranians. There was no difference between groups regarding age, gender, and ethnicity [Supplementary Table 1]. Participants with HCV, Hepatitis E virus, HAV, HIV, alcohol consumption, drug abuse, liver diseases were excluded from the study. Between July and December 2015, the study population was recruited, and they were enrolled in the outpatient clinic of the blood transfusion organization clinics in Zahedan, Iran. The study was approved by the Institutional Ethics Committee of the Zahedan University of Medical Sciences (IR. ZAUMS. REC.1395.159, grant number: 6809) and carried out in Infectious Diseases and Tropical Medicine Research Center, Zahedan, Iran. Written informed consent was obtained from each participant.

Analysis of the serum laminin

Biochemical parameters and serum level of laminin were determined by the sensitive sandwich ELISA technique (EASTBIOPHARM, China) using 3 ml of blood.

Polymorphism genotyping

Genomic DNA was extracted from blood using the salting-out method. The LAMC*1* polymorphisms rs20563 and rs10911193 were detected by PCR–restriction fragment-length polymorphism (PCR-RFLP). The *rs20558, rs10911251,* and *rs1413390* were analyzed by amplification-refractory mutation system (ARMS-PCR). The sequence of the primers, restriction enzymes, and the fragment length after digestion is shown in Supplementary Table 2.

PCR amplification was carried out in a total volume of 20 μL containing 1 μl of each primer, 100 ng of template DNA and 10 μl of 2X Prime Taq Premix (Genet Bio, Korea) and 7 μl ddH$_2$O. The annealing temperature was 60°C for *rs20563,* 59°C for *rs10911193* polymorphisms of the LAMC1. Digestion was done by the restriction enzymes (Fermentas, Vilnius, Lithuania) and products were checked by electrophoresis (2% agarose gel and ethidium bromide).

ARMS-PCR reactions were performed using 2X Prime Taq Premix (Genet Bio, Korea). For each sample, we used two tubes, one for wild allele and another tube for the mutant allele. In each 0.20 ml reaction, 1 μl of each primer (allele-specific and inner control primers), 1 μl of genomic DNA (~100 ng/ml) and 10 μl of 2X Prime Taq Premix (Genet Bio, Korea) and 5 μl ddH2O were added. One tube contained forward primer (F) and reverse primer (R1) for wild allele and the other tube contained forward primer (F) and reverse primer (R2) for the mutant allele. All tubes had forward primer and reverse primer for inner control (F, R) which was part of the LAMC1 gene sequence. PCR reaction was performed according to the following protocol: initial denaturation: 95°C for 5 min, 35 cycles of

denaturation: 95°C for 30 s, annealing: 57°C *rs20558*, 59°C for *rs10911251*, and 60°C for *rs1413390* for 30 s, extension: 72°C for 30 s and final extension: 72°C for 5 min. The amplified products were resolved by electrophoresis in 2% agarose gel and stained with ethidium bromide.

Statistical analysis

Descriptive statistics were conducted using SPSS 20.0 (IBM, Armonk, NY, United States of America) and assuming an alpha level of 0.05. Data were expressed as a mean ± standard deviation. The comparing the data were done by Chi-square analyses and Student's *t*-test. To test for association of single SNPs with disease, we constructed logistic regression models.

RESULTS

Summary results of the clinical characteristics are shown in Supplementary Table 1. The population of the study was comprised chronic HBV-infected, SR and healthy controls. The gender, ages, and ethnicities were not significantly different between the three groups ($P = 0.427$, $P = 0.125$, and $P = 0.292$).

Genotype analysis

Genotyping findings of *rs20558*, *rs20563*, *rs10911193*, *rs10911251*, and *rs1413390* in the laminin gene are listed in Tables 1 and 2.

The distribution of polymorphisms in control group was based on the Hardy–Weinberg equilibrium ($P = 0.152$ for rs20558, $P = 0.101$ for rs20563, $P = 0.180$ for *rs10911193*, $P = 0.074$ for *rs10911251*, and $P = 0.0681$ for rs1413390 $P > 0.05$).

