Research Article

Associations between Extracellular Matrix Protein 1 Gene Polymorphism and Progression of Liver Disease

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Background. Our study aimed to investigate the relationship between extracellular matrix 1 (ECM1) gene polymorphism and progression of liver fibrosis in the Chinese population.

Methods. A total of 656 patients with hepatitis B virus (HBV) infection and 298 healthy individuals of the Chinese Han population were recruited for a retrospective case-control study. Of the disease group, 104 cases had chronic hepatitis B (CHB), 266 had LC, and 286 had hepatocellular carcinoma (HCC). Subjects were frequency-matched according to age and gender. Polymorphisms of the ECM1 gene were examined using the MassARRAY SNP genotyping method.

Results. There were no associations between genotype and allele frequencies of ECM1 rs3737240 and rs13294 loci with the risk of CHB and CHB-related HCC. After adjustment for age, sex, smoking status, and drinking habits, the GT genotype was dramatically related to a reduced risk of chronic HBV infection in both non-HCC (OR = 0.68, 95% CI: 0.49-0.94) and total chronic HBV infection patients (OR = 0.75, 95% CI: 0.56-1.00). Haplotype analyses revealed twelve protective haplotypes against total chronic HBV infection and four against non-HCC chronic HBV infection. Conclusion. ECM1 gene polymorphism in rs3834087 and rs3754217 loci is associated with a reduced risk of chronic HBV infection but not with liver fibrosis development and the occurrence of HCC.

1. Introduction

Approximately 240 million people, especially those in developing countries, are infected with hepatitis B virus (HBV), and nearly half of these have chronic liver disease [1, 2]. Liver fibrosis is a pathological consequence of chronic liver damage and extracellular matrix (ECM) protein accumulation, which may further result in liver diseases such as liver cirrhosis (LC), liver failure, and hepatocellular carcinoma (HCC) [3–5]. Liver fibrosis and its associated liver diseases have been considered the most important causes of HBV-induced death [6]. Although the molecular mechanisms of liver fibrosis have been well documented, more effective drugs for liver fibrosis need to be explored [7, 8].

Regardless of whether a toxic or metabolic pathway ensues, a hepatitis virus infection involves the induction of immune cell infiltration and hepatocyte injury, triggering the trans-differentiation of hepatic stellate cells (HSCs) into collagen-producing myofibroblasts [9, 10], and causing deposition of ECM which leads to liver tissue damage [11, 12]. Human ECM1 gene, located on chromosome 1q21 [13, 14], encodes for a glycoprotein which promotes ECM protein binding and regulates the integrity and barrier function of epidermal ECM in a variety of tissues [13, 15, 16].

A recent study on patients and mouse models has shown an inverse correlation between the ECM1 production in chronic hepatitis liver tissues with transforming growth factor-β activation which is responsible for promoting fibrosis and inducing the activation of static HSCs [17]. Several studies identified genetic variants that are associated with inflammatory bowel disease, such as ulcerative colitis [18, 19] and Crohn’s disease [20]. However, the correlation between ECM1 gene polymorphisms and fibrogenic disease formation has not been reported. In this study, we screened the ECM1 gene polymorphisms of both
Genetics Research

regulatory and coding regions that could modify ECM1 transcription or amino acid sequence and analyzed their association with LC progression in Chinese Han population. Our study might aid in understanding the effects of ECM1 gene polymorphism on the initiation and progression of liver fibrosis and on the occurrence of HCC.

2. Methods

2.1. Subjects. A total of 656 patients with chronic HBV infection, diagnosed as chronic hepatitis B (CHB) \((n = 104)\), LC \((n = 266)\), and HCC \((n = 286)\) based on the Guideline of Prevention and Treatment for CHB [21], were enrolled from the First Hospital of Jilin University [22]. Patients within each group were frequency-matched based on age and sex. CHB was defined as a persistent or intermittent elevation of serum alanine aminotransferase (ALT) \((\geq 2x\text{UTL})\) level, with increased HBV-DNA level \((\geq 500 \text{IU/mL})\) for at least 6 months, and the absence of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. CHB/LC was diagnosed as active necroinflammatory liver disease with/without fibrosis by imageological inspection. The diagnosis of HCC was confirmed with histopathology. The exclusion criteria involved the presence of HAV/HEV infection, autoimmune diseases, alcoholic liver disease, silt hemorrhagic liver disease, autoimmune liver disease, and intra- and extrahepatic bile duct stones. Another 298 liver disease-free individuals were selected as healthy controls, and they were negative for HBeAg, HBsAg, anti-HBc, anti-HBe, and anti-HCV. Demographic information, including drinking habits and smoking status, was collected for analysis. Drinkers were classified as those who consumed alcohol more than once per week for more than six months, while smokers were those who smoke daily for over a year. All subjects provided informed consent, and the research was approved by the ethical committee of The First Hospital of Jilin University (Approval number: 2020-Linshen No. 2020-355). All methods were carried out in accordance with relevant guidelines and regulations.

