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Identification of Novel TERT Promoter Mutations in Chronic Hepatitis B-Induced HCC Patients in Burkina Faso

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Abstract

Introduction: Hepatocellular carcinoma (HCC) is one of the serious complications of chronic hepatitis B virus (HBV) infection and constitutes a major global public health problem. Several factors contribute to the development of this cancer, the genetic aspect of which is linked to mutations in genes such as the human telomerase reverse transcriptase (TERT) gene. This gene plays a crucial role in maintaining telomeres. Genetic alterations in this gene can result in a defect in the continuous synthesis of telomeres, reducing the sensitivity of cells to apoptosis. **Method:** This was a descriptive study in which 97 patients were enrolled. TERT promoter mutations were characterized using Sanger sequencing technology and the functionality of the mutations identified was validated using cell culture techniques. **Results:** Our study showed that patients with unknown etiology status were mainly affected by mutations

at positions G176A, G113T and C123G (100%). Patients with cirrhosis were mainly affected by these SNPs. **Conclusion**: This study mainly identified 21 new SNPs in the TERT promoter and identified the function of certain mutations. Only the G228T or G228A mutation in our cohort is functional.

Keywords

CHC, Chronic Hepatitis, Cirrhosis, TERT Promoter, Burkina Faso

1. Introduction

Liver cancer is a significant public health concern, ranking sixth in most diagnosed cancers and third in cancer-related deaths globally [1] [2]. Liver cancer, particularly hepatocellular carcinoma (HCC), has shown a rise in both the number of new cases and deaths worldwide [3]. HCC is the predominant form of primary liver cancer and is the third leading cause of cancer-related deaths in Burkina Faso [1] [4]. Based on WHO projections, there will be a 58% increase in new cases by 2040, with 1,400,000 new cases and 1,000,000 deaths expected in 2030 [5]. The primary etiological factors for HCC vary depending on the geographical location and the underlying risk factors for liver diseases. For example, in areas with high prevalence rates of hepatitis B virus (HBV) infection, such as in Asia and sub-Saharan Africa, chronic HBV infection is the leading cause of HCC [5] [6]. In contrast, hepatitis C virus (HCV) infection is the primary etiological factor for HCC in many developed countries, including the United States, Japan, and Western Europe [7]. In addition to viral infections, other factors such as alcohol consumption, non-alcoholic fatty liver disease (NAFLD), and exposure to environmental toxins also contribute to the development of HCC [8]. Furthermore, there are disparities in access to healthcare and treatment options for HCC, which can lead to differences in survival rates across populations. Addressing these disparities in HCC etiology, incidence, and mortality requires a comprehensive understanding of the underlying risk factors and effective public health interventions to prevent and manage the liver disease.

In addition to these many risk factors, changes such as mutations within the human genome have been identified as integral to the development of HCC. Among these mutations, mutations in the tumor protein P53 (TP53), β-catenin (CTNNB1), and telomerase reverse transcriptase (TERT) genes are frequently found in HCC and have been studied extensively in relation to the development and progression of the disease [9] [10].

Telomerase activity is weakly absent in somatic cells, although strongly expressed in embryonic and stem cells [9]. TERT plays a central role in cell immortality and tumorigenesis by maintaining telomere homeostasis and integrity and TERT promoter mutations are found in about 50% of HCC [11]. Two common single-nucleotide polymorphisms (SNPs), C228T and C250T, have been identified

in the promoter of TERT. They are known to create potential binding sites for transcription factors and increase promoter activity, ultimately leading to TERT transcription and cell immortality [12]. These SNPs are located at two hotspots, corresponding to positions 124 (C228T) and (C250A)146 bp upstream of the TERT translation start site. The mutant TERT promoter activity requires the "de novo" ETS motif, and ETS candidate factors may regulate TERT expression directly through the C228T and C250T mutations [10] [11].

The association of TERT promoter mutations with HCC in African patients with chronic HBV infection is not well studied, as most of the research has been conducted in East Asia and Europe. However, some studies have suggested that TERT promoter mutations are also prevalent in HCC cases in Africa. For example, a study conducted in South Africa found that TERT promoter mutations were present in 53.3% of HCC cases, and the prevalence of these mutations was higher in cases with chronic HBV infection [13].

The present study aims to investigate the prevalence and functional significance of TERT promoter mutations in HCC patients from Burkina Faso, where chronic HBV infection is a major risk factor for the disease with a prevalence of 14,47% [14]. By understanding how these mutations contribute to HCC development and progression in African populations, this investigation may provide important insights into the molecular mechanisms. These findings could help guide more effective prevention and treatment strategies.

2. Materials and Methods

2.1. Ethical Consideration

This study receive permission from national comity for health science research. In addition, free and informed consent from all participants, or the parents/legal guardians for patients in critical condition. This study was approved by the Institutional Review Board of the Burkina Faso National Ethics Committee for Health Research (CERS) by deliberation number 2019-5-067.