Distribution of the rs20558C allele had higher frequency in HBV patients compare to the *rs20558T* allele and increased risk of disease ($P = 0.001$, odds ratio [OR] = 2.062, 95% confidence interval [CI] = 1.360–3.125). Distribution of the *rs20563G* allele had higher frequency in HBV patients compared to the rs20563A allele and increased risk of disease ($P = 0.001$, OR = 2.062, 95% CI = 1.360–3.125). Distribution of the rs10911193T allele had higher frequency in HBV patients compare to the rs10911193C allele and increased risk of disease ($P = 0.008$, OR = 1.826, 95% CI = 1.171-2.849) [Table 1].

This study showed that the existence of *rs20558C,* allele significantly increased risk of chronic HBV infection compared to the rs20558T allele ($P = 0.001$, OR = 2.062, 95% CI = 1.360–3.125). In addition the results showed that the *rs20563G* allele significantly increased risk of chronic HBV infection compared to the rs20563A allele ($P = 0.001$, OR = 2.062, 95% CI = 1.360–3.125). On the other hand, the

results revealed that rs10911193T allele significantly increased risk of chronic HBV infection compared to the *rs10911193C* allele ($P = 0.008$, OR = 1.826, 95% CI = 1.171–2.849) [Table 1].

For *rs10911251* and rs1413390 polymorphisms, no significant differences in allele or genotype frequencies between groups were found [Table 1]. The results showing that there is not any relationship between the rs10911251A ($P = 0.974$) and *rs1413390T* ($P = 0.116$) and elimination of HBV [Table 2].

We calculated the rs20558, *rs20563, rs10911193, rs10911251, rs1413390* haplotype association analysis, and found 5 genotypes. As shown in Tables 3 and 4, the distribution frequency of haplotypes showed a significant association between chronic HBV infection compared to the C and SR groups. In our study, CGTAT haplotype had a higher frequency in HBV patients than C and SR groups. It means that this haplotype might relate to the natural history of the infection. Linkage disequilibrium (LD) analysis indicated that the rs20558 variant was in complete LD with the rs20563 variant ($R^2 = 1$) [Table 3].

Analysis of serum laminin level

Serum levels of the laminin were significantly increased in the chronic HBV patients compared to the healthy groups (C + SR) (48.16 ± 14.19, 51.56 ± 11.53, 83.13 ± 15.43, in C, SR and HBV groups, respectively, $P < 0.001$). Statistical analysis indicated a significant association between laminin genotypes and serum levels of the laminin in HBV patients [Table 5]. Chronic HBV patients with *rs20558CC, rs2056GG,* and *rs10911193TT* genotypes had higher levels of laminin compared to rs20558TT ($P = 0.038$), *rs2056AA* ($P = 0.038$), and *rs10911193CC* ($P = 0.039$) genotypes, respectively. The results showed that subjects with at least one *rs20558C, rs2056G,* and *rs10911193T* alleles had increased levels of laminin, compared to the other alleles [Supplementary Table 3].

DISCUSSION

In this case–control analysis of LAMC1 genetic variance, we provide important evidence that LAMC1 is a potential candidate gene for increased susceptibility to chronic HBV infection in Iranian populations. Although we did not find an association between the rs10911251, rs1413390 SNPs and chronic infection, we found a relationship between *rs20558, rs20563, and rs10911193* SNPs and disease that strictly indicated a trend toward significance. The *rs20558* and *rs20563* were in the complete LD and confirmed the association between CGTAT haplotype and risk of chronic infection. In addition, we found that the serum level of laminin was significantly associated with susceptibility and risk of the chronic HBV infection.

Table 1: The frequency of genotypes and alleles of the *rs20558, rs20563, rs10911193, rs10911251, rs1413390* polymorphisms between chronic hepatitis B patients, spontaneously recovered subjects and control group

