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Association Between DEPDC5 and PNPLA3 Variants and the Risk of Hepatocellular Carcinoma in HCV-Infected Patients in Egypt

Osama M. Roshdy[1,2], Mai M. El-Daly[3], Mohamed Gamal Mohamed[4], Mohamed Abdel-Hamid[5], Mohamed I. Zanaty[6]*

[1] Department of Molecular Biology, Nasser Institute for Research and Treatment, Ministry of Health, Cairo, Egypt
[2] Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt
[3] Department of Clinical Pathology, National Liver Institute, Menoufia University, Shebin El-Kom, Egypt
[4] Internal Medicine Department, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt
[5] Department of Microbiology, Faculty of Medicine, Minia University, Minia, Egypt
[6] Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt

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Abstract:

Hepatocellular carcinoma (HCC) is the most prevalent and life-threatening form of liver cancer worldwide. In Egypt, HCC ranks as the second most common cancer in men and the sixth most common in women. This study investigated the association between DEPDC5 and PNPLA3 variants and the risk of developing HCC. One hundred HCC patients related to HCV infection and 100 healthy controls were enrolled. Single nucleotide polymorphisms (SNPs) for DEPDC5 (rs1012068) and PNPLA3 (rs738409) were analyzed using real-time PCR. The DEPDC5 variants were significantly associated with the development of HCC. Mutant and heterozygous genotypes of DEPDC5 were significantly associated with HCC (OR = 15.98 [95% CI: 5.53-46.13], $p < 0.001$; OR = 6.04 [95% CI: 3.08-11.84], $p < 0.001$, respectively). However, the PNPLA3 polymorphism was not associated with HCC risk. Nevertheless, the PNPLA3 SNP was significantly correlated with HCC in individuals with elevated AFP levels ($p = 0.026$). These findings suggest that the DEPDC5 could serve as an indicator for diagnosing the progression of liver diseases to HCC in HCV patients in Egypt. Additionally, PNPLA3 polymorphisms may play a role in the progression and severity of HCC, especially in cases with elevated AFP levels.

Keywords: HCC, HCV, DEPDC5, PNPLA3, Egypt.

Introduction

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer, accounting for more than 90% of cases. HCC affects approximately 85% of individuals with cirrhosis and is currently the sixth most common cancer worldwide. HCC is the second leading cause of cancer-related deaths among men, following lung cancer. HCC has a five-year survival rate of only 18%, which ranks second only to pancreatic cancer in terms of poor prognosis. Major risk causes of HCC include viral hepatitis B, hepatitis C, and alcoholic liver disease [1]. Hepatitis C virus (HCV) infection is a leading cause of cirrhosis worldwide, resulting in significant societal and economic burdens. HCV infects approximately 170 million people globally (about 3% of the population), putting them at increased risk for cirrhosis and

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hepatocellular carcinoma. Recent studies have noted an increase in the proportion of HCV-related cirrhosis [2].

Egypt has faced a high prevalence of HCV infection for many years, once having the highest rates in the world. The disease posed a major public health challenge, impacting millions of Egyptians and causing significant economic and social issues. In the early 2000s, it was estimated that around 10% of the Egyptian population was infected with HCV. However, in recent years, with the introduction of direct-acting antiviral medications, the country has made substantial strides in addressing this public health crisis. Egypt successfully developed a model of care for HCV management and became the first country to eliminate hepatitis C. This achievement set a precedent for other countries, thanks to a combination of innovative health initiatives and political commitment. In 2023, Egypt became the first country to meet the World Health Organization's programmatic requirements for reducing hepatitis C incidence and mortality to levels near disease eradication, attaining the "gold tier" designation on the path to disease elimination [3,4].

Genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) linked to susceptibility to HCC. While these findings are intriguing, using SNPs to predict HCC risk poses practical challenges. Firstly, the results of these studies can vary among different ethnic groups. More importantly, each susceptibility SNP identified so far has an odds ratio of less than 1.5, which is considered the threshold for significance [5]. The significance of viral infections in the development of HCC is an important scientific topic that is gaining increasing attention. Current understanding of the molecular mechanisms underlying cancer suggests that SNPs are critical in tumor growth [6]. Recently, variants of DEPDC5 (rs1012068) in Japanese individuals and variants of PNPLA3 (rs738409) in European individuals have been associated with HCC [7].