2.2. SNP Selection. SNPs from the promoter, 5’ untranslated regions (UTRs), exons, and 3’ UTR of the ECM1 gene (minor allele frequency [MAF] >5% in the Northern Chinese Han population database) were screened using GeneView of NCBI. SNP function prediction was carried out using the online tool (https://snipinfo.niehs.nih.gov/). Linkage disequilibrium (LD) analysis was performed for the selected SNPs using the “Linkage Disequilibrium Calculator” (https://asia.ensembl.org/Homo_sapiens/Tools/LD?db=core). One of the complete linkage sites were chosen if \(R^2 = 1\). Validated and hot SNPs with reported relationship with the disease on Google Scholar were also selected and verified for MAF >5% in Beijing Chinese Han population. Of the SNPs found in the promoter region \((rs3834087 \text{ and } rs3754217)\), the latter has been predicted to be located in the transcription factor binding site (TFBS) and may have effects on genetic transcription. SNPs found in the exon-missense region \((rs3737240 \text{ and } rs13294)\) may affect the expression and function of the gene. The ECM1 gene location and the selected SNPs are exhibited in Figure 1.

2.3. Genotype Analysis. Genomic DNA was isolated from whole blood of subjects. SNP genotypes of rs3834087, rs3754217, rs3737240, and rs13294 were analyzed by using the MassARRAY SNP genotyping method (BioMiao Biological Technology, Beijing, CHN). Polymerase chain reaction (PCR) primers and the amplification program are shown in Table 1.

2.4. Statistical Methods. All data were analyzed using the SPSS 18.0 software (SPSS, Chicago, USA). The differences in clinical data and demographic information among groups were calculated using \(\chi^2\) test or Wilcoxon rank-sum test. Independent segregation of alleles was tested by the Hardy–Weinberg equilibrium (H-WE) analysis. Haplotype analysis of polymorphism was performed by using the UNPHASED 3.1.4 software. P-value, odds ratios (ORs), and 95% confidence intervals (CIs) after adjusting for age, gender, and environmental factors were calculated by the bivariate logistic regression analysis. Genotype distributions and allele frequencies were analyzed by the \(\chi^2\) test or Fisher’s exact test. \(P\)-value < 0.05 indicated statistical significance.

3. Results

Demographic information of patients including gender, smoking status, age, and alcohol intake is summarized in Tables 2 and 3. No statistical differences were found in gender \((\chi^2\) test) and age (Wilcoxon rank-sum test) among the groups \((P > 0.05)\) except between non-HCC and HCC groups \((P = 0.0482 \text{ and } P = 0.0220, \text{respectively})\). However, significant differences were observed in smoking status \((\chi^2\) test) between HCC and healthy controls \((P = 0.0023)\), HCC and LC \((P = 0.0001)\), as well as HCC and non-HCC \((P = 0.0001)\). The same was observed in drinking habits \((\chi^2\) test) between non-HCC and healthy controls \((P = 0.0416)\) and non-HCC and HCC \((P = 0.0229)\). Furthermore, the H-WE test determined that all four SNPs \((rs3834087, rs3754217, rs3737240, \text{and } rs13294)\) of the controls were in equilibrium \((P = 0.0916, P = 0.0963, P = 0.5324, \text{and } P = 0.7512, \text{respectively})\).

3.1. Analysis in Chronic HBV Infection Patients and Healthy Controls. The allele and genotype frequencies of the ECM1 SNPs in HBV infected-patients and the control group are displayed in Table 3. There were no correlations between the allele and genotype frequencies of the ECM1 gene polymorphisms at rs3737240 and rs13294 loci of patients with chronic HBV infection. There was an obvious association between the GAG/- genotype of rs3834087 with reduced risk of chronic HBV infection \((OR = 0.65, 95\% CI: 0.45-0.95)\). After adjustment for age, smoking status, sex, and drinking habits, bivariate logistic regression analyses showed that the GAG/- and -/- combined genotypes were markedly correlated with a declined risk of chronic HBV infection.
Figure 1: Location of the extracellular matrix 1 gene and single nucleotide polymorphisms.