2.2. Patients' Selection

Patients were recruited and tested for HBV viral load from March 3, 2015, through February 2, 2017, at the Pietro Annigoni Biomedical Research Center (CERBA) in Ouagadougou, Burkina Faso, West Africa. The study population consisted of HBV-infected patients, with a subset having HCC with or without cirrhosis. A total of 97 patients diagnosed with HBV infection were included in our study, among whom 19 had HCC without cirrhosis, 30 had HCC with cirrhosis, 39 had cirrhosis only, and 9 had an unknown cirrhosis status.

2.3. DNA Extraction

Genomic DNA was extracted from 200 µL of plasma using the Genomic Pure Link DNA Extraction Mini Kit (INVITROGEN) following the manufacturer's protocol. The extracted genomic DNA was stored at −20˚C until use.

2.4. Direct Sequencing of TERT Promoter SNPs: Amplification and Analysis

To analyze the TERT promoter region, we used specific primers, TERT-F (5'-acgaacgtggccagcggcag-3') and TERT-R (5'-ctggcgtccctgcaccctgg-3'), to amplify a 474-bp fragment covering the −124 and −146-bp hotspots of the ATG start codon in the TERT promoter. The PCR reactions were carried out using the GeneAmp PCR System 9700 instrument, and the resulting products were purified and sent for sanger sequencing at the Arizona State University (ASU) Genomics Core Facilities, Tempe, Arizona, USA. PCR was run in a total reaction volume of 25 µL containing 7 µL of molecular biology grade water, 10 µL of 2X master mix, 0.5 µL of each primer at a concentration of 0.2 µM and 5 µL of each DNA extract at 10 ng/µL. PCR steps were initial denaturation at 94˚C for 3 min, followed by 45 cycles of denaturation at 94˚C for 30 s, hybridization and amplification at 65˚C for 30 s, elongation at 72˚C for 1 min, and final elongation for 10 min at 72˚C. We specifically looked for mutations in the promoter region of the TERT gene (hg38/NG_055467.1 chr5, position 1,295,113 C>T and 1,295,135 C>T) to further understand their potential impact on HCC development and progression in our study population.

2.5. Data Analysis

The sequence data obtained from sanger sequencing were processed for the mutation analysis. Briefly, 110 sequences from 97 patients were mapped to reference HG38 with BWA (Burrows-Wheeler Aligner) and reviewed by IGV (Integrative Genomics Viewer). With the alignment to HG38, 36 sequences were removed from the data set due to failed mapping to target sequence regions. Fifty-eight sequences were processed to count mutations in the final data set, followed by filtering sequences with low mapping quality. Detected mutations were then compared to dbSNP and COSMIC DB database, unique mutations in the cohort were then identified. Based on the clinical information available for the patients, corresponding sequences were then differentiated into 4 groups (cirrhosis, HCC & cirrhosis, HCC and others), mutations among groups were further compared and characterized.

2.6. Cell Culture

Human HCC cell lines, Hep3B [ATCC HB-8064], Huh7 [ATCC HB-8065], SNU182 [ATCC CRL-2235], SNU387 [ATCC CRL-2237], PLC [ATCC CRL-8024] used in this study were purchased from American Type Culture Collection (ATCC) and were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. Normal hepatocytes, HH, were obtained from ScienCell, USA Research Laboratories, Inc., and grow according to the manufacturer. Details of these cancer cells are contained in Table 1. All cells were tested for mycoplasma and passaged in a tissue culture incubator at 37˚C and 5% CO_2.

Table 1. Characteristic of the cell lines.

Cells lines	Type of cells	diseases	Gender/Age	Property of growth
HH	Lymphoblast T	Lymphom	White Men/61 years old	Suspension
SNU-387	Liver	HCC	Correan Women/41 days old	Adherent
SNU-182	Liver	Primary HCC	Correan men/21 years old	Adherent
Hep3B	Liver	HCC with integrated genome of the HBV	Black men/8 years old	Adherent
PLC/PRF/5	Liver	HCC		Adherent
HuH-7	Liver	Well differenced HCC	Men/57 years old	Adherent

2.7. Luciferase Activity

The study utilized the dual luciferase reporter system to examine the impact of TERT promoter mutation on gene expression in six cultured cell lines. The luciferase reporters (pNL1.1/TERTmut, pNL1.1/CMV, and pNL1.1/TERT/wt) were created by inserting the TERT promoter or CMV into the pNL1.1[Nluc] vector in the potential TEAD binding site. After transfection with the appropriate luciferase reporter, cells were lysed, and luciferase activity was measured using the Dual-Luciferase® Reporter Assay System kit (Catalog# E1980 Promega, USA). Promoter luciferase activity was calculated by normalizing firefly to Renilla luciferase activity. Cell culture and transfection were performed according to the manufacturer's instructions.

