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Original Article

Association between extracellular matrix protein 1 (*ECM1*) gene polymorphisms (rs3834087 and rs3754217) and Hepatitis B Virus evolution in an African cohort

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Abstract

Hepatitis B virus (HBV) is a significant cause of liver disease and cancer worldwide. Understanding the genetic factors influencing HBV evolution is crucial for developing effective prevention and treatment strategies. Host genetic and environmental factors particularly influence the evolution of this infection. Recent studies have implicated the *ECM1 gene* in HBV pathogenesis, mainly two specific polymorphisms (rs3834087 and rs3754217). In an African cohort, we comprehensively analyzed these *ECM1 gene* polymorphisms and their association with HBV evolution.In this case-control analysis, 167 samples, consisting of 59 controls and 108 cases, were examined. The cases included 50 patients with Chronic Hepatitis B(CHB), 16 with cirrhosis, and 42 with hepatocellular carcinoma (HCC). Genomic DNA extraction was executed using INVITROGEN and FAVORGEN kits. Genotyping of rs3834087 and rs3754217 polymorphisms in the *ECM1 gene* was accomplished via real-time PCR on the QuantStudioTM 5 Real-Time instrument, followed by allelic discrimination using TaqMan Genotyper Software. Data was interpreted using SPSS version 20 and Epi info version 7.5.2.0. Odds ratios (OR), confidence intervals (CI), and p-values were derived for risk and significance evaluation.In our study, the heterozygous genotype (GT) of rs3754217 could confer protection to controls against the onset of chronic hepatitis in the event of infection (OR=0.05; CI=0.006-0.46; p=0.002). In addition, carriage of mutated *alleles* of the two (2) polymorphisms was associated with the course of infection and may influence the appearance of severe forms at certain stages of the disease.Our study is the first to assess the association between polymorphisms (rs3834087 and rs3754217) in the *ECM1 gene* and the course of HBV infection in Burkina Faso. It showed that combining specific genotypes of the two (2) polymorphisms would be associated with protection against chronic hepatitis.

Keywords: Polymorphisms, *gene*, *ECM1*, HBV, Burkina Faso

1. Introduction

Hepatitis B, induced by the hepatitis B virus (HBV), stands as a global public health concern. It primarily progresses through an acute phase, leading to recovery in 90% of cases, and a chronic phase characterized by a persistent infection beyond six months [1]. Chronic infection substantially raises the risk of fatality from complications like cirrhosis and hepatocellular carcinoma (HCC). With 81 million individuals chronically infected, Africa remains particularly affected [2].

In fact, in 2020, the African region bore 26% of the global disease burden from hepatitis B and C, resulting in 125,000 deaths. Strikingly, nearly 70% of global hepatitis B cases are clustered in Africa [3].

Burkina Faso is a country with high HBV endemicity. HBV prevalence in the general population was 14.5%

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in 2014 [4], and 9.1% in 2018 [5]. Since 2006, children in Burkina Faso have received HBV vaccinations within eight weeks post-birth as a segment of the Expanded Program on Immunization [6].

The shift of the HBV infection to chronicity and severe stages like cirrhosis and HCC is driven by the interplay between the host's immune response and the different viral genotypes involved [7]. To date, there are 10 HBV genotypes, graded from A to J [8]. HBV genotype E (HBV-E) is the most common strain in West Africa [9] and Burkina Faso [10,11].

Numerous human *genes*, including *p53* and *RB1* (Retinoblastoma 1) [12,13], *KIR* [14], and notably, ECM1 (extracellular matrix protein 1) [15], have been documented to influence disease progression. The extracellular matrix (ECM) facilitates cellular cohesion, proliferation, and intercellular signaling [16]. ECM deposition and stiffness have been found to be correlated with desmoplasia, which limits drug delivery and immune cell infiltration in the tumor microenvironment [17]. ECM1, a glycoprotein that was first documented in 1994, is essential in cell-ECM binding, and studies have shown that elevated ECM1 levels are associated with various cancers, including HCC [18–20]. HBV infection triggers immune responses that lead to hepatocyte damage, which in turn activates hepatic stellate cells (HSCs) to produce collagen via fibrogenic cytokines such as TGF-β1 [21]. This results in increased extracellular matrix protein synthesis [22]. The primary role of ECM1 is to facilitate binding with ECM proteins, and when its production diminishes, liver fibrosis severity increases [15]. This research aims to investigate the correlation between the rs3834087 and rs3754217 *ECM1 gene* polymorphisms and HBV progression. The rs3834087 involves a three-base insertion/deletion (GAG) in the *ECM1 gene*, while the rs3754217 results from guanine (G) to thymine (T) substitution on chromosome 1. The impact of *ECM1 gene* polymorphisms on the progression of HBV in Burkina Faso remains unknown. This study aims to investigate the frequency of these polymorphisms in a cohort of HBV-infected individuals in Burkina Faso, in order to evaluate their effect on HBV infection and enable timely intervention for those at risk of severe disease manifestations.