Laminin polymorphisms	C (%)	SR (%)	P (C vs. SR)	OR (C vs. SR)	HBV (%)	P (C vs. HBV)	OR (C vs. HBV)
rs20558							
TT	51 (51.0)	19 (47.5)	Reference=1	-	33 (33.0)	Reference=1	-
TC	42 (42.0)	16 (40.0)	0.955	1.023 (0.469-2.232)	45 (45.0)	0.119	1.623 (0.884-2.983)
CC	7 (7.0)	5 (12.5)	0.312	1.917 (0.542-6.778)	22 (22.0)	0.001	4.762 (1.828-12.404)
TC + CC	49 (49.5)	21 (52.5)	0.708	1.150 (0.552-2.397)	67 (67.0)	0.013	2.072 (1.167-3.676)
T	144 (72.0)	54 (67.5)	Reference=1	-	111 (55.5)	Reference=1	-
C	56 (28.0)	26 (32.5)	0.455	1.238 (0.707-2.169)	89 (44.5)	0.001	2.062 (1.360-3.125)
rs20563							
AA	51 (51.0)	19 (47.5)	Reference=1	-	33 (33.0)	Reference=1	-
AG	42 (42.0)	16 (40.0)	0.955	1.023 (0.469-2.232)	45 (45.0)	0.119	1.623 (0.884-2.983)
GG	7 (7.0)	5 (12.5)	0.312	1.917 (0.542-6.778)	22 (22.0)	0.001	4.762 (1.828-12.404)
GG + AG	49 (49.5)	21 (52.5)	0.708	1.150 (0.552-2.397)	67 (67.0)	0.013	2.072 (1.167-3.676)
A	144 (72.0)	54 (67.5)	Reference=1	-	111 (55.5)	Reference=1	-
G	56 (28.0)	26 (32.5)	0.455	1.238 (0.707-2.169)	89 (44.5)	0.001	2.062 (1.360-3.125)
rs10911193							
CC	61 (61.0)	26 (65.0)	Reference=1	-	44 (44.0)	Reference=1	-
CT	34 (34.0)	13 (32.5)	0.787	0.897 (0.408-1.970)	44 (44.0)	0.053	1.794 (0.992-3.243)
TT	5 (5.0)	1 (2.5)	0.499	0.469 (0.052-4.216)	12 (12.0)	0.034	3.327 (1.093-10.126)
CT + TT	39 (39.0)	14 (35.0)	0.660	0.842 (0.392-1.808)	56 (56.0)	0.017	1.991 (1.133-3.497)
C	156 (78.0)	65 (81.2)	Reference=1	-	132 (66.0)	Reference=1	-
T	44 (22.0)	15 (18.8)	0.547	0.818 (0.426-1.573)	68 (34.0)	0.008	1.826 (1.171-2.849)
rs10911251							
AA	46 (46.0)	22 (55.0)	0.797	1.136 (0.431-2.996)	49 (49.0)	0.975	1.012 (0.480-2.133)
AC	35 (35.0)	10 (25.0)	0.483	0.679 (0.229-2.007)	31 (31.0)	0.669	0.841 (0.381-1.858)
CC	19 (19.0)	8 (20.0)	Reference=1	-	20 (20.0)	Reference=1	-
AA + CC	81 (81.0)	32 (80.0)	0.892	0.938 (0.373-2.359)	80 (80.0)	0.858	0.938 (0.466-1.889)
A	127 (63.5)	54 (67.5)	0.527	1.194 (0.689-2.068)	129 (64.5)	0.835	1.044 (0.694-1.571)
C	73 (36.5)	26 (32.5)	Reference=1	-	71 (35.5)	Reference=1	-
rs1413390							
CC	22 (22.0)	7 (17.5)	Reference=1	-	20 (20.0)	Reference=1	-
CT	40 (40.0)	17 (42.5)	0.579	1.336 (0.480-3.713)	28 (28.0)	0.508	0.770 (0.335-1.671)
TT	38 (38.0)	16 (40.0)	0.595	1.323 (0.472-3.713)	52 (52.0)	0.276	1.505 (0.721-3.142)
CT + TT	78 (78.0)	33 (82.5)	0.554	1.330 (0.518-3.414)	80 (80.0)	0.729	1.128 (0.571-2.229)
C	84 (42.0)	31 (38.8)	Reference=1	-	68 (34.0)	Reference=1	-
T	116 (58.0)	49 (61.2)	0.618	1.145 (0.674-1.945)	132 (66.0)	0.100	1.406 (0.937-2.109)

Data are given as percentage of cases (%). C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B; OR=Odds ratio

LAMC1 gene is of great interest due to the important role of laminin in ECM normal function. There are numerous supporting evidence that indicating the particular interest of LAMC1. LAMC1 gene may involve in chronic HBV infection, and further investigations can describe its possible involvement in disease pathology.