3. Results

3.1. Socio-Demographic Characterization of HBV-HCC Patients

For this study, a total of 97 HBV-infected patients were selected for the study, with varying types of liver diseases, including 19 cases of HCC without cirrhosis, 30 cases of HCC with cirrhosis, 39 cases of cirrhosis and 9 undetermined cases. The undetermined cases are those with liver disease but unknown etiology. The study population ranged in age from 20 to 76 years, with an average age of 42.29 years. Of the total patients, 55 (56.7%) were male (Table 2). Interestingly, 70% of the patients in the study belonged to the Mossi ethnic group, the principal ethnic group from Burkina Faso. Table 1 summarizes the socio-demographic and clinical characteristics of the study population, including age, gender, ethnicity, and types of liver diseases.

Table 2. Socio-demographic characteristics of patients.

Variables	Cirrhosis *N* = 39 (%)	HCC + Cirrhosis *N* = 30 (%)	*HCC* 19 = (%)	Other N = 9 (%)
Gender				
Male	22 (56.40)	18 (60)	9 (47.36)	6 (66.66)
Female	17 (43.58)	12(40)	10 (52.63)	3 (33.33)

Continued

Age (years)				
<20	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
20 - 50	34 (87.17)	23(76.66)	14 (73.68)	8 (88.88)
>50	5 (12.82)	7 (23.33)	5 (26.31)	1(11.11)
Ethnic group				
Mossi	29 (74.36)	21 (70)	12 (63.16)	8 (90)
Bissa-gourounsi	6 (15.38)	3 (10)	3 (15.79)	0
Other	4 (10.26)	6 (20)	4 (21.05)	1 (10)

3.2. Identification of SNPs in the Promoter of TERT in Plasma cfDNA

The analysis conducted using Integrative Genomics Viewer software on 110 Sanger sequences from HBV-infected patients, including 40 homozygous sequences, resulted in the generation of 70 TERT promoter sequences that were classified into four different disease groups (Figure 1). The analysis identified several mutations between positions 1295102-1295177, which were found upstream and downstream of highly recurrent mutations (C228T and C250T). To confirm our findings, we compared them with the Catalogue of Somatic Mutations in Cancer (COSMIC) database and the database for single nucleotide polymorphisms (dbSNP). This analysis revealed that some mutations were present in both databases, while others were only present in the COSMIC database or absent from both. Interestingly, we found that only the −228 mutation (Rs1242535815) was present in this cohort, despite both mutations being commonly found in the literature. Table 3 displays the SNP sets in the cohort, with novel ones marked in red. Our results showed that we identified a total of 22 novel SNPs at 17 different sites, strengthening the understanding of TERT promoter SNPs in HBV-infected

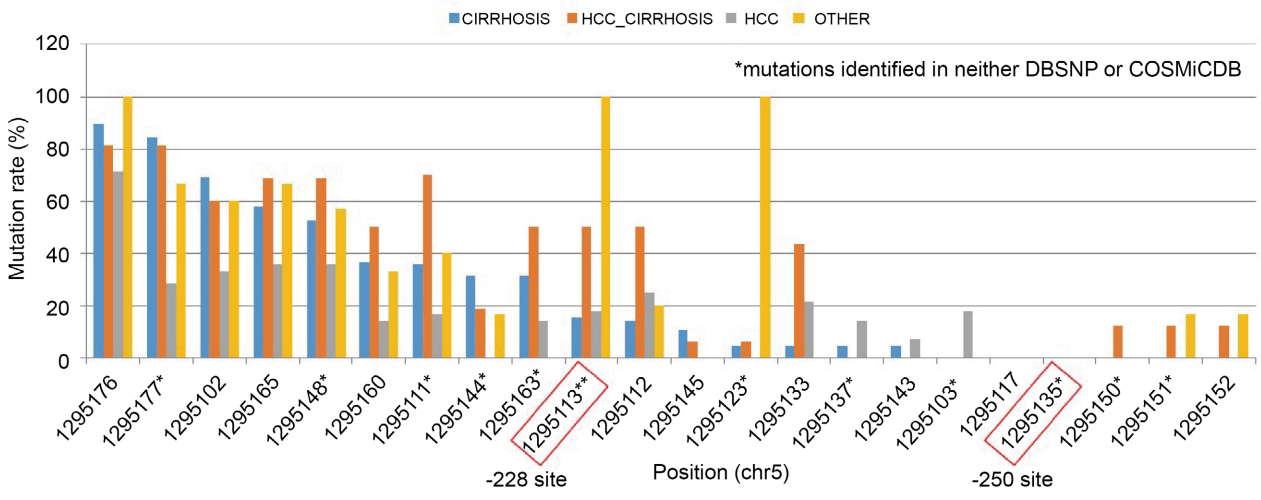

Figure 1. Frequencies of SNPs in HBV-induced HCC with or without cirrhosis.

Table 3. SNPs detected in the TERT promoter from HBV-infected patients.