2. Materials and method

2.1. Study Design, Setting, and Population

An analytical case-control study was undertaken between August and December 2022 in Ouagadougou. The study enrolled two distinct cohorts: cases and controls. The case-cohort consisted of individuals diagnosed with chronic hepatitis B (CHB), viral cirrhosis B, and hepatocellular carcinoma attributed to HBV infection. Conversely, the control cohort included individuals who tested negative for HBsAg, anti-HCV, and HIV. Of the 167 participants in the study, there were 108 cases and 59 healthy controls. Participants with hepatocellular carcinoma and cirrhosis were sourced from the hepato-gastroenterology departments of Yalgado OUEDRAOGO (CHU-YO) Teaching Hospital and Paul VI Hospital. Meanwhile, CHB participants were recruited from the Biomolecular Research Center Pietro Annigoni (CERBA) and the control groups from the National Blood Transfusion Center (CNTS).

2.2. Inclusion Criteria

- Chronic hepatitis B: Participants in this group had a confirmed HBV infection for over six months, evidenced by HBsAg positivity, and ultrasound results showing no significant liver abnormalities.
- Cirrhosis: Participants in this category had a clinically confirmed cirrhotic liver condition, with HBV being the sole etiological agent.
- Hepatocellular carcinoma: Enrollment was based on alpha-fetoprotein (AFP) assay results, CT scan findings, and/or histological liver examination. Only individuals with HBV as the sole exposure factor were considered.
- Control group: Participants tested for HBsAg, anti-HCV, and HIV were categorized as controls.
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2.3. Non-Inclusion Criteria

Exclusions encompassed HBV-negative cases, HIV-positive cases, HBV-positive and/or HIV-positive controls, and individuals unwilling to partake in the study. Also excluded were individuals who did not provide explicit informed written consent.

2.4. Sample Collection

Sampling began with patient interviews employing a structured questionnaire, capturing socio-demographic data, dietary inclinations, and liver disease history. Subsequent to the interview, whole blood was procured and apportioned into two labeled tubes (EDTA and dry tube) for subsequent serological and molecular analyses. Following centrifugation, samples were segmented and stored at -20°C pending analysis.

2.5. DNA Extraction and Quantification

Genomic DNA extraction was conducted employing INVITROGEN and FAVORGEN kits, strictly adhering to the manufacturers' guidelines. DNA concentration and purity were verified using the "Biodrop" spectrophotometer.

2.6. Genotyping of *ECM1 gene* Polymorphisms (rs3834087 and rs3754217)

Real-time PCR, utilizing the QuantStudioTM 5 Real-Time PCR System, facilitated the genotyping of the *ECM1 gene* polymorphisms rs3834087 and rs3754217. Each genotyping reaction (25 µL total volume) consisted of 17.5 µL of distilled water, 3 µL of HOT FIREPol® Probe Universal qPCR Mix (5X concentration), 1.5 µL of TaqMan® SNP Genotyping Assays (diluted 1:5), and 3 µL of genomic DNA.

The PCR conditions entailed an initial 10-minute denaturation step at 95°C, followed by 40 cycles: 15 seconds of denaturation at 95°C, 1-minute hybridization/extension at 60°C, and a concluding 30-second extension at 60°C. The specific primers employed for amplification are detailed in Table 1.

2. 7. Statistical Analysis

Data were inputted into Excel 2019 and subsequently analyzed employing the Statistical Package for the Social Sciences (SPSS) version 20, in conjunction with EPI info 7.2.5.0. To ascertain risk levels, odds ratios (OR) along with their corresponding 95% confidence intervals (95% CI) were computed. A statistical difference was deemed significant when $P < 0.05$.

Table 1. Primer and probe sequences.