LAMC1 gene encodes the ϒchain of laminin which is one of the ECM glycoproteins and produce the noncollagenous portion of the basement membranes.[13] Nikolova *et al*.[17] reported that the rs10911193 minor *T* allele was more prevalent among patients with early-onset prolapse. Chen *et al*.[18] indicated that LAMC1 *rs10911192, rs20563*, and *rs20558* SNPs did not associate with prolapse in Caucasians and African–Americans patients with prolapse, which might be due to their small sample size.

The current study showed that *rs20563* and *rs20558* SNPs had a trend toward significance. The *rs20563* G allele causes substitution of isoleucine to valine. This phenomenon is somehow conservative shift because both isoleucine and valine have hydrophobic side chains. The *rs20558* C allele leads to insufficient conservative substitution from leucine to proline. These changes associated with tolerated and benign effects on the protein expression and function.[19]

The *rs10911193* polymorphism is located upstream of the start site of the gene and may play a role in causing the chronic infection. This polymorphic region can affect the efficiency of the binding site for NFIL3. NFIL3 is transcriptional repressor and simultaneously expressed with LAMC1.[20] This function of NFIL3 can increase expression of laminin gene which has polymorphism. The

Table 2: The frequency of genotypes and alleles of the rs20558, rs20563, rs10911193, rs10911251, rs1413390 polymorphisms between chronic hepatitis B patients and spontaneously recovered subjects/control group (control + spontaneously recovered)

Laminin polymorphisms	HBV (%)	Healthy (C + SR) (%)	*P*	OR
rs20558				
TT	33 (33.0)	70 (50.0)	Reference=1	-
TC	45 (45.0)	58 (41.4)	0.086	1.646 (0.932–2.905)
CC	22 (22.0)	12 (8.6)	0.001	3.889 (1.719–8.795)
TC + CC	67 (67.0)	70 (50.0)	0.009	2.030 (1.192–3.458)
T	111 (55.5)	198 (70.7)	Reference=1	-
C	89 (44.5)	82 (29.3)	0.001	1.936 (1.325–2.830)
rs20563				
AA	33 (33.0)	70 (50.0)	Reference=1	-
AG	45 (45.0)	58 (41.4)	0.086	1.646 (0.932–2.905)
GG	22 (22.0)	12 (8.6)	0.001	3.889 (1.719–8.795)
GG + AG	67 (67.0)	70 (50.0)	0.009	2.030 (1.192–3.458)
A	111 (55.5)	198 (70.7)	Reference=1	-
G	89 (44.5)	82 (29.3)	0.001	1.936 (1.325–2.830)
rs10911193				
CC	44 (44.0)	87 (62.1)	Reference=1	-
CT	44 (44.0)	47 (33.6)	0.028	1.851 (1.070–3.203)
TT	12 (12.0)	6 (4.3)	0.10	3.955 (1.391–11.243)
CT + TT	56 (56.0)	53 (37.9)	0.006	2.089 (1.240–3.521)
C	132 (66.0)	221 (78.9)	Reference=1	-
T	68 (34.0)	59 (21.1)	0.002	1.930 (1.281–2.908)
rs10911251				
AA	49 (49.0)	68 (48.6)	0.937	0.973 (0.490–1.930)
AC	31 (31.0)	45 (32.1)	0.847	0.930 (0.445–1.944)
CC	20 (20.0)	27 (19.3)	Reference=1	-
AA + CC	80 (80.0)	113 (80.7)	0.891	0.956 (0.501–1.822)
A	129 (64.5)	181 (64.6)	0.974	0.994 (0.680–1.452)
C	71 (35.5)	99 (35.4)	Reference=1	-
rs1413390				
CC	20 (20.0)	29 (20.7)	Reference=1	-
CT	28 (28.0)	57 (40.7)	0.361	0.712 (0.344–1.474)
TT	52 (52.0)	54 (38.6)	0.340	1.396 (0.704–2.771)
CT + TT	80 (80.0)	111 (79.3)	0.892	1.045 (0.552–1.978)
C	68 (34.0)	115 (41.1)	Reference=1	-
T	132 (66.0)	165 (58.9)	0.116	1.353 (0.928–1.973)