Table 1: Primer sequences and reaction conditions for genotyping ECM1 polymorphisms.

| SNP         | Sequence of the primer | Annealing temperature (°C) |
|-------------|------------------------|---------------------------|
| Rs3834087   | F:5′-ACGTGGATGAGACCTAGATGGAATCAGCC-3′ | 47.9                      |
|             | R:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ |                           |
| Rs3754217   | F:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ | 55.5                      |
|             | R:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ |                           |
| Rs3737240   | F:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ | 50.8                      |
|             | R:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ |                           |
| Rs13294     | F:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ | 51.6                      |
|             | R:5′-ACGTGGATGGAATCAGCCACACATGGCAG-3′ |                           |

Table 2: Baseline characteristics of 954 study subjects.

| Group                      | Healthy control n = 298 | Non-HCC n = 370 | P -valuea | HBV-related HCC n = 286 | P -valueb | P -valuec |
|----------------------------|-------------------------|-----------------|------------|-------------------------|------------|------------|
| Male, n (%)                | 246 (82.6)              | 295 (79.7)      | 0.3734     | 245 (85.7)              | 0.3112     | 1.0000     |
| Age, M (P25, P75)          | 50 (45.55)              | 49 (42.55)      | 0.0885     | 50 (46.56)              | 0.5214     | 0.4585     |
| Smoking                    |                         |                 | 0.4654     |                         | 0.0023     | 0.2574     |
| Ever, n (%)                | 112 (37.6)              | 128 (34.6)      | 0.0416     | 119 (41.6)              | 0.8052     | 0.2542     |
| Never, n (%)               | 186 (62.4)              | 242 (65.4)      | 144 (50.3) |                         |            |            |
| Drinking                   |                         |                 |            |                         |            |            |
| Ever, n (%)                | 121 (40.6)              | 122 (33.0)      |            |                         |            |            |
| Never, n (%)               | 177 (59.4)              | 248 (67.0)      |            |                         |            |            |

P-valuea,b,c represents the non-HCC, HCC, and chronic HBV patients compared to the healthy controls. The rank-sum test or \( \chi^2 \) test was used to evaluate continuous numeric variables and grouping variables in demographic data among the groups. HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

Table 3: Baseline characteristic of chronic HBV infection subgroups.

| Group                      | Non-HCC n = 104 | CHB n = 104 | P -valuea | LC n = 266 | P -valueb | HCC n = 286 | P -valuec |
|----------------------------|-----------------|------------|-----------|------------|-----------|------------|-----------|
| Male, n (%)                | 84 (80.8)       | 47 (43.53) | 0.7558    | 211 (79.3) | 0.0495    | 245 (85.7) | 0.0482    |
| Age, M (P25, P75)          |                 |            | 0.3404    | 49 (41.56) | 0.1443    | 50 (46.56) | 0.0220    |
| Smoking                    | 0.8039          |            |           | 0.0001     |           | <0.0001    |           |
| Ever, n (%)                | 37 (35.6)       | 67 (64.4)  |           | 91 (34.2)  |           | 144 (50.3) |           |
| Never, n (%)               | 47 (64.4)       |            |           | 175 (65.8) |           | 142 (49.7) |           |
| Drinking                   | 0.1217          |            |           | 0.1305     |           | 0.0229     |           |

P-valuea,b,c represents the CHB vs. LC, LC vs. HCC, and non-HCC vs. HCC, respectively. The rank-sum test or \( \chi^2 \) test was used to evaluate continuous numeric variables and grouping variables in demographic data among the groups. CHB, chronic hepatitis B; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; OR, odds ratio.
(OR = 0.66, 95% CI: 0.45-0.96). The GT genotype of rs3754217, as compared to the wild GG genotype, was closely related to a reduced risk of chronic HBV infection (adjusted OR = 0.75, 95% CI: 0.56-1.00).

3.2. Analysis in HCC Patients and Healthy Controls. The genotype and allele frequencies of the ECM1 SNPs in HCC patients and the control group are presented in Table 4. There were no differences in the frequencies of all alleles and genotypes (rs3834087, rs3754217, rs3737240, and rs13294).

3.3. The Genotype and Allele Frequencies of ECM1 SNPs in Non-HCC Patients. The genotype and allele frequencies of ECM1 SNPs in non-HCC patients and the control group are presented in Table 4. No obvious effects were identified between the genotype and allele frequencies of ECM1 SNPs at rs3737240 and rs13294 loci and in the risk of non-HCC after adjusting for smoking status, drinking habits, sex, and age.