Polymorphism	Primers and probes
rs 3834087	**Primers: F: 5′**-ACGTTGGATGAGACCTAGATGGAATCAGCC-**3′** **R: 5′**-ACGTTGGATGTGAAAAAGGGAGCATGGCAG-**3′** **Probes: 5'-VIC-** ATGGAATCAGCCCTAAGGGATGAG-**MGB-NFQ-3'** **5'-FAM-** AAAGGCCTTAGGGAGAAATTCTG-**MGB-NFQ-3'**
rs 3754217	**Primers: F**: 5′ACGTTGGATGGGGACTGATTAGAGGAGAAC-**3′** **R**: 5′-ACGTTTGGATGAACTGAGGCACAAACTAGGG-**3′** **Probes: 5'-VIC**- AGGGGCTCAAACACCTCTTGCTCCT-**MGB-NFQ-3'** **5'-FAM**- GATTCTCTGAATCAGTTTCTCTTGA -**MGB-NFQ-3'**

2. 8. Ethical Considerations

The Ethics Committee for Health Research of Burkina Faso granted approval for this study (reference: deliberation N° 2022-02-027). All participants, including patients and donors, provided written informed consent prior to their inclusion in the study. Rigorous measures were adopted to maintain data confidentiality, with the database securely stored on a password-protected computer.

3. Results

3. 1. Socio-demographic Characteristics of the Study Population

Of the total 167 participants, 97 (58.1%) were male and 70 (41.9%) were female, leading to a sex ratio of 1.38. Age distribution ranged from 12 to 75 years, averaging 35.31 ± 11.96 years. Females reported an average age of 33.80 ± 10.67 years, whereas the males averaged 36.40 ± 12.75 years.

Considering the clinical subgroups, the mean ages were as follows:

- Chronic Hepatitis B (CHB): 35.82 ± 11.70 years
- Cirrhosis: 41.75 ± 11.77 years
- Hepatocellular Carcinoma (HCC): 41.05 ± 13.67 years
- Controls: 29.05 ± 7.09 years

3.2. Distribution by clinical status

Our study population comprised 59 controls and 108 cases, including 50 patients with CHB, 16 with cirrhosis and 42 with HCC. Figure 1 shows the distribution of the study population by clinical status.

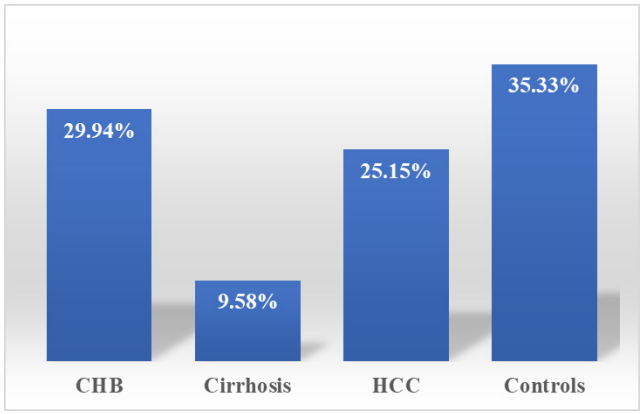

Fig. 1. Distribution of study population by clinical status. *CHB: Chronic Hepatitis B; HCC: Hepato-Cellular Carcinoma.*

3.3. Genotypic and Allelic Frequencies of *ECM1 gene* Polymorphisms (rs3834087 and rs3754217) Stratified by Gender

- For rs3834087:

Females: In cases, the genotypic frequencies were 1.75% for GAG/GAG homozygotes, 77.2% for GAG/- heterozygotes, and 21.05% for -/- *mutated homozygotes*. In controls, these were 0% for both GAG/GAG homozygotes and -/- *mutated homozygotes*, and 13% for GAG/- heterozygotes. The mutated *allele* was represented in the case group with a frequency of 59.65% versus 50% in the control group. This difference was not statistically significant (p-value>0.05).

Males: The genotypic frequencies were 1.96% for GAG/GAG homozygotes, 80.4% for GAG/- heterozygotes, and 17.64% for -/- *mutated homozygotes* in cases. In controls, these values were 0% for both GAG/GAG homozygotes and -/- *mutated homozygotes*, and 46% for GAG/- heterozygotes. The mutated *allele* frequency was 57.84% in cases versus 50% in controls, which was not a significant difference (p-value>0.05). (Table 2).

- For rs3754217:

Females: Genotypic frequencies in cases were 10.53% for GG homozygotes, 56.14% for GT heterozygotes, and 33.33% for TT *mutated homozygotes*. In controls, these were 0% for both GG homozygotes and TT *mutated homozygotes*, and 13% for GT heterozygotes. The mutated *allele* frequency was 61.4% in cases versus 50% in controls, showing no significant difference (p-value>0.05).