A genome-wide association study (GWAS), utilizing high-throughput genomic technology, identified an intronic single nucleotide polymorphism (SNP) in the DEPDC5 (Dishevelled, Egl-10, and Pleckstrin domain-containing 5) gene region, which was strongly linked to the risk of HCC progression in Japanese patients with HCV. In 2011, a GWAS discovered a common variant in the DEPDC5 locus on chromosome 22, which affected the susceptibility of individuals with chronic HCV infection in Japan to developing HCC [8]. The DEPDC5 gene is located on chromosome 22 at 22q12.3 and encodes a cytoplasmic protein that has recently been shown to play a critical role in focal epilepsy, a neurological disorder [9].

The Patatin-like phospholipase domain-containing 3 (PNPLA3), commonly referred to as adiponectin, encodes a transmembrane protein composed of 481 amino acids. This protein is expressed on the membranes of hepatocytes and plays a role in lipid metabolism and the regulation of inflammatory mediators [10, 11]. PNPLA3 is located on the long arm of human chromosome 22 (22q.13.31) and is part of the Patatin-like phospholipase family. It is predominantly expressed in the liver and adipose tissue [12]. Research has shown that PNPLA3 is independently associated with the accumulation of fat (steatosis) and fibrosis in various liver diseases. It also increases the risk of developing HCC in patients with cirrhosis, particularly those with a background of fatty liver conditions [13].

1. Patients and Methods

2.1 Patients:

This case-control study included a total of 200 participants, comprising 100 hepatocellular carcinoma (HCC) patients from the National Cancer Institute and 100 healthy individuals. Samples were collected between January 2017 and December 2018, with ethical approval obtained from the Cairo University Ethics Committee.

2.2. Samples Collection:

Venous blood samples were collected from cases and controls, all aged 18 years or older, using EDTA vacutainer tubes. The samples were centrifuged to separate the plasma and buffy coat, which were then aliquoted and stored at −80°C until testing.

1.3 Methodology

All samples were tested for HCV antibodies (HCV-Ab) and hepatitis B surface antigen (HBsAg) to exclude co-infection with hepatitis B. Additionally, alpha-fetoprotein (AFP) levels were measured using the microparticle enzyme immunoassay (MEIA) AxSYM® (EIA, Abbott Laboratories, USA), following the manufacturer's instructions. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), and albumin (ALB) levels were assessed using the Beckman CX4 chemistry analyzer (NY, USA). Hemoglobin (HB) and platelet counts were measured using the Sysmex XT-1800i (Japan).

RNA was extracted from plasma using the QIAamp Viral RNA Kit (Qiagen, Santa Clarita, USA) for in-house RT-PCR to detect HCV, based on the method described by [14]. Genomic DNA was isolated from the buffy coat of all samples using the QiAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was amplified to detect genetic polymorphisms of DEPDC5 (rs1012068) [7], and PNPLA3 (rs738409)[15, 16]. Allelic discrimination probes for each gene were labeled with FAM or VIC fluorescent dyes and analyzed using a real-time PCR system (Applied Biosystems, Foster City, CA, USA).

The PCR reaction was carried out using a TaqMan Universal Master Mix (Applied Biosystems) at a 1X primer/probe concentration, in a 96-well format with a total volume of 25 µl containing 20 ng of genomic DNA. The reaction conditions included an initial hold at 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1.5 minutes. Fluorescence intensity was measured in each cycle, generating an amplification plot through the real-time PCR machine. Allelic discrimination was automatically evaluated using SDS Software v2.3 (USA).