However, the DEL allele of rs3834087 was markedly correlated with a decreased risk of CHB (OR = 0.60, 95% CI: 0.39-0.92). Compared to GAG/GAG, the GAG/- and -/- genotypes of rs3834087 were greatly associated with a decreased risk of CHB (OR = 0.54, 95% CI: 0.35-0.85; and OR = 0.55, 95% CI: 0.35-0.86, respectively). Additionally, with adjustment for age, sex, smoking status, and drinking habits, the GT and GT plus TT genotypes of rs3754217, when compared to the wild GG genotype, correlated with a reduced risk of CHB (OR = 0.68, 95% CI: 0.49-0.94; OR = 0.71, 95% CI: 0.52-0.98, respectively).

3.4. Distribution Difference between HBV Infection Subgroups. The allele and genotype frequencies of the ECM1 SNPs in non-HCC (including CHB and LC) and HCC patients are summarized in Table 5. There were no differences in the frequencies of all alleles and genotypes (rs3834087, rs3754217, rs3737240, and rs13294) between the LC and CHB, LC and HCC, and non-HCC and HCC patients.

3.5. Haplotype Analysis. The haplotype distributions of healthy individuals against those of chronic HBV infection, non-HCC, and HCC patients were analyzed, and positive results are shown in Table 6. Between chronic HBV infection patients and controls, the following haplotypes greatly correlated with a decreased risk of chronic HBV infection when compared to their wild type alleles: G-T and T-C alleles of rs3754217-rs3737240 (OR = 0.54, 95% CI: 0.31-0.96; OR = 0.64, 95% CI: 0.44-0.93, respectively) and G-G-T and Del-T-C alleles of rs3834087-rs3754217-rs3737240 (OR = 0.54, 95% CI: 0.31-0.96; OR = 0.65, 95% CI: 0.45-0.96, respectively).

Between non-HCC chronic HBV infection patients and healthy controls, the following haplotypes were related to a decreased risk of non-HCC chronic HBV infection: the T-C alleles of rs3754217-rs3737240 (OR = 0.51; 95% CI: 0.33-0.81) and Del-T alleles of rs3834087-rs3754217 (OR = 0.61; 95% CI: 0.40-0.95) (Table 7).

Liver stiffness measurement (LSM, kPa) scores collected using FibroScan on CHB patients with the different genotypes of rs3834087 and rs3754217 are shown in Supplemental Table 1. Patients with the GT and the GT plus TT genotypes demonstrated lower LSM scores as than that of wild GG genotype patients (Supplemental Table 1). No significant differences were found in the Child-Pugh scores of LC between the different genotypes (Supplemental Table 2).

4. Discussion

Mutation of ECM1 may lead to various genetic diseases such as lipid proteinosis and autosomal recessive genodermatosis [23, 24]. ECM1 has also been identified to be involved in the differentiation and function of immune cells. Its important role in fibrosis was only recently discovered, but published results have been controversial. In a research by Fan et al. on liver fibrosis in mouse models, liver damage was shown to reduce the levels of ECM1 production during fibrogenesis, and re-expression of ECM1 prevented liver fibrosis progression [17]. In contrast, another study on heart fibrosis showed that ECM1 led to cardiac fibrosis in myocardial infarction by acting as an intermediary between inflammation and fibrosis [25]. We therefore conducted this large case-control study to evaluate the effects of ECM1 on the progression of liver fibrosis in vivo. To our knowledge, our study is the first to have investigated the relationships between ECM1 SNPs and the development of chronic liver fibrosis; other articles on ECM1 SNPs have only involved extrahepatic diseases [18, 20, 26, 27].