Males: In cases, the frequencies were 1.96% for GG homozygotes, 52.94% for GT heterozygotes, and 45.1% for TT *mutated homozygotes*. For controls, 2.17% were GG homozygotes, 97.83% were GT heterozygotes, and 0% were TT *mutated homozygotes*. The mutated allele had a frequency of 71.57% in cases compared to 48.9% in controls, a statistically significant difference (p-value = 0.002). The data insinuates that in males, the mutated *allele* might increase the risk of the infection advancing to severe stages (OR=2.62; CI=1.45-4.75) (Table 2).

3. 4. Distribution of Genotypic and Allelic Frequencies of *ECM1 gene* Polymorphisms (rs3834087 and rs3754217) Based on Clinical Status

To understand the potential relationship between the genotypic and allelic frequencies of the *ECM1 gene* polymorphisms (rs3834087 and rs3754217) and the progression of hepatitis B virus (HBV) infection, we examined their distribution in the four clinical statuses: chronic hepatitis B (CHB), cirrhosis, HCC and controls. Upon analysis, the distribution of genotypes for the two polymorphisms showed no specific pattern or correlation related to any of the clinical groups. However, only the mutated *allele*

of the rs3754217 polymorphism was associated with the progression or manifestation of HBV infection in the participants (p=0.003). In fact, patients carrying the mutated rs3754217 *allele* were 2 times more likely to progress to severe forms of the disease (OR= OR=2.02; CI=1.28-3.2) (Table 3).

3. 5. **Comparison of Genotypic and Allelic Frequencies of *ECM1 gene* Polymorphisms (rs3834087 and rs3754217) Between Cases and Controls**

The rs3834087 and rs3754217 polymorphisms of the *ECM1 gene* appear to be closely linked to the progression and manifestation of hepatitis B virus (HBV) infections. Carriage of the *homozygous mutated* rs3834087 (p=0.03) and *heterozygous* rs3754217 (p=0.02) genotypes was associated with the progression of chronic infection to cirrhosis and hepatocellular carcinoma respectively. More specifically, individuals with the rs3834087 *mutated allele* showed protection against HCC at the chronic infection stage, with an associated odds ratio (OR) of 0.5, a confidence interval (CI) of 0.28-0.94 and a p-value of 0.044 (Table 4). Simultaneously, the same allele showed

Table 2. Distribution of genotype and allele frequencies by sex.

		Women				Men			
		Cases N=57(%)	**Controls N=13(%)**	**OR (95% CI)**	**p-value**	**Cases N=51(%)**	**Controls N=46(%)**	**OR (95% CI)**	**p-value**
				rs 38 34 087					
Genotype	**GAG/GAG**	1 (1.75)	0	**Reference**		1 (1.96)	0	**Reference**	
	GAG/-	44 (77.2)	13 (100)	0	1	41 (80.4)	46 (100)	0	0.96
	-/ -	12 (21.05)	0	0	1	9 (17.64)	0	0	1
Alleles	**GAG**	46 (40.35)	13 (50)	**Reference**		43 (42.16)	46 (50)	**Reference**	
	Mutated *allele*	68 (59.65)	13 (50)	0.47 (0.62-3.4)	0.49	59 (57.84)	46 (50)	1.37 (0.77-2.41)	0.34
				rs 37 54 217					
Genotypes	**GG**	6 (10.53)	0	**Reference**		1 (1.96)	1 (2.17)	**Reference**	
	GT	32 (56.14)	13 (100)	0	0.3	27 (52.94)	45 (97.83)	0.6 (0.03-9.99)	1
	TT	19 (33.33)	0	0	1	23 (45.1)	0	NA	0.11
Alleles	**G**	44 (38.6)	13 (50)	**Reference**		29 (28.43)	47 (51.1)	**Reference**	
	T	70 (61.4)	13 (50)	1.59 (0.67-3.74)	0.39	73 (71.57)	45 (48.9)	2.62 (1.45-4.75)	**0.002**

Table 3. Genotype and allele frequencies by clinical status.