2.4 Statistical analyses

The statistical software for the social sciences was used to undertake data statistical analysis (SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA). The number of cases (%) for categorical variables was recorded, and Pearson's chi-square ($\chi 2$) test or Fisher's exact test, if suitable, was used for comparison. The non-parametric Mann-Whitney U test or the Kruskal-Wallis test was used when comparing continuous variables that were not normally distributed. Continuous variables were reported as median (interquartile range, IQR: 25th quartile to 75th quartile or minimum–maximum as applicable). In the bivariate analysis, we included a set of characteristics (age, gender, degree of HCC, and etiology). A univariate and multivariate logistic regression analysis was used to account for any potential known confounders. We contrasted the wild type with the heterozygous and mutant genotypes in the analysis.

2. Results

3.1 Demographic and Clinical Characteristics of the Case-Control Populations

The demographic and clinical characteristics of the HCC patients (n = 100) and the control group (n = 100) are summarized in Table 1. No statistically significant difference in gender was observed between the cases and controls (p = 0.626). All HCC cases tested positive for HCV-Ab and HCV RNA, while all healthy controls were negative. Additionally, both groups were negative for HBsAg. The levels of AFP, ALT, AST, and TBIL were significantly higher in the HCC cases compared to the controls. Conversely, ALB levels, platelet counts, and HB concentrations were significantly lower in the HCC cases than in the control group.

Table 1: Demographic Characteristics of the study subjects

Variable	Control (n=100)	HCC (n=100)	*p-value*
Age	55.0(47.25-60.00)	58.5(51.00-64.00)	0.002*
Gender: Male Female	 76(76%) 24(24%)	 73(73%) 27(27%)	0.626
AFP (ng/ml)	3.77(2.26-12.85)	441.30(20.30-1276.75)	<0.0001*
ALT (IU/L)	16.00(11.25-21.00)	49.50(26.00-74.00)	<0.0001*
AST (IU/L)	15.50(12.00-19.00)	49.00(33.00-69.75)	<0.0001*
TBIL (mg/dL)	0.50(0.40-0.70)	1.80(1.30-2.48)	<0.0001*
DBIL (mg/dL)	0.10(0.10-0.10)	1.00(0.50-1.60)	<0.0001*
ALB (g/dL)	4.10(3.83-4.40)	3.10(2.90-3.40)	<0.0001*
HB (g/dL)	13.50(12.80-14.10)	11.05(10.20-12.00)	<0.0001*
Platelets (103/µL)	224.50(199.00-266.00)	118.00(95.75-148.75)	<0.0001*

3.2 DEPDC5 and PNPLA3 and HCC risk according to the severity and etiologies

In our investigation, we examined the relationship between DEPDC5 in HCC patients and the control group. The genotyping results for DEPDC5 showed GG as the mutant genotype, TG as the heterozygous genotype, and TT as the wild type (Figure 1). We found that 27% of HCC patients had the GG genotype, compared to 5% in the control group, indicating an association between DEPDC5 and HCC risk (OR = 15.98 [CI95%: 5.53-46.13], p < 0.001*). Additionally, 49% of HCC patients had the TG genotype, compared to 24% in the control group, showing an association with HCC risk (OR = 6.04 [CI95%: 3.08-11.84], p < 0.001*). The wild-type TT genotype was present in 24% of HCC patients, compared to 71% in the control group, further indicating an association with HCC risk (Table 2).

The genotyping results for PNPLA3, with mutant genotype (GG), heterozygous genotype (CG), and wild type (CC), are displayed in Figure 1. The distribution of genotypes in HCC patients and controls showed no significant difference. The frequency of the GG genotype was 6% in HCC patients and 7% in controls (p = 0.705, OR = 0.80 [CI95%: 0.25-2.53], p = 0.610). For the CG genotype, the frequency was 33% in HCC patients and 36% in controls, indicating no association with HCC risk (OR = 0.86 [CI95%: 0.47-1.55], p = 0.610). The wild type (CC) genotype, used as a reference, had a frequency of 61% in HCC patients and 57% in controls, as shown in Table 2.