We found that the GAG/- and GAG/- plus -/- genotypes of rs3834087 were correlated with a reduced risk of chronic HBV infection both in non-HCC (OR = 0.54, 95% CI: 0.35-0.85; OR = 0.55, 95% CI: 0.35-0.86, respectively) and total chronic HBV infection patients (OR = 0.65, 95% CI: 0.45-0.95; OR = 0.66, 95% CI: 0.45-0.96, respectively). The Del allele of rs3834087 was also related to a lower risk of non-HCC chronic HBV infection (OR = 0.60, 95% CI: 0.39-0.92). After adjustment for age, sex, smoking status, and drinking habits, the GT genotype of rs3754217 was significantly related to a declined risk of chronic HBV infection both in non-HCC (OR = 0.68, 95% CI: 0.49-0.94) and in total chronic HBV infection patients (OR = 0.75, 95% CI: 0.56-1.00). The GT plus TT genotype of rs3754217 was also related to a decreased risk of non-HCC chronic HBV infection (OR = 0.71, 95% CI: 0.52-0.98). Haplotype analyses showed significant association of several haplotypes with a reduced risk of chronic HBV infection, including the T-C allele of rs3754217-rs3737240, DE-T-G allele of rs3834087-rs3754217-rs13294, and the T-C-G allele of rs3754217-rs3737240-rs13294. The Del allele of rs3834087 and the T allele of rs3754217 may be the influencing factors of chronic HBV infection. The mechanisms of these genotype differences on HBV susceptibility remain unclear and warrant further investigations. The rs3834087 and rs3754217 loci in the promoter region of ECM1 and the rs3754217 locus initially predicted as the TFBS were speculated to affect the functions of other associated genes. Previous studies have
| SNP                     | Healthy control | Non-HCC (N = 370) | Chronic HBV infection patient | HBV-related HCC (N = 286) | Chronic HBV infection patient (N = 656) vs. healthy control (N = 298) |
|-------------------------|-----------------|-------------------|-------------------------------|--------------------------|---------------------------------------------------------------|
|                         | N (%)           | N (%)             | OR (95% CI)                    | P-value$^{a}$             | OR (95% CI)                              | P-value$^{b}$             | OR (95% CI)                              | P-value$^{c}$ |
| Rs3834087 genotype and allele | Detected number | N = 298           | N = 370                       | N = 286                   | N = 656 vs. N = 298                                   |
| GAG/GAG                 | 245 (82.2)      | 330 (89.2)        | 1                             | 243 (85.0)                | 1                                                      | 0.65 (0.45-0.95)          | 0.0248                                    |
| GAG/-                   | 53 (17.8)       | 39 (10.5)         | 0.54 (0.35-0.85)               | 0.0068                    | 0.79 (0.50-1.23)                                       | 0.2921                    | 0.0428                                    |
| GAG/+ -                 | 0 (0.0)         | 1 (0.3)           | 1                             | 1.0000                    | —                                                      | —                         | 1.0000                                    |
| GAG allele              | 543 (91.1)      | 699 (94.5)        | 1                             | 529 (92.5)                | 1                                                      | 0.66 (0.45-0.96)          | 0.0287                                    |
| DEL allele              | 53 (8.9)        | 41 (5.5)          | 0.60 (0.39-0.92)               | 0.0172                    | 0.83 (0.55-1.27)                                       | 0.3922                    | 0.70 (0.49-1.00)                          | 0.0514                                    |
| Rs3754217 genotype and allele | Detected number | N = 294           | N = 369                       | N = 284                   | N = 653 vs. N = 294                                   |
| GG                      | 144 (49.0)      | 210 (56.9)        | 1                             | 146 (51.4)                | 1                                                      | 0.75 (0.56-1.00)          | 0.0481                                    |
| GT                      | 132 (44.9)      | 134 (36.3)        | 0.68 (0.49-0.94)               | 0.0209                    | 0.83 (0.59-1.17)                                       | 0.2782                    | 1.11 (0.63-1.98)                          | 0.7152                                    |
| TT                      | 18 (6.1)        | 25 (6.8)          | 0.95 (0.50-1.82)               | 0.8850                    | 1.31 (0.68-2.55)                                       | 0.4180                    | 0.79 (0.60-1.04)                          | 0.0981                                    |
| CT                      | 150             | 554 (75.1)        | 0.71 (0.52-0.98)               | 0.0351                    | 0.88 (0.63-1.23)                                       | 0.4642                    | 0.66 (0.45-0.96)                          | 0.2877                                    |
| G allele                | 420 (71.4)      | 554 (75.1)        | 0.60 (0.39-0.92)               | 0.0172                    | 0.83 (0.55-1.27)                                       | 0.3922                    | 0.70 (0.49-1.00)                          | 0.0514                                    |
| T allele                | 168 (28.6)      | 184 (24.9)        | 0.65 (0.45-1.06)               | 0.1358                    | 1.00 (0.77-1.29)                                       | 0.9847                    | 0.90 (0.73-1.12)                          | 0.3469                                    |
| Rs3737240 genotype and allele | Detected number | N = 296           | N = 369                       | N = 285                   | N = 653 vs. N = 296                                   |
| CC                      | 176 (59.5)      | 224 (60.7)        | 1                             | 169 (59.3)                | 1                                                      | 0.99 (0.74-1.33)          | 0.9411                                    |
| CT                      | 102 (34.5)      | 127 (34.4)        | 0.96 (0.69-1.34)               | 0.8270                    | 1.02 (0.71-1.44)                                       | 0.9335                    | 0.86 (0.47-1.56)                          | 0.6069                                    |
| TT                      | 18 (6.1)        | 18 (4.9)          | 0.79 (0.40-1.57)               | 0.4982                    | 1.6 (5.6)                                              | 0.91 (0.47-1.86)          | 0.7979                                    | 0.8282                                    |
| CT + TT                 | 120             | 145              | 0.94 (0.68-1.29)               | 0.6870                    | 1.16 (7.1-1.40)                                        | 0.8231                    | 0.97 (0.73-1.28)                          | 0.8272                                    |
| C allele                | 454 (76.7)      | 575 (77.9)        | —                             | 438 (76.8)                | 1                                                      | 0.98 (0.76-1.26)          | 0.8509                                    |
| T allele                | 138 (23.3)      | 163 (22.1)        | 0.94 (0.64-1.37)               | 0.7415                    | 1.00 (0.76-1.31)                                       | 0.9783                    | 0.98 (0.76-1.26)                          | 0.8509                                    |