Position (hg38/NG_055467.1)	Reference base	dbSNP (reference)	COSMIC (RC: NP_937983.2)	Sample alleles	Function
1295102/575	G	Rs796849347 (A)	c.1-113C>T	G (18)/C(22)	217
1295103/576	A	Rs796849347 (G)		A(38)/T(2)	218
1295111/584	G			G(27)/T(10)/C(4)/+C(10)	226
1295112/585	A	Rs1327649395 (G)	c.1-123T>C	A(30)/T(10)	227
1295113/586	G	Rs1242535815 (A)	c.1-124C>A c.1-124C>T c.1-125_1-124CC>TT	G(27)/T(10)/−G(6)	−228 site
1295117/590	G	Rs1176096403 (C)	c.1-128C>A c.1-128C>G c.1-128C>T	G(57)/+T(2)	232
1295123/596	C			C(55)/G(2)	238
1295133/606	G	Rs1445328796 (-G)	c.1-144C>T	G(46)/T(11)	248
1295135/608	G		c.1-146C>T	G(57)	−250 site
1295137/610	G			G(54)/C(3)	252
1295143/616	G	Rs990361282 (A/C)	c.1-154C>A c.1-154C>G c.1-154C>T	G(55)/C(2)	258
1295144/617	G			G(45)/A(7)/T(3)	259
1295145/618	G	Rs796188588 (A)	c.1-156C>T	G(52)/T(3)	260
1295148/621	G			G(25)/A(13)/T(17)	263
1295150/623	G			G(53)/T(2)	265
1295151/624	G			G(52)/T(3)	266
1295152/625	C		c.1-163G>C	C(52)/T(3)	267
1295160/633	G		c.1-171C>T	G(36)/A(19)	275
1295163/636	C	Rs914835735 (T)		C(39)/T(16)	278
1295165/638	G		c.1-176C>T	G(24)/A(31)	280
1295176/649	G	Rs796451171 (A)	c.1-187C>T	G(9)/C(46)	291
1295177/650	C	Rs559772340 (A)		C(12)/A(43)	292

patients. The SNPs identified were then grouped according to the clinical cases studied. In addition to the SNPs, we identified a number of insertion and/or deletion mutations at positions 1 295 111, 1 295 113 and 1 295 117 (**Table 4**).

3.3. Uncovering the Relationship between SNPs and Clinical Cases of Hepatocellular Carcinoma

We identified several single nucleotide polymorphisms (SNPs) in the TERT promoter of HBV-infected patients and investigated their relationship to different clinical cases. To categorize the SNPs according to their association with specific clinical cases, we grouped them into four categories: HCC without cirrhosis, HCC

Table 4. Mutations and relationship to clinical cases.

hg38.chr5	reference	CIRRHOSIS								HCC_CIRRHOSIS								HCC								OTHER							
position	base	A	C	G	T	Indel	Total	Muts	%	A	C	G	T	indel	Total	Muts	%	A	C	G	T	indel	Total	Muts	%	A	C	G	T	indel	Total	Muts	%
1295102	G	0	9	4	0	0	13	9	69.2	0	6	4	0	0	10	6	60	0	4	8	0	0	12	4	33.3	0	3	2	0	0	5	3	60
1295103	A	13	0	0	0	0	13	0	0	10	0	0	0	0	10	0	0	9	0	0	2	0	11	2	18.2	5	0	0	0	0	5	0	0
1295111	G	0	2	9	3	3C1T	14	5	35.7	2	5	3	0	1C	10	7	70	0	0	10	2	2C	12	2	16.7	0	0	3	2	4C	5	2	40
1295112	A	12	0	0	2	0	14	2	14.3	5	0	0	5	0	10	5	50	9	0	0	3	0	12	3	25	4	0	0	1	0	5	1	20
1295113	G	0	0	11	2	−1G	13	2	15.4	0	0	3	3	−3G	6	3	50	0	0	9	2	−1G	11	2	18.2	4	0	0	1	0	5	5	100
1295117	G	0	0	21	0	0	21	0	0	0	0	16	0	0	16	0	0	0	0	14	0	1T	14	0	0	0	0	6	0	1T	6	0	0
1295123	C	0	20	1	0	0	21	1	4.76	0	15	1	0	0	16	1	6.25	0	0	0	0	0	0	0	0	0	0	6	0	0	6	6	100
1295133	G	0	0	20	1	0	21	1	4.76	0	0	9	7	0	16	7	43.8	0	0	11	3	0	14	3	21.4	0	0	6	0	0	6	0	0
1295135	G	0	0	21	0	0	21	0	0	0	0	16	0	0	16	0	0	0	0	14	0	0	14	0	0	0	0	6	0	0	6	0	0
1295137	G	0	1	20	0	0	21	1	4.76	0	0	16	0	0	16	0	0	0	2	12	0	0	14	2	14.3	0	0	6	0	0	6	0	0
1295143	G	0	1	20	0	0	21	1	4.76	0	0	16	0	0	16	0	0	0	1	13	0	0	14	1	7.14	0	0	6	0	0	6	0	0
1295144	G	4	0	13	2	0	19	6	31.6	2	0	13	1	0	16	3	18.8	0	0	14	0	0	14	0	0	1	0	5	0	0	6	1	16.7
1295145	G	0	0	17	2	0	19	2	10.5	0	0	15	1	0	16	1	6.25	0	0	14	0	0	14	0	0	0	0	6	0	0	6	0	0
1295148	G	6	0	9	4	0	19	10	52.6	3	0	5	8	0	16	11	68.8	1	0	9	4	0	14	5	35.7	3	0	3	1	0	7	4	57.1
1295150	G	0	0	19	0	0	19	0	0	0	0	14	2	0	16	2	12.5	0	0	14	0	0	14	0	0	0	0	6	0	0	6	0	0
1295151	G	0	0	19	0	0	19	0	0	0	0	14	2	0	16	2	12.5	0	0	14	0	0	14	0	0	0	0	5	1	0	6	1	16.7
1295152	C	0	19	0	0	0	19	0	0	0	14	0	2	0	16	2	12.5	0	14	0	0	0	14	0	0	0	5	0	1	0	6	1	16.7
1295160	G	7	0	12	0	0	19	7	36.8	8	0	8	0	0	16	8	50	2	0	12	0	0	14	2	14.3	2	0	4	0	0	6	2	33.3
1295163	C	0	13	0	6	0	19	6	31.6	0	8	0	8	0	16	8	50	0	12	0	2	0	14	2	14.3	0	6	0	0	0	6	0	0
1295165	G	11	0	8	0	0	19	11	57.9	11	0	5	0	0	16	11	68.8	5	0	9	0	0	14	5	35.7	4	0	2	0	0	6	4	66.7
1295176	G	0	17	2	0	0	19	17	89.5	0	13	3	0	0	16	13	81.3	0	10	4	0	0	14	10	71.4	0	6	0	0	0	6	6	100
1295177	C	16	3	0	0	0	19	16	84.2	13	3	0	0	0	16	13	81.3	0	10	4	0	0	14	4	28.6	4	2	0	0	0	6	4	66.7