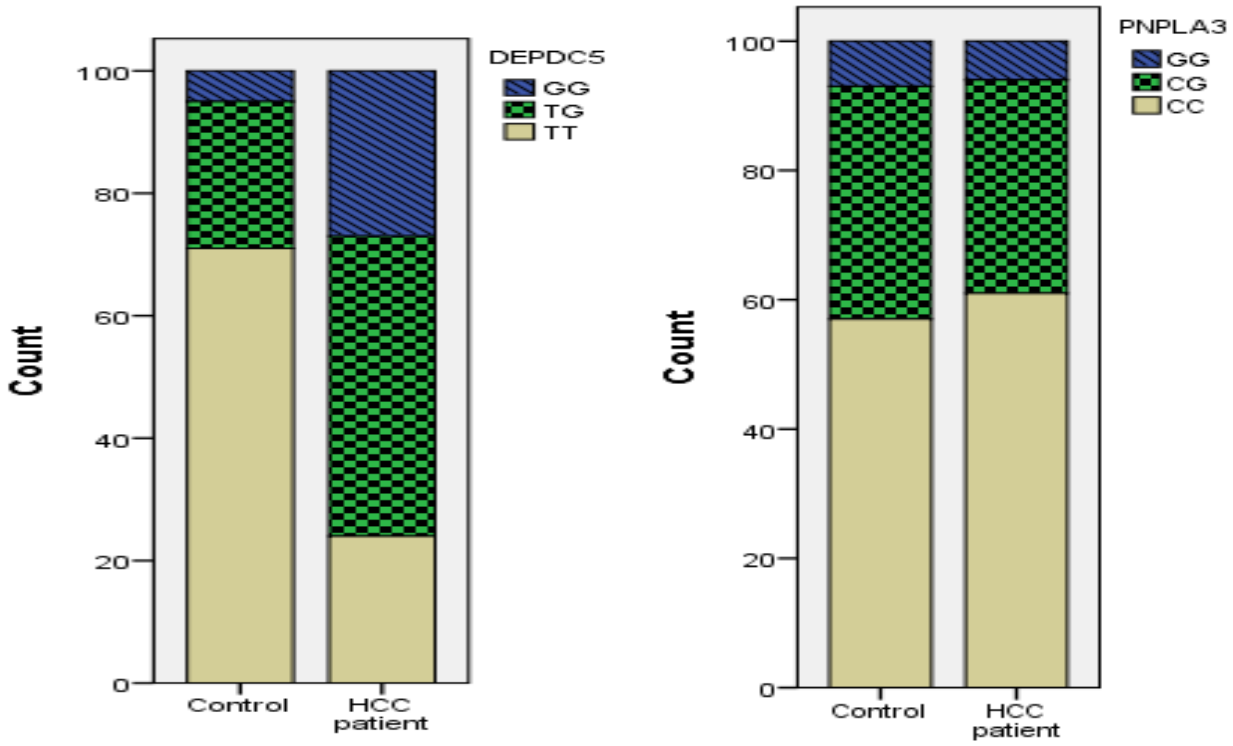

Figure 1: Genotypes of DEPDC5, and PNPLA3 in control HCC.

Table 2: Genotype of DEPDC5 and PNPLA3 in HCC cases and control group

SNP	Univariate Logistic Regression Analysis			Multivariate Logistic Regression Analysis		
	OR	CI	*p-value*	OR	CI	*p-value*
DEPDC5						
GG	15.98	5.53-46.13	<0.001*	16.13	5.57-46.68	<0.001*
TG	6.04	3.08-11.84	<0.001*	6.13	3.11-12.10	<0.001*
TT	**Reference**			**Reference**		
PNPLA3						
GG	0.80	0.25-2.53	0.705	0.66	0.18-2.42	0.529
CG	0.86	0.47-1.55	0.610	0.99	0.50-1.97	0.981
CC	**Reference**			**Reference**		

3.3 Comparison of Demographic and Clinical Characteristics with Autophagy-related SNP

Table 3 illustrates the classification of patients as high or low expressers of autophagy-related genes based on their median expression values.