			General population N= 167(%)	**CHB N=50 (%)**	**Cirrhosis N=16 (%)**	**HCC N=42 (%)**	**Controls N=59 (%)**	**OR (95% CI)**	***p-value***
rs3834087	**Genotypes**	**GAG/GAG**	2 (1.2)	0	1 (6.2)	1 (2.4)	0	**Reference**	
		GAG/-	144 (86.2)	32 (64.0)	15 (93.8)	38 (90.5)	59 (100.0)	0	0.65
		-/-	21 (12.6)	18 (36.0)	0	3 (7.1)	0	NA	NA
	Alleles	**GAG**	148 (44.3)	32 (32)	17 (53.13)	40 (47.62)	59 (50)	**Reference**	
		Mutated *allele*	186 (55.7)	68 (68)	15 (46.87)	44 (52.38)	59 (50)	1.42 (0.9-2.24)	0.15
rs 3754217	**Genotypes**	**GG**	8 (4.8)	7 (14,0)	0	0	1 (1.7)	**Reference**	
		GT	117 (70.1)	22 (44,0)	11 (68.8)	26 (61.9)	58 (98.3)	0.14 (0.01-1.21)	0.095
		TT	42 (25.1)	21 (42,0)	5 (31.2)	16 (38.1)	0	NA	0.34
	Alleles	**G**	133 (39.8)	36 (36)	11 (34.37)	26 (30.95)	60 (50.85)	**Reference**	
		T	201 (60.2)	64 (64)	21 (65.63)	58 (69.05)	58 (49.15)	2.02 (1.28-3.2)	**0.003**

CHB: Chronic Hepatitis B; ***HCC:*** Hepatocellular Carcinoma; ***OR:*** Odds Ratio; ***CI:*** confidence interval; ***NA:*** Not applicable.

Table 4. Comparison of genotypic and allelic frequencies of CHB, cirrhosis, HCC and controls.

Genotypic and allelic frequencies in CHB and cirrhosis						
			CHB N =50(%)	**Cirrhosis N =16 (%)**	**OR (95% CI)**	***p-value***
rs 3834087	**Genotypes**	**GAG/GAG**	0	1 (6.2)	**Reference**	
		GAG/-	32 (64)	15 (93.8)	NA	0.72
		-/-	18 (36)	0		**0.03**
	Alleles	**GAG**	32 (32)	17 (53.13)	**Reference**	
		Mutated *allele*	68 (68)	15 (46.87)	0.41 (0.19-0.97)	0.05
rs 3754217	**Genotypes**	**GG**	7 (14)	0	**Reference**	
		GT	22 (44)	11 (68.8)	NA	0.18
		TT	21 (42)	5 (31.2)		0.5
	Alleles	**G**	36 (36)	11 (34.37)	**Reference**	
		T	64 (64)	21 (65.63)	1.07 (0.46-2.47)	1
Genotypic and allelic frequencies in CHB and HCC						
			CHB N = 50 (%)	**HCC N = 42 (%)**	**OR (95% CI)**	***p-value***
rs 3834087	**Genotypes**	**GAG/GAG**	0	1 (2.4)	**Reference**	
		GAG/-	32 (64)	38 (90.5)	0	1
		-/-	18 (36)	3 (7.1)		0.39
	Alleles	**GAG**	32 (32)	40 (47.62)	**Reference**	
		Mutated *allele*	68 (68)	44 (52.38)	0.5 (0.28-0.94)	**0.044**
rs 3754217	**Genotypes**	**GG**	7 (14)	0	**Reference**	
		GT	22 (44)	26 (61.9)	NA	**0.02**
		TT	21 (42)	16 (38.1)		0.07
	Alleles	**G**	36 (36)	26 (30.95)	**Reference**	
		T	64 (64)	58 (69.05)	1.25 (0.67-2.32)	0.57
Genotypic and allelic frequencies in CHB and Controls						
			CHB N =50(%)	**Controls N =59(%)**	**OR (95% CI)**	***p-value***
rs 3834087	**Genotypes**	**GAG/GAG**	0	0	**Reference**	
		GAG/-	32 (64)	59 (100)	NA	NA
		-/-	18 (36)	0		
	Alleles	**GAG**	32 (32)	59 (50)	**Reference**	
		Mutated *allele*	68 (68)	59 (50)	2.12 (1.22-3.69)	**0.01**
rs 3754217	**Genotypes**	**GG**	7 (14)	1 (1.7)	**Reference**	
		GT	22 (44)	58 (98.3)	0.05 (0.006-0.46)	**0.002**
		TT	21 (42)	0	NA	0.6
	Alleles	**G**	36 (36)	60 (50.85)	**Reference**	
		T	64 (64)	58 (49.15)	1.83 (1.06-3.17)	**0.03**

CHB: Chronic Hepatitis B; ***HCC:*** Hepatocellular Carcinoma; ***OR:*** Odds Ratio; ***CI:*** confidence interval; ***NA:*** Not applicable

an association with a high probability of progression of the manifestation of HBV infection in participants, with an OR of 2.12, a CI of 1.22-3.69 and a p-value of 0.01, between chronic HBV carriers and controls. Conversely, in the same group, the heterozygous genotype (GT) of the rs3754217 polymorphism indicates potential protection against the chronic phase of infection, supported by an OR of 0.05, a CI of 0.006-0.46 and a p-value of 0.002, and the *mutated allele* could present a reduced progression of developing chronic hepatitis in HBV infection with an OR of 1.83, a CI of 1.06-3.17 and a p-value of 0.03. These results underline the importance of these polymorphisms as potential prognostic markers in the HBV infection landscape and call for further in-depth studies to confirm and expand on these associations (Table 4).