with cirrhosis, cirrhosis, and other unknown etiology. Our results showed that patients with HCC of undetermined origin had the highest percentage of SNPs, with 100% at positions G176A, G113T, C123G, followed by 57% to 66% at positions C177A, G102C, G165A and G148A/T (Figure 1). Cirrhotic patients had the second highest percentage of SNPs, with 89.5% and 84.2% of G176C, C177A, respectively, followed by 69%, 57.9% and 52.6% of SNPs at positions G102C, G165A and G148A/T. HCC patients on cirrhosis occupied the third place, with two positions (G176C, C177A) having a frequency of 81%, followed by several other positions with a frequency of 50% to 70% (Figure 1). Finally, the predominant mutation in the group of HCCs was 71% (G176C).

3.4. Functional Characterization of New TERT Promoter Mutations

The luciferase reporter assay revealed that the newly identified TERT promoter mutations are not only functional but also increase TERT promoter activity. Specifically, the G228A and G228T mutations generated a "de novo" TERT promoter capable of initiating gene transcription in four cell lines: Hep3b, SNU-387, SNU-182 and HuH-7, with a transcription frequency 2-4 times higher than that of the wild-type TERT promoter (Figure 2). Interestingly, the G225C mutation also induced significant replication in PLC/PRF/5 and Hep3b cells, indicating its potential role in promoting cancer cell proliferation.

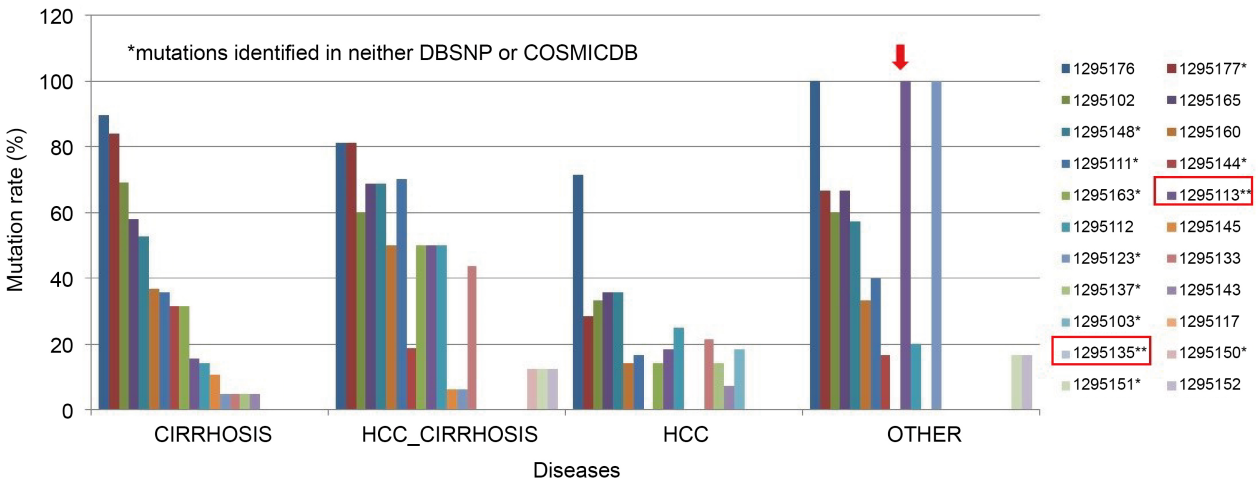

Figure 2. Functional validation of novel SNPs found in the TERT promoter.