Regarding the DEPDC5 gene, the difference in genotype distribution between males and females in the wild type (TT) and the mutant or heterozygous genotype groups (GG, TG) was not statistically significant (p=0.063). This indicates no apparent link between gender and the DEPDC5 genotype. Additionally, there was no significant age difference between the groups (p=0.64), suggesting that age does not influence the distribution of DEPDC5 genotypes. Notably, individuals with the mutant genotype exhibited significantly higher levels of AFP compared to the other group (p<0.001*), implying a strong association between DEPDC5 mutations and elevated AFP levels. Furthermore, a significant relationship was found between the HCC stage and DEPDC5 genotypes (p<0.001*), with the mutant genotype correlating with more advanced stages of HCC.

For the PNPLA3 gene, there was no statistically significant difference between males and females in the wild type (CC) and the mutant or heterozygous genotype groups (CG, GG) (p=0.109). Moreover, age did not significantly correlate with PNPLA3 genotypes (p=0.838). However, there was a significant difference regarding AFP levels between the genotypes (p=0.024), suggesting that PNPLA3 may have a moderate association with AFP levels. Regarding HCC classification, no statistically significant difference was observed between PNPLA3 genotypes and HCC stages (p=0.898), indicating no clear association between PNPLA3 and HCC classification.

3.4 Expression of Autophagy-related SNP, based on AFP Levels and HCC Grade

DEPDC5 gene AFP Levels: Individuals with the wild type (TT) had significantly lower AFP levels compared to those with the mutant genotype (GG), with a p-value of less than 0.001.

HCC Classification: The wild-type (TT) individuals also had a higher likelihood of exhibiting a lower HCC grade (I+II) compared to the mutant genotype group, again with a significant p-value (p < 0.001*).

PNPLA3 gene and AFP Levels: A statistically significant difference (p = 0.047) was observed in AFP levels between the wild-type and mutant genotypes, suggesting a possible relationship between PNPLA3 and AFP. However, no significant difference (p = 0.289) was found between PNPLA3 genotypes and HCC grade, indicating no substantial link between PNPLA3 and HCC progression.

3.5 Chi-square Test for SNPs and Clinicopathological Features of HCC Patients

Table 5 illustrates the analysis comparing wild-type genotypes to mutant and heterozygous genotypes. In DEPDC5 Patients with mutant and heterozygous genotypes showed significantly higher levels of AFP compared to those with the wild-type genotypes (p = 0.001*).

DEPDC5 with HCC Classification: Individuals with the mutant and heterozygous genotypes were more likely to be classified with advanced stages of HCC (grades III-IV) than those with the wild-type genotypes (p < 0.001*). PNPLA3 Gene and AFP Levels: There were significant differences in AFP levels among the PNPLA3 genotypes (p = 0.024). In the HCC classification, no significant relationship was found between PNPLA3 genotypes and the stages of HCC (p = 0.558).

Table 3: Comparison of demographic and clinical characteristics with autophagy-related SNP

Characteristic	DEPDC5			PNPLA3		
	Entire series of patients (n=100)			Entire series of patients (n=100)		
	Wild type (n=24)	Mutant and heterozygous genotype (n= 76)	*p*-value	Wild type (n=61)	Mutant and heterozygous genotype (n= 39)	*p*-value
Gender: Male Female	 14(58.33) 10(41.67)	 59(77.63) 17(22.37)	0.063	 48(78.69) 13(21.31)	 25(64.1) 14(35.9)	0.109
Age (years): Low High	 11(45.83) 13(54.17)	 39(51.32) 37(48.68)	0.640	 31(50.82) 30(49.18)	 19(48.72) 20(51.28)	0.838
AFP (ng/ml): Low High	 19(79.17) 5(20.83)	 31(40.79) 45(59.21)	<0.001*	 36(59.02) 25(40.98)	 14(35.9) 25(64.1)	0.024*
HCC classification: I + II vs. III + IV	14(58.33) 4(16.67) 5(20.83) 1(4.17)	9(11.84) 13(17.11) 22(28.95) 32(42.11)	<0.001*	13(21.31) 10(16.39) 18(29.51) 20(32.79)	10(25.64) 7(17.95) 9(23.08) 13(33.33)	0.898
ALT (IU/L): Low High	 19(79.17) 5(20.83)	 31(40.79) 45(59.21)	0.001*	 28(45.9) 33(54.1)	 22(56.41) 17(43.59)	0.305
AST (IU/L): Low High	 19(79.17) 5(20.83)	 33(43.42) 43(56.58)	0.002*	 32(52.46) 29(47.54)	 20(51.28) 19(48.72)	0.909
DBIL (mg/dL): Low High	 17(70.83) 7(29.17)	 36(47.37) 40(52.63)	0.045*	 34(55.74) 27(44.26)	 19(48.72) 20(51.28)	0.493
TBIL (mg/dL): Low High	 16(66.67) 8(33.33)	 35(46.05) 41(53.95)	0.078	 34(55.74) 27(44.26)	 17(43.59) 22(56.41)	0.236
ALB (g/dL): Low High	 6(25) 18(75)	 44(57.89) 32(42.11)	0.005*	 31(50.82) 30(49.18)	 19(48.72) 20(51.28)	0.838
HB (g/dL): Low High	 12(50) 12(50)	 45(59.21) 31(40.79)	0.427	 33(54.1) 28(45.9)	 24(61.54) 15(38.46)	0.464
Platelets ($10^3/\mu L$): Low High	 5(20.83) 19(79.17)	 45(59.21) 31(40.79)	0.001*	 29(47.54) 32(52.46)	 21(53.85) 18(46.15)	0.539