$P$-value$^{a,b,c}$ represents the non-HCC, HCC, and chronic HBV infection patients compared to the healthy control groups adjusted for age, gender, smoking, and drinking by logistic regression analysis. The two-sided $\chi^2$ test or Fisher's exact test was used in allele distribution comparison. CI, confidence interval; ECM, extracellular matrix; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; SNP, single nucleotide polymorphism.
Table 5: Genotype and allele frequencies of SNPs in the ECM1 gene in chronic HBV infection subgroups.

|                      | CHB N (%) | LC N (%) | CHB vs. LC OR (95% CI) a | P-value a | HCC N (%) | LC vs. HCC OR (95% CI) b | P-value b | Non-HCC N (%) | Non-HCC vs. HCC OR (95% CI) c | P-value c |
|----------------------|-----------|----------|--------------------------|-----------|-----------|--------------------------|-----------|----------------|-------------------------------|-----------|
| Rs3834087 genotype and allele |           |          |                          |           |           |                          |           |                |                               |           |
| Detected number      | 96 (92.3) | 234 (88.0) | 1                       |           | 243 (85.0) | 1                       |           | 330 (89.2) | 1                               |           |
| GAG/GAG              | 8 (7.7)   | 31 (11.7)  | 1.63 (0.72-3.68)         | 0.2442    | 43 (15.0)  | 1.24 (0.75-2.05)         | 0.4121    | 39 (10.5)   | 1.40 (0.87-2.25)                | 0.1622    |
| GAG/-                | 0 (0.0)   | 1 (0.4)    | 1.00 (0.99-1.01)         | 1.0000    | 0 (0.0)    | 1.00 (0.99-1.00)         | 0.4922    | 1 (0.3)      | 1.00 (0.99-1.00)                | 1.0000    |
| GAG/-/GAG            | 8         | 32        | 1.67 (0.74-3.78)         | 0.2162    | 43         | 1.19 (0.72-1.97)         | 0.4884    | 40           | 1.36 (0.85-2.18)                | 0.1963    |
| GAG allele           | 200 (96.2) | 499 (93.8)  | 1                       |           | 529 (92.5) | 1.23 (0.77-1.97)         | 0.3984    | 699 (94.5) | 1.39 (0.89-2.16)                | 0.1472    |
| Del allele           | 8 (3.8)   | 33 (6.2)   | 1.65 (0.75-3.64)         | 0.2082    | 43 (7.5)   | 1.23 (0.77-1.97)         | 0.3992    | 41 (5.5)     | 1.39 (0.89-2.16)                | 0.1472    |
| Rs3754217 genotype and allele |           |          |                          |           |           |                          |           |                |                               |           |
| Detected number      | 61 (58.7) | 149 (56.2)  | 1                       |           | 146 (54.1) | 1                       |           | 210 (56.9) | 1                               |           |
| GG                   | 37 (35.6) | 97 (36.6)   | 1.15 (0.70-1.88)         | 0.5791    | 114 (40.1) | 1.18 (0.82-1.69)         | 0.3693    | 134 (36.3) | 1.20 (0.86-1.68)                | 0.2749    |
| GT                   | 6 (5.8)   | 19 (7.2)    | 1.35 (0.51-3.56)         | 0.5512    | 24 (8.5)   | 1.13 (0.59-2.19)         | 0.7068    | 25 (6.8)     | 1.24 (0.67-2.29)                | 0.4901    |
| GT + TT              | 43        | 116        | 1.18 (0.78-1.88)         | 0.4592    | 138        | 1.17 (0.83-1.65)         | 0.3642    | 159          | 1.21 (0.88-1.66)                | 0.2381    |
| G allele             | 159 (76.4) | 395 (74.5)  | 1                       |           | 406 (71.5) | 1                       |           | 554 (75.1) | 1                               |           |
| T allele             | 49 (23.6) | 135 (25.5)  | 1.11 (0.76-1.61)         | 0.5891    | 162 (28.5) | 1.17 (0.89-1.53)         | 0.2559    | 184 (24.9) | 1.20 (0.94-1.54)                | 0.1449    |
| Rs373240 genotype and allele |           |          |                          |           |           |                          |           |                |                               |           |
| Detected number      | 62 (59.6) | 162 (61.1)  | 1                       |           | 169 (59.3) | 1                       |           | 224 (60.7) | 1                               |           |
| CC                   | 38 (36.5) | 89 (33.6)   | 0.95 (0.58-1.55)         | 0.8401    | 100 (35.1) | 0.961 (0.74-1.53)        | 0.7572    | 127 (34.4) | 1.03 (0.73-1.44)                | 0.8821    |
| TT                   | 4 (3.8)   | 14 (5.3)    | 1.35 (0.42-4.27)         | 0.6151    | 16 (5.6)   | 1.04 (0.49-2.24)         | 0.9132    | 18 (4.9)    | 1.12 (0.55-2.29)                | 0.7623    |
| CT + TT              | 42        | 103        | 0.90 (0.62-1.59)         | 0.9697    | 116        | 1.06 (0.75-1.50)         | 0.7546    | 145          | 1.04 (0.75-1.43)                | 0.8229    |
| C allele             | 162 (77.9) | 413 (77.9)  | 1                       |           | 438 (76.8) | 1                       |           | 575 (77.9) | 1                               |           |
| T allele             | 46 (22.1) | 117 (22.1)  | 0.99 (0.68-1.47)         | 0.9914    | 132 (23.2) | 0.93 (0.70-1.23)         | 0.6223    | 163 (22.1) | 1.06 (0.73-1.55)                | 0.7585    |
| Rs13294 genotype and allele |           |          |                          |           |           |                          |           |                |                               |           |
| Detected number      | 62 (59.6) | 162 (61.1)  | 1                       |           | 169 (59.3) | 1                       |           | 224 (60.7) | 1                               |           |
| GA                   | 38 (36.5) | 89 (33.6)   | 0.95 (0.58-1.55)         | 0.8401    | 100 (35.1) | 1.06 (0.74-1.53)         | 0.7572    | 127 (34.4) | 1.03 (0.73-1.44)                | 0.8822    |
| AA                   | 4 (3.8)   | 14 (5.3)    | 1.35 (0.42-4.27)         | 0.6145    | 16 (5.6)   | 1.04 (0.49-2.24)         | 0.9127    | 18 (4.9)    | 1.12 (0.55-2.29)                | 0.7615    |
| GA + AA              | 42        | 103        | 0.99 (0.62-1.59)         | 0.9682    | 116        | 1.06 (0.75-1.50)         | 0.7550    | 145          | 1.04 (0.75-1.43)                | 0.8230    |
| G allele             | 162 (77.9) | 413 (77.9)  | 1                       |           | 438 (76.8) | 1                       |           | 575 (77.9) | 1                               |           |
| A allele             | 46 (22.1) | 117 (22.1)  | 1.00 (0.68-1.47)         | 0.9911    | 132 (23.2) | 1.06 (0.80-1.41)         | 0.6682    | 163 (22.1) | 1.06 (0.82-1.38)                | 0.6462    |