4. Discussion

This study provides new insights into TERT promoter mutations in a population samples from Burkina Faso, which have not been extensively studied in the literature. The results of the study demonstrate the presence of multiple mutations in four groups of patients, some of which have not been previously reported. Despite the small sample size, the findings are an important contribution to the under-

standing of HCC in this region and emphasize the need for continued investigation into the molecular abnormalities associated with the disease.

Our study showed a relatively young age of onset, with a mean age of approximately 42,29 years, which is in contrast to the typical occurrence of HCC in elderly (~55 years) individuals reported in developed countries [15] [16]. However, this finding is consistent with the demographic profile of Burkina Faso, where nearly 80% of the population is under 35 years of age, according to the National Institute of Statistics and Demography [17]. To effectively prevent and treat HCC in Burkina Faso, it is crucial to understand the unique characteristics of this population. As etiologic factors leading to HCC can vary between countries, further investigation into the specific risk factors for HCC in Burkina Faso is needed. Moreover, the higher incidence of HCC in men globally may indicate a potential role for gender-specific risk factors, emphasizing the need for gender-specific research in this area. Additionally, environmental factors such as high incidence of viral infections (e.g., hepatitis B and C), alcohol consumption, and exposure to aflatoxins can also contribute to the development of HCC. These factors can further exacerbate the accumulation of genetic and epigenetic changes in the liver cells, increasing the risk of developing HCC [18]. Therefore, genetic profiling and understanding of the molecular mechanisms underlying HCC development remain crucial for developing effective prevention and treatment strategies.

In this respect, previous studies have already characterized the main risk factor in Burkina Faso, the hepatitis B virus, as well as some genetic characteristics of infected individuals. Genotype E of the hepatitis B virus has been identified as the main healing variant [19]. Various studies have also looked at the role of certain genes in the progression of infection. Compaoré *et al.,* showed that the APOBEC3 gene polymorphism rs35228531 had a protective effect in HBV/HIV coinfection [20]; KIR3DL1, KIR3DL2, KIR2DS1 also had a protective effect against chronic hepatitis B infection [21].

The G228T and G250T mutations in the TERT promoter are known to be mutually exclusive, meaning that they tend to occur independently of each other [22] [23]. The G228T mutation is more common than the G250T mutation, and it has been found to be associated with HCC (31.4% [23], 65.4% [16]) and other types of cancer [24]. Recent research suggests that both mutations may be associated with rare cancer cases, but more studies are needed to confirm these findings.

Our study identified the G228T mutation in the promoter region of TERT as a common mutation in HCC cases (18.18%), HCC with cirrhosis (50%), cirrhosis only (15.38%), and indeterminate cases (100%). In addition to G228T and G250T, we also identified other previously unreported SNPs in the TERT promoter in our cohort. Notably, our study found the G228T mutation to be the only TERT promoter mutation present in our cohort, which differs from previous studies that have reported both G228T and C250T mutations. This may suggest a regional or population difference in the prevalence of these mutations. However, it is important to note that our sample size was relatively small and further studies are

needed to confirm these findings.

Our luciferase experiments provided additional support for the importance of the G>A/T transition in TERT gene activation, as this mutation is believed to create a new binding site for ETS transcription factors, which in turn activated TERT transcription and telomerase. Our study confirms the findings of a previous study on glioblastoma [10], which showed that mutations induced at specific positions did not increase promoter activity, highlighting the necessity of the G>A/T transition for TERT gene activation. Studies have also suggested that GA-binding proteins (GABPA and GABPB1), members of the ETS family of transcription factors, are specifically recruited to the TERT mutant promoter but not to the wild-type promoter in cancer cells, thereby activating TERT transcription and telomerase [25] [26].

While the G228T mutation in the TERT promoter region is a crucial factor in activating TERT transcription and telomerase, it is not the only factor involved in telomere maintenance and HCC development. TERT promoter methylation, which can affect the binding of transcription factors, has been reported to be associated with TERT expression and telomere maintenance in HCC. Moreover, genomic structural rearrangements and TERT copy number alterations have also been implicated in HCC development and telomere maintenance [27]. Finally, some HCCs can maintain telomere length through an alternative mechanism known as alternative lengthening of telomeres (ALT) that involves homologous recombination-mediated telomere synthesis [27]. Thus, a comprehensive understanding of the mechanisms of telomere maintenance in HCC requires considering multiple factors, including the G228T mutation, TERT promoter methylation, genomic structural rearrangements, TERT copy number, and alternative telomere lengthening.