C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B; OR=Odds ratio

Table 3: Haplotype frequencies (*rs20558, rs20563, rs10911193, rs10911251, rs1413390*) in chronic hepatitis B patients, spontaneously recovered subjects and control group

Haplotypes	C group, *n* (%)	SR group, *n* (%)	*P* (C vs. SR)	OR (C vs. SR)	HBV group, *n* (%)	*P* (C vs. HBV)	OR (C vs. HBV)
CGTAT	10 (10.0)	7 (17.5)	0.322	1.808 (0.559–5.847)	39 (39.0)	<0.001	10.075 (3.847–26.383)
CGCAC	29 (29.0)	7 (17.5)	0.383	0.624 (0.216–1.801)	26 (26.0)	0.053	2.316 (0.989–5.424)
TACCT	13 (13.0)	6 (15.0)	0.769	1.192 (0.368–3.859)	7 (7.0)	0.569	1.391 (0.447–4.328)
TATAT	17 (17.0)	8 (20.0)	0.721	1.216 (0.416–3.553)	16 (16.0)	0.068	2.431 (0.937–6.312)
TACCC	31 (31.0)	12 (30.0)	Reference=1	-	12 (12.0)	Reference=1	-
Total	100 (100.0)	40 (100.0)			100 (100.0)		

C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B; OR=Odds ratio

interaction between NFIL3 and LAMC1 also can affect the signaling pathway of IL three which is a regulatory cytokine in the host immune system.[21] Since we also found the significantly different frequency of *rs10911193* genotypes in chronic HBV patients and found an increased serum level of laminin in patients compared to the healthy controls, we hypothesized that it has a role in the existence of the chronic HBV infection. Therefore, it is recommended to analysis the

association of LAMC1 polymorphisms with other outcomes of the HBV infection.

Table 4: Haplotype frequencies (rs20558, rs20563, rs10911193, rs10911251, rs1413390) in chronic hepatitis B patients and spontaneously recovered subjects/control group (control+spontaneously recovered)

Haplotypes	Healthy (C + SR), n (%)	HBV group, n (%)	P	OR
CGTAT	17 (12.1)	39 (39.0)	0.000	8.221 (3.490–19.362)
CGCAC	36 (25.7)	26 (26.0)	0.022	2.588 (1.146–5.846)
TACCT	19 (13.6)	7 (7.0)	0.613	1.320 (0.450–3.877)
TATAT	25 (17.9)	16 (16.0)	0.069	2.293 (0.936–5.619)
TACCC	43 (30.7)	12 (12.0)	Reference=1	-
Total	140 (100.0)	100 (100.0)		

C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B; OR=Odds ratio

The effective role of LAMC1 in encoding the laminin Υ subunit attracted our attention. Collagen IV, laminin, nidogen, and perlecan are the main glycoproteins of the basement membranes. Laminin, as a noncollagenous member, is a heterogenous glycoprotein, which, can regulate various biological processes such as proliferation, differentiation, and migration of the cells.[22] Laminin molecules contain α, β and Υ chains which produce 15 distinct isoforms. Laminin Υ1 subunit has a widespread distribution between laminin isoforms and basement membranes.[23] Furthermore, laminin has important role in the interaction of ECM molecules.[24] Therefore, changes in gene structure or expression can affect the host immune responses against pathogens. In addition, it has been reported that laminin may contribute to liver fibrosis during liver inflammations. Laminin has also been associated with normal tissue architecture and function through CT remodeling, and deficiency in gene function leads to progressive organ failure.[16] Laminin deficiency

Table 5: Association of genotypes of rs20558, rs20563, rs10911193, rs10911251, rs1413390 polymorphisms with serum laminin levels between chronic hepatitis B patients and spontaneously recovered subjects/control group (control+spontaneously recovered)