P-valuea,b,c represents the comparison adjusted for age, gender, smoking, and drinking by logistic regression analysis. The two-sided χ² test or Fisher’s exact test was used in allele distribution comparison. CHB, chronic hepatitis B; CI, confidence interval; ECM, extracellular matrix; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; OR, odds ratio; SNP, single nucleotide polymorphism.
found that the rs3737240 and rs13294 loci exhibited strong associations with the occurrence of ulcerative colitis [20]. However, we did not find the role of these two SNPs in chronic hepatitis B occurrence, progression of liver fibrosis, and HCC.

In the analysis of the subgroups, no effects on the progression from chronic hepatitis to cirrhosis and HCC were found in the four SNP variants (rs3834087, rs3754217, rs3737240, and rs13294). However, LSM, which has displayed excellent diagnostic accuracy in the identification of HBV-associated fibrosis and cirrhosis [6, 28], revealed significant differences between the GT, GT plus TT, and wild TT genotypes of rs3754217. Mutant genotypes showed low LSM scores, which reflected the lower degree of fibrosis. Due to the limited samples, further studies with expanded sample number are required for further confirmation.

5. Limitations

The primary limitation of this study is that this case-control study was a hospital-based study. Thus, selection bias may have occurred. In addition, due to technical problems in functional studies, we did not find direct evidence on whether these two polymorphisms (rs3834087 and rs3754217) located in promoter regulate or influence ECM1 expression. Therefore, further functional studies are warranted.