5. Conclusion

In summary, our study identified new TERT promoter mutations in HCC patients from Burkina Faso and validated the function of some of these mutations, particularly G228A/T, which creates a novel binding site for the transcription factor ETS. Our findings support the role of TERT promoter mutations in the development of HCC and cirrhosis in this population. However, further research is needed to confirm our results in larger HCC samples and to investigate the association of TERT promoter mutations with other tumor-associated mutations, such as TP53 and CTNNB1.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Okeke, E., Davwar, P.M., Roberts, L., Sartorius, K., Spearman, W., Malu, A., *et al*. (2020) Epidemiology of Liver Cancer in Africa: Current and Future Trends. *Seminars in Liver Disease*, **40**, 111-123. https://doi.org/10.1055/s-0039-3399566

[2] Rumgay, H., Arnold, M., Ferlay, J., Lesi, O., Cabasag, C.J., Vignat, J., *et al*. (2022) Global Burden of Primary Liver Cancer in 2020 and Predictions to 2040. *Journal of Hepatology*, **77**, 1598-1606. https://doi.org/10.1016/j.jhep.2022.08.021

[3] Samant, H., Amiri, H.S. and Zibari, G.B. (2021) Addressing the Worldwide Hepatocellular Carcinoma: Epidemiology, Prevention and Management. *Journal of Gastrointestinal Oncology*, **12**, S361-S373. https://doi.org/10.21037/jgo.2020.02.08

[4] Wongjarupong, N., Yonli, A.T., Nagalo, B.M., Djigma, F.W., Somda, S.K., Hassan, M.A., *et al*. (2020) Characteristics of Patients with Chronic Hepatitis B Virus Infection with Genotype E Predominance in Burkina Faso. *Hepatology Communications*, **4**, 1781-1792. https://doi.org/10.1002/hep4.1595

[5] Maloberti, T., De Leo, A., Sanza, V., Gruppioni, E., Altimari, A., Riefolo, M., *et al*. (2022) Correlation of Molecular Alterations with Pathological Features in Hepatocellular Carcinoma: Literature Review and Experience of an Italian Center. *World Journal of Gastroenterology*, **28**, 2854-2866. https://doi.org/10.3748/wjg.v28.i25.2854

[6] Fujiwara, N., Liu, P., Athuluri-Divakar, S.K., Zhu, S. and Hoshida, Y. (2019) Risk Factors of Hepatocellular Carcinoma for Precision Personalized Care. In: Hoshida, Y., Ed., *Molecular and Translational Medicine*, Springer International Publishing, 3-25. https://doi.org/10.1007/978-3-030-21540-8_1

[7] de Martel, C., Maucort-Boulch, D., Plummer, M. and Franceschi, S. (2015) Worldwide Relative Contribution of Hepatitis B and C Viruses in Hepatocellular Carcinoma. *Hepatology*, **62**, 1190-1200. https://doi.org/10.1002/hep.27969

[8] Gomaa, A.I., Khan, S.A., Toledano, M.B., Waked, I. and Taylor-Robinson, S.D. (2008) Hepatocellular Carcinoma: Epidemiology, Risk Factors and Pathogenesis. *World Journal of Gastroenterology*, **14**, 4300-4308. https://doi.org/10.3748/wjg.14.4300

[9] Avin, B.A., Umbricht, C.B. and Zeiger, M.A. (2016) Human Telomerase Reverse Transcriptase Regulation by DNA Methylation, Transcription Factor Binding and Alternative Splicing (Review). *International Journal of Oncology*, **49**, 2199-2205. https://doi.org/10.3892/ijo.2016.3743

[10] Bell, R.J.A., Rube, H.T., Kreig, A., Mancini, A., Fouse, S.D., Nagarajan, R.P., *et al*. (2015) The Transcription Factor GABP Selectively Binds and Activates the Mutant TERT Promoter in Cancer. *Science*, **348**, 1036-1039. https://doi.org/10.1126/science.aab0015

[11] Hirai, M., Kinugasa, H., Nouso, K., Yamamoto, S., Terasawa, H., Onishi, Y., *et al*. (2021) Prediction of the Prognosis of Advanced Hepatocellular Carcinoma by TERT Promoter Mutations in Circulating Tumor DNA. *Journal of Gastroenterology and Hepatology*, **36**, 1118-1125. https://doi.org/10.1111/jgh.15227

[12] Kang, S.Y., Kim, D.G., Kim, H., Cho, Y.A., Ha, S.Y., Kwon, G.Y., *et al*. (2022) Direct Comparison of the Next-Generation Sequencing and iTERT PCR Methods for the Diagnosis of TERT Hotspot Mutations in Advanced Solid Cancers. *BMC Medical Genomics*, **15**, Article No. 25. https://doi.org/10.1186/s12920-022-01175-2

[13] Pezzuto, F., Buonaguro, L., Buonaguro, F.M. and Tornesello, M.L. (2017) Frequency and Geographic Distribution of TERT Promoter Mutations in Primary Hepatocellular Carcinoma. *Infectious Agents and Cancer*, **12**, Article No. 27.

https://doi.org/10.1186/s13027-017-0138-5

[14] Tao, I., Compaoré, T.R., Diarra, B., Djigma, F., Zohoncon, T.M., Assih, M., *et al*. (2014) Seroepidemiology of Hepatitis B and C Viruses in the General Population of Burkina Faso. *Hepatitis Research and Treatment*, **2014**, 1-5. https://doi.org/10.1155/2014/781843