Genotype	HBV (n)	HBV Laminin (ng/ml)	Healthy (C+SR) (n)	Healthy (C+SR) Laminin (ng/ml)
rs20558 (T/C)				
TT	33 (33.0)	78.46±11.29	70 (50.0)	48.36±13.64
TC	45 (45.0)	83.78±15.16	58 (41.4)	48.56±13.57
CC	22 (22.0)	88.34±16.69*	12 (8.6)	53.84±9.93
P		0.044		0.410
F		3.227		0.896
rs20563 (A/G)				
AA	33 (33.0)	78.46±11.29	70 (50.0)	48.36±13.64
AG	45 (45.0)	83.78±15.16	58 (41.4)	48.56±13.57
GG	22 (22.0)	88.34±16.69**	12 (8.6)	53.84±9.93
P		0.044		0.410
F		3.227		0.896
rs10911193 (C/T)				
CC	44 (44.0)	81.11±13.14	87 (62.1)	49.37±12.88
CT	44 (44.0)	82.80±15.10	47 (33.6)	47.32±13.60
TT	12 (12.0)	92.53±14.05***	6 (4.3)	54.20±11.28
P		0.049		0.445
F		3.104		0.814
rs10911251 (A/C)				
AA	49 (49.0)	84.27±16.22	68 (48.6)	50.54±12.62
AC	31 (31.0)	82.51±15.37	45 (32.1)	46.53±14.61
CC	20 (20.0)	81.29±10.78	27 (19.3)	48.80±12.73
P		0.730		0.296
F		0.316		1.228
rs1413390 (C/T)			29 (20.7)	
CC	20 (20.0)	79.24±10.66	57 (40.7)	67.61±19.65
CT	28 (28.0)	82.64±15.67	54 (38.6)	59.23±23.21
TT	52 (52.0)	84.69±15.77		64.56±21.34
P		0.377		0.073
F		0.985		2.642

*P=0.038, compared with genotype TT, **P=0.038, compared with genotype AA, ***P=0.039, compared with genotype CC. C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B

related-inflammation can affect numerous organs in the human body and causes serious diseases such as liver chronic inflammation, cardiovascular diseases, pulmonary fibrosis and chronic kidney disease.[25] According to the important role of laminin and specifically LAMC1, it seems that LAMC1 gene might have an effect in the predisposition of chronic HBV infection. *In vivo* studies indicated that deficiency of ϒ1 subunit could change the polymerization of the laminin molecule and also, other ECM proteins could not compensate the laminin production. Therefore, basement membranes were unable to cell migration, specifically, immune regulatory cells.[26] Moreover, the studies have been reported that genetic variation in LAMC1 gene might alter the interaction between LAMC1 and nidogen during cell development, which is crucial for the development of hepatic cells in the liver.[16] The role of LAMC1 in liver development emphasizes that the gene may have a role in physiology and maintenance of hepatic organs.

It has been identified that a number of severe tissue integrity disorders caused by mutations in the coding sequence of laminin gene. Bonneau *et al.*[27] implicated that LAMB1 gene deficiency was related to the cutis laxa. Helbling-Leclerc *et al.*[28] indicated that LAMA2 gene variation was associated with the muscular dystrophy. The study of Pulkkinen *et al.*[29] and Vidal *et al.*[30] found that genetic variation in LAMB3 and LAMA3 genes were associated with Herlitzjunctional Epidermolysis bullosa. Based on the present study, the LAMC1 polymorphism in the promoter region is obvious mutation which can explain the risk of HBV infection and disease severity. It is likely that the polymorphism in LAMC1 gene is the risk factor for the existence of HBV infection and also is in LD with other mutations in laminin gene.

Further studies with regard to the functional and population association between LAMC1 and chronic HBV infection can better explain the role of LAMC1 in the prediction of disease. In the current study frequency of the *rs20558C*, *rs20563G*, and *rs10911193T* alleles significantly increased in a sample of Iranian patients with HBV infection. This finding supports the predictive capacity of LAMC1.