6. Conclusion

In conclusion, our study preliminarily demonstrated that the ECM1 locus may mediate the chronicity of HBV infections in vivo. The mechanisms involved in inflammation and fibrosis should be further explored. It should be noted that identification of new therapeutic targets is still required to promote the development of new antifibrotic drugs and fibrotic biomarkers to improve the management of fibrosis.

**Abbreviations**

- **SNPs**: Single nucleotide polymorphisms
- **ECM1**: Extracellular matrix protein 1
- **CHB**: Chronic hepatitis B
- **LC**: Liver cirrhosis
- **HCC**: Hepatocellular carcinoma
- **HBV**: Hepatitis B virus
- **ECM**: Extracellular matrix
- **HSCs**: Hepatic stellate cells
- **HCV**: Hepatitis C virus

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**Table 6: Haplotype distributions between healthy controls and chronic HBV infection patients.**

| Haplotype                  | Healthy controls (%) | Chronic HBV infection (%) | χ²  | P       | OR (95% CI) |
|----------------------------|----------------------|---------------------------|-----|---------|-------------|
| rs3754217-rs3737240        |                      |                           |     |         |             |
| G-C                       | 397 (67.7)           | 931 (71.4)                | 8.47| 0.0373  | 1           |
| G-T                       | 22 (3.8)             | 28 (2.1)                  | 0.54| 0.31-0.96|             |
| T-C                       | 52 (8.9)             | 80 (6.1)                  | 0.64| 0.44-0.93|             |
| T-T                       | 115 (19.6)           | 265 (20.3)                | 0.99| 0.77-1.27|             |
| *rs3834087-rs3754217-rs3737240* |                   |                           |     |         |             |
| G-G-C                     | 395 (67.2)           | 931 (71.4)                | 17.2| 0.0086  | 1           |
| G-G-T                     | 22 (3.7)             | 28 (2.6)                  | 0.54| 0.31-0.96|             |
| G-T-C                     | 3 (0.5)              | 4 (0.4)                   | 0.55| 0.12-2.49|             |
| G-T-T                     | 115 (19.6)           | 259 (19.8)                | 0.95| 0.74-1.22|             |
| D-G-C                     | 4 (0.7)              | 0 (0.0)                   |     |         |             |
| D-T-C                     | 49 (8.3)             | 76 (5.8)                  | 0.65| 0.45-0.96|             |
| D-T-T                     | 0 (0.0)              | 6 (0.3)                   |     |         |             |

*rs3834087’s GAG allele was simply marked with G; DEL allele was simply marked with D. CI, confidence interval; HBV, hepatitis B virus; OR, odd ratio.

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**Table 7: Haplotype distributions between healthy controls and non-HCC patients.**

| Haplotype                  | Healthy controls (%) | Non-HCC (%) | χ²  | P       | OR (95% CI) |
|----------------------------|----------------------|-------------|-----|---------|-------------|
| *rs3834087-rs3754217       |                      |             |     |         |             |
| G-G                       | 416 (70.7)           | 554 (75.1)  | 10.42| 0.0153  | 1           |
| G-T                       | 119 (20.2)           | 144 (19.5)  | 0.91| 0.69-1.20|             |
| D-G                       | 4 (0.7)              | 0 (0.0)     | 1.00| 0.99-1.02|             |
| D-T                       | 49 (8.3)             | 40 (5.4)    | 0.61| 0.40-0.95|             |
| *rs3834087’s GAG allele was simply marked with G; DEL allele was simply marked with D. CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio. |
HIV: Human immunodeficiency virus
UTR: Untranslated regions
LD: Linkage disequilibrium
TFBS: Transcription factor binding site
PCR: Polymerase chain reaction
H-WE: Hardy–Weinberg equilibrium
ORs: Odds ratios
CIs: Confidence intervals
LSM, kPa: Liver stiffness measurement.

Data Availability
The data are available from the corresponding author on reasonable request.

Ethical Approval
This study was approved by the Ethics Committee of The First Hospital of Jilin University (Approval number: 2020-Linshen No. 2020-355). Written informed consent for participation in the study was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent
All patients provided written informed consent for the publication of any associated data and accompanying images.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
XL conceived and designed the study; XTH acquired the SNP data and was a major contributor of manuscript writing; TL analyzed and interpreted the patient data; RZ interpreted the SNP data and performed the statistical analysis; and XL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Supplementary Materials
Supplemental Table 1. The liver stiffness measurement (LSM, kPa) score’s distribution in FibroScan of rs3834087 and rs3754217 polymorphisms in chronic hepatitis B (CHB) group patients. Supplemental Table 2. The Child–Pugh scores and level’s distribution of rs3834087 and rs3754217 polymorphisms in liver cirrhosis. (Supplementary Materials)

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