[15] Pezzuto, F., Izzo, F., De Luca, P., Biffali, E., Buonaguro, L., Tatangelo, F., *et al*. (2021) Clinical Significance of Telomerase Reverse-Transcriptase Promoter Mutations in Hepatocellular Carcinoma. *Cancers*, **13**, Article 3771. https://doi.org/10.3390/cancers13153771

[16] Kawai-Kitahata, F., Asahina, Y., Tanaka, S., Kakinuma, S., Murakawa, M., Nitta, S., *et al*. (2016) Comprehensive Analyses of Mutations and Hepatitis B Virus Integration in Hepatocellular Carcinoma with Clinicopathological Features. *Journal of Gastroenterology*, **51**, 473-486. https://doi.org/10.1007/s00535-015-1126-4

[17] Institut national de la statistique et de la démographie (INSD) (2020) Annuaire Statistique National 2019. https://www.insd.bf/sites/default/files/2021-12/Annuaire_Statistique_National_2019.pdf

[18] Nault, J.C., Calderaro, J., Di Tommaso, L., Balabaud, C., Zafrani, E.S., Bioulac-Sage, P., *et al*. (2014) Telomerase Reverse Transcriptase Promoter Mutation Is an Early Somatic Genetic Alteration in the Transformation of Premalignant Nodules in Hepatocellular Carcinoma on Cirrhosis. *Hepatology*, **60**, 1983-1992. https://doi.org/10.1002/hep.27372

[19] Diarra, B., Yonli, A.T., Sorgho, P.A., Compaore, T.R., Obiri-Yeboah, D., Ouattara, A.K., *et al*. (2018) Occult Hepatitis B Virus Infection and Associated Genotypes Among HBsAg-Negative Subjects in Burkina Faso. *Mediterranean Journal of Hematology and Infectious Diseases*, **10**, e2018007. https://doi.org/10.4084/mjhid.2018.007

[20] Compaore, T.R., Diarra, B., Assih, M., Obiri-Yeboah, D., Soubeiga, S.T., Ouattara, A.K., *et al*. (2016) HBV/HIV Co-Infection and APOBEC3G Polymorphisms in a Population from Burkina Faso. *BMC Infectious Diseases*, **16**, Article No. 336. https://doi.org/10.1186/s12879-016-1672-2

[21] Sorgho, A.P., Martinson, J.J., Djigma, F.W., Yonli, A.T., Nagalo, B.M., Compaore, R.T., *et al*. (2018) Insights into the Interplay between KIR Gene Frequencies and Chronic HBV Infection in Burkina Faso. *Mediterranean Journal of Hematology and Infectious Diseases*, **10**, e2018060. https://doi.org/10.4084/mjhid.2018.060

[22] Nault, J.C., Mallet, M., Pilati, C., Calderaro, J., Bioulac-Sage, P., Laurent, C., *et al*. (2013) High Frequency of Telomerase Reverse-Transcriptase Promoter Somatic Mutations in Hepatocellular Carcinoma and Preneoplastic Lesions. *Nature Communications*, **4**, Article No. 2218. https://doi.org/10.1038/ncomms3218

[23] Huang, D., Wang, Z., He, X., Diplas, B.H., Yang, R., Killela, P.J., *et al*. (2015) Recurrent TERT Promoter Mutations Identified in a Large-Scale Study of Multiple Tumour Types Are Associated with Increased TERT Expression and Telomerase Activation. *European Journal of Cancer*, **51**, 969-976. https://doi.org/10.1016/j.ejca.2015.03.010

[24] Barger, C.J., Suwala, A.K., Soczek, K.M., Wang, A.S., Kim, M.Y., Hong, C., *et al*. (2022) Conserved Features of TERT Promoter Duplications Reveal an Activation Mechanism That Mimics Hotspot Mutations in Cancer. *Nature Communications*, **13**, Article No. 5430. https://doi.org/10.1038/s41467-022-33099-x

[25] Yuan, X., Dai, M. and Xu, D. (2020) TERT Promoter Mutations and GABP Transcription Factors in Carcinogenesis: More Foes than Friends. *Cancer Letters*, **493**, 1-9. https://doi.org/10.1016/j.canlet.2020.07.003

[26] Yuan, X., Larsson, C. and Xu, D. (2019) Mechanisms Underlying the Activation of TERT Transcription and Telomerase Activity in Human Cancer: Old Actors and New Players. *Oncogene*, **38**, 6172-6183. https://doi.org/10.1038/s41388-019-0872-9

[27] Yasir, S., Thompson, S., Chen, Z.E., Knudson, R., Knutson, D., Kloft-Nelson, S., *et al*. (2023) Alternative Lengthening of Telomeres in Primary Hepatic Neoplasms. *Human Pathology*, **131**, 79-86. https://doi.org/10.1016/j.humpath.2022.11.003