CONCLUSIONS

To the best of our knowledge, some SNPs studied have important effects on the susceptibility and outcomes of HBV infection. Furthermore, we found that laminin levels probably be dependent on the severity of the infection. Determination of the real effects of laminin polymorphisms needs more detailed studies. The restrictions of the current study, likely include sample size, different cultural backgrounds, epidemiological and geographical factors, study conditions and different gene-gene interactions. The results have shown the role of genetic polymorphisms of *rs20558*, *rs20563*, *rs10911193*, *rs10911251*, and *rs1413390* of LAMC1 gene in the chronic HBV infection, and elucidated an association between chronic HBV infection and LAMC1 gene polymorphisms.
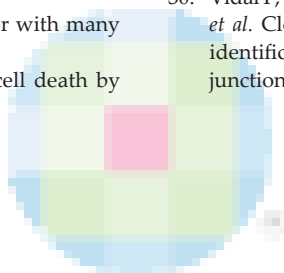
Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Demographic data of chronic hepatitis B patients, spontaneously recovered subjects and control group

Parameters	C, n (%)	SR, n (%)	HBV, n (%)	P
Age (years)	30.44±4.539	29.72±5.517	29.03±5.710	0.125
Sex				
Male	51 (51.0)	25 (62.5)	57 (57.0)	0.427
Female	49 (49.0)	15 (37.5)	43 (43.0)	
Ethnicities				
Sistani	46 (46.0)	18 (45.0)	41 (41.0)	0.292
Baluch	18 (18.0)	13 (32.5)	22 (22.0)	
Others	36 (36.0)	9 (22.5)	37 (37.0)	

Data are given as mean±SD, or as percentage of cases (%). C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B; OR=Odds ratio; SD=Standard deviation

Supplementary Table 2: polymerase chain reaction–restriction fragment-length polymorphism and amplification-refractory mutation system-restriction fragment-length polymorphism based assay of *rs20558, rs20563, rs10911193, rs10911251, rs1413390* single nucleotide polymorphisms

Polymorphism	Primers	PCR	Annealing temperature (°C)	Restriction enzyme	PCR product (bp)	Allele phenotype (bp)
rs20563	F: TGTTGGCTGGGCGCCTGTG R: GTAATCTCACAGTTCTAACAGG	RFLP	60	Ssp I	757	A: 428/329 G: 757
rs10911193	F: TGACACATTTCCATAAGCAAG R: TTTCATACATGAGTTGAAGCG	RFLP	59	Mae III	473	C: 384/89 T: 473
rs20558	R1:TGCTGCTTCATGGTCCCATACA R2:TGCTGCTTCATGGTCCCATACG F: TGCAAAGGTAATCAGCCTTTGATC	ARMS	57	-	564	T: 564 C: 564
rs10911251	R1:TCGGGGTTTCTGCCAATAATTTT R2: TCGGGGTTTCTGCCAATAATTTG F: ACATTGTTTATCCCTTCATAATGTC	ARMS	59	-	524	A: 524 C: 524
rs1413390	R1:TTTAAGAACAGGGACCACATCTAC R2:TTTAAGAACAGGGACCACATCTAT F: ATCCTTGAAGTGCATGAAACGAG	ARMS	60	-	595	T: 595 C: 595
Inner control	Control F: ACATTGTTTATCCCTTCATAATGTC Control R: CTGATGTTCTGAGACGGTAAAGC	-	-	-	1128	-

PCR=Polymerase chain reaction; RFLP=Restriction fragment-length polymorphism; ARMS=Amplification-refractory mutation system

Supplementary Table 3: Association of haplotypes of *rs20558/rs20563/rs10911193/rs10911251/rs141339* polymorphisms with serum laminin levels between chronic hepatitis B patients and spontaneously recovered subjects/control group (control + spontaneously recovered)

Haplotypes	HBV (n)	HBV Laminin (ng/ml)	Healthy (C + SR) (n)	Healthy (C + SR) Laminin (ng/ml)
CGTAT	39 (39.0)	90.48±14.84*	17 (12.1)	47.42±14.99
CGCAC	26 (26.0)	80.90±11.12	36 (25.7)	50.22±13.10
TACCT	7 (7.0)	80.94±13.61	19 (13.6)	49.89±15.91
TATAT	16 (16.0)	80.31±14.72	25 (17.9)	47.36±12.70
TACCC	12 (12.0)	75.25±18.05	43 (30.7)	49.58±13.12
P		0.007		0.907
F		3.775		0.253
Total	100 (100.0)		140 (100.0)	

*P=0.015, Compared with genotype TACCC. C=Control; SR=Spontaneously recovered; HBV=Chronic hepatitis B