3. 6. **Comparison of Genotypic and Allelic Frequencies of the rs3834087 and rs3754217 Polymorphisms of the *ECM1 gene* between Cirrhosis, HCC and Controls.**

The analysis of genotypic and allelic frequencies between cirrhosis, HCC, and controls for the polymorphisms rs3834087 and rs3754217 of the *ECM1 gene* showed some interesting findings. For the rs3834087 polymorphism, while no significant association was identified between the various genotypes and the progression from cirrhosis to HCC, the mutated alleles seemed to confer a

reduced risk for progression to HCC. When examining the different genotypes of both polymorphisms in relation to the course of infection among the cirrhosis, HCC, and control groups, no statistically significant association was detected (p>0.05) (Table 5), but mutated alleles of both polymorphisms could be linked to a lower risk of progression to more severe forms of the disease. However, only the T mutated allele of rs3754217 with an OR of 2.3 and a CI of 1.28-4.14 was associated with a high risk of progression to HCC (p=0.007). These observations, as outlined in Table 5, underline the importance of understanding genetic factors in the progression of HBV infections and their potential prognostic implications.

4. Discussion

In our pursuit to elucidate the role of rs3834087 and rs3754217 polymorphisms of the ExtraCellular Matrix protein 1 (*ECM1*) *gene* in the progression of hepatitis B virus infection in Burkina Faso, several patterns were discovered. Socio-demographically, the mean age of our study subjects was relatively young, echoing the age distribution of Burkina Faso and the higher prevalence of HBV in sub-Saharan Africa. [23]. It was evident that HCC patients were often diagnosed in the middle age bracket,

Table 5. Comparison of genotypic and allelic frequencies of cirrhosis, HCC and controls.

Genotypic and allelic frequencies in cirrhosis and HCC						
			Cirrhosis N =16(%)	**HCC N =42(%)**	**OR (95% CI)**	***p-value***
rs 3834087	**Genotypes**	**GAG/GAG**	1 (6.2)	1 (2.4)	**Reference**	
		GAG/-	15 (93.8)	38 (90.5)	2.53(0.14-43.17)	1
		-/-	0	3 (7.1)	NA	0.81
	Alleles	**GAG**	17 (53.13)	40 (47.62)	**Reference**	
		Mutated allele	15 (46.87)	44 (52.38)	1.24(0.55-2.81)	0.74
rs 3754217	**Genotypes**	**GG**	0	0	**Reference**	
		GT	11 (68.8)	26 (61.9)	NA	NA
		TT	5 (31.2)	16 (38.1)		
	Alleles	**G**	11 (34.37)	26 (30.95)	**Reference**	
		T	21 (65.63)	58 (69.05)	1.16(0.49-2.77)	0.89
Genotypic and allelic frequencies in cirrhosis and controls						
			Cirrhosis N=16(%)	**Controls N=59(%)**	**OR (95% CI)**	***P-value***
rs 3834087	**Genotypes**	**GAG/GAG**	1 (6.2)	0	**Reference**	
		GAG/-	15 (93.8)	59 (100)	0	0.48
		-/-	0	0	NA	NA
	Alleles	**GAG**	17 (53.13)	59 (50)	**Reference**	
		Mutated allele	15 (46.87)	59 (50)	0.88(0.4-1.92)	0.9
rs 3754217	**Genotypes**	**GG**	0	1 (1.7)	**Reference**	
		GT	11 (68.8)	58 (98.3)	NA	1
		TT	5 (31.2)	0		0.32
	Alleles	**G**	11 (34.37)	60 (50.85)	**Reference**	
		T	21 (65.63)	58 (49.15)	1.97(0.87-4.45)	0.14
Genotypic and allelic frequencies in HCC and Controls						
			HCC N=42(%)	**Controls N=59(%)**	**OR (95% CI)**	***p-value***
rs 3834087	**Genotypes**	**GAG/GAG**	1 (2.4)	0	**Reference**	
		GAG/-	38 (90.5)	59 (100)	0	0.83
		-/-	3 (7.1)	0	NA	NA
	Alleles	**GAG**	40 (47.62)	59 (50)	**Reference**	
		Mutated allele	44 (52.38)	59 (50)	1.1(0.62-1.92)	0.84
rs 3754217	**Genotypes**	**GG**	0	1 (1.7)	**Reference**	
		GT	26 (61.9)	58 (98.3)	NA	1
		TT	16 (38.1)	0		0.05
	Alleles	**G**	26 (30.95)	60 (50.85)	**Reference**	
		T	58 (69.05)	58 (49.15)	2.3(1.28-4.14)	**0.007**

CHB: Chronic Hepatitis B; ***HCC:*** Hepatocellular Carcinoma; ***OR:*** Odds Ratio; ***CI:*** confidence interval; ***NA:*** Not applicable.

and our results aligned with previous findings within the region [14], but differed considerably from data in developed countries, where patients were relatively older.[24].
The analysis of *ECM1 gene* polymorphism data in the general study population revealed interesting results. For rs3834087, we found that the GAG/GAG, GAG/- and -/- genotypes had frequencies of 1.2%, 86.2%, and 12.6% respectively. Furthermore, the frequency of the wild-type GAG allele was 44.3% whereas the mutated allele had a frequency of 55.7%. These results are different from those of a study conducted by Xiuting He et al. in China, where the wild GAG/GAG genotype and the GAG allele were found to be predominant [25]. On the other hand, we observed a predominance of the GAG/- heterozygote. Moving on to rs3754217 polymorphisms, we found that the GG, GT, and TT genotypes had frequencies of 4.8%, 70.1%, and 25.1%, respectively. Moreover, the frequency of the wild-type T allele was 39.8%, while the mutated T allele had a frequency of 60.2%. However, there was a difference between the genotypic frequencies of the two studies. The Xiuting He et al. study showed that the wild GG and heterozygote GT genotypes were more prevalent, with frequencies of around 45-50%, and the mutated genotype was about 6%. Additionally, the wild G allele was found to be predominant compared to the mutated allele. Such disparities emphasize the influence of geographical and racial factors on genetic compositions.
Analysing of our data by gender, we found intriguing patterns. The mutated T in rs3754217 appeared to increase the risk of severe viral hepatitis B, especially in men. This could be explained by the fact that sex hormones play a role in chronic HBsAg carriage and the severity of infection. Indeed, according to some studies, the virus genome contains a particular DNA sequence that interacts with the androgen receptor. This could explain why men infected with the virus are likely to progress to severe forms of the infection [26,27].
In line with the findings of our study, a potential protective effect against the progression of severe infection was associated with the mutated allele of rs3834087 in the progression from chronic infection to HCC (OR=0.5; IC=0.28-0.94; p=0.044). Our results differ from those of Xiuting He et al. who found no difference in the allelic frequency of this polymorphism and progression to HCC. This protection against severe forms of infection could be explained by the fact that mutation within the *ECM1 gene* would lead to an increase in ECM1 protein. Indeed, ECM1 has been shown to be systematically down-regulated during liver injury, and strategies to re-express ECM1 in hepatocytes could be used to treat liver fibrosis [15].
In the group of chronic carriers and controls, the T-mutated allele of rs3754217 was associated with a low risk of progression to severity (OR=1.83; CI=1.06-3.17; p=0.03). Our results differ from those of Xiuting He et al. who found no difference in the allelic frequency of this polymorphism and the chronicity of infection.
In the same group, the GAG mutated allele of rs3834087 was associated with a 2-fold increased risk of progressing to chronic hepatitis (OR=2.12; CI=1.22-3.69; p=0.01). Our results differ from those of Xiuting He et al. who found instead a protection of the mutated allele against chronic infection (OR=0.6; CI=0.39-0.92; p=0.01).
In the group of HCC patients and controls, there was also an association between the T-mutated allele of the rs3754217 of the *ECM1 gene* and liver cancer. Indeed, the T allele could be associated with an elevated risk of developing severe forms of the infection (OR=2.3; CI=1.28-4.14; p=0.007). Our results differ from those of Xiuting He et al. who found no difference in the allelic frequency of this polymorphism and HCC in these 2 clinical groups.
There was a significant association between chronic HBV carriers and those with cirrhosis for the homozygous mutated genotype of rs3834087 (p=0.03), and the heterozygous GT genotype of rs3754217 between chronic carriers and HCC (p=0.02). Our results differ from those of Xiuting He et al. who found no association.
Based on the associations observed across our study groups and the discrepancies with findings from Xiuting He et al., we can speculate on the influence of regional epigenetic differences or environmental determinants. Our study was conducted in Burkina Faso, and the observed differences in genotypic and allelic frequencies may be due to regional epigenetic differences or differences in environmental exposures. These could include dietary, lifestyle, and environmental exposures unique to Burkina Faso that may have influenced the expression of the *ECM1 gene*. Given these findings, further research could be undertaken to explore the potential influence of these factors on *ECM1 gene* expression. Such research could provide valuable insights into the underlying mechanisms that contribute to the observed differences in genotypic and allelic frequencies and could help explain the discrepancies with findings from Xiuting He et al. It could also contribute to the development of personalized interventions and treatments tailored to specific populations based on their unique genetic and environmental profiles.
In the chronic carrier and control groups, there was an association between the heterozygous GT genotype of the rs3754217 polymorphism of the *ECM1 gene* and the course of infection. The GT heterozygous genotype could have a protective effect against severity (OR=0.05; CI= 0.006-0.46; p=0.002). Our results corroborate those of Xiuting He et al.
The implications of these genetic polymorphisms, especially in the backdrop of liver health and the pivotal role of ECM1 in liver fibrosis, provide a rich area for further exploration.

5. Conclusion

Our study was the first to investigate the association between the rs3834087 and rs3754217 polymorphisms of the *ECM1 gene* and the occurrence of severe forms of HBV infection in the population of Burkina Faso. We found that the frequencies of the GAG/GAG, GAG/-, and -/- genotypes of rs3834087 were 1.2%, 86.2%, and 12.6%, respectively, with allelic frequencies of 43.3% for the wild-type GAG allele and 55.7% for the mutated allele. For rs3754217 polymorphisms, the frequencies of the GG, GT, and TT genotypes were 4.8%, 70.1%, and 25.1%, respectively. Moreover, the frequency of the wild-type G allele was 39.8% compared to 60.2% for the mutated T allele.
This study has shown that the rs3834087 and rs3754217 polymorphisms of the *ECM1 gene* may be involved in the progression of chronic hepatitis to cirrhosis and hepatocellular carcinoma in HBV-infected patients or contribute to a protective effect.
However, no link between these two polymorphisms and

the onset of cirrhosis, or its progression to HCC, has been observed.

It is possible that an interaction between several factors could better explain the emergence of severe forms of HBV infection in Burkina Faso. Therefore, further research in this area is necessary to identify other factors that may contribute to the development of severe forms of HBV infection in this population. This could help improve the prevention, diagnosis, and treatment of HBV infection, especially in regions with a high prevalence of the disease like Burkina Faso.

Abbreviations

ALAT: Alanine Amino Transferase; CHB: Chronique Hepatitis B; DNA: Deoxyribonucleic acid; ECM1: Extracellular Matrix Protein 1; HBsAg: HBs Antigen; HBV: Hepatitis B Virus; HCC: HepatoCellular Carcinoma; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; HSC: Hepatic Stellate Cells; KIR: Killer-cell Immunoglobulin-like Receptor; WHO: World Health Organization; p53: tumor protein 53; RB1: Retinoblastoma 1; Rs: Reference of SNP; SNP: Single Nucleotide Polymorphism; TGF-β1: Transforming growth factor beta 1

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not Applicable

Ethics approval and consent to participate

This study was approved by the Ethics Committee for Health Research **CERS N° 25 48 89 37**. Written informed consent was obtained from patients and donors. We ensured the confidentiality of our database by storing it on a password-protected computer.

Protection of Human Subjects and Animals in Research

Written informed consent was obtained from patients and donors. The blood samples were taken by health professionals under appropriate conditions

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Study concept and design: LT, SVZ, FWD and JS. Sampling and laboratory analysis: LT, MNT, SVZ, AKO, MS, MST, TRC, ATB, RAS. Statistical analysis and data interpretation: LT, MNT, TRC and AKO. Drafting of the manuscript: LT, MNT and AKO. Critical revision of the manuscript for important intellectual content: SVZ, AKO, MS, MST, BD, ATY, BMN, FWD and JS. Administrative, technical, and material support: LT, SVZ, TRC, FWD and JS. Study supervision: BLN, ATY, FWD and JS. The corresponding author declares that the manuscript has been read and approved by all named authors and that the order of authorship in the manuscript has been approved by all of us.

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