Qualitative data are represented as case numbers (%), whereas quantitative data is represented as median (range or interquartile range, IQR: 25th quartile to 75th quartile) if non-normally distributed. *Indicates a statistical significance.

Table 4: Expression of Autophagy-related SNP according to AFP and HCC grade

SNP	Genotype	AFP Low N (%)	AFP High N (%)	*p*-value	HCC classification (I+II) N (%)	HCC classification (III+IV) N (%)	*p*-value
DEPDC5	TT	19(38%)	5(10%)	<0.001*	18(45%)	6(10%)	<0.001*
	TG	25(50%)	24(48%)		22(55%)	27(45%)	
	GG	6(12%)	21(42%)		0(0%)	27(45%)	
PNPLA3	CC	36(72%)	25(50%)	0.047*	23(57.5%)	38(63.33%)	0.289
	CG	13(26%)	20(40%)		16(40%)	17(28.33%)	
	GG	1(2%)	5(10%)		1(2.5%)	5(8.33%)	

- **DEPDC5:** TT (wild type), TG (heterozygous genotype), GG (mutant genotype)
- **PNPLA3:** CC (wild type), CG (heterozygous genotype), GG (mutant genotype)

Table 5. Genotypes of SNPs (wild-type vs mutant and heterozygous genotypes) and clinic-pathologic features of HCC

SNP	Number	Serum AFP levels, ng/mL			Grade of HCC (stage).		
		Low N (%)	High N (%)	*p-value*	I-II N (%)	III-IV N (%)	*p-value*
DEPDC5							
GG&TG	76	31(40.8%)	45(59.2%)	0.001*	22(28.9%)	54(71.1%)	<0.001*
TT	24	19(79.2%)	5(20.8%)		18(75.0%)	6(25.0%)	
PNPLA3							
GG&CG	39	14(35.9%)	25(64.1%)	0.024*	17(43.6%)	22(56.4%)	0.558
CC	61	36(59.0%)	25(41.0%)		23(37.7%)	38(62.3%)	

3.6 The relationship between the SNPs of DEPDC5 and PNPLA3 and the molecular and pathological characteristics in HCC patients

Table 6 shows the Logistic Regression of Clinical Characteristics and SNPs Associated with HCC Occurrence. This analysis compares wild-type genotypes to mutant and heterozygous genotypes. We employed logistic regression models to calculate odds ratios (OR) with 95% confidence intervals, along with corresponding p-values for each SNP site and other clinical characteristics, using SPSS. We conducted a single-factor regression that included the following variables: gender, age, AFP, HCC grade, HCV PCR, HCV-Ab, HBsAg, ALT, AST, DBIL, TBIL, ALB, HB, platelets, as well as the DEPDC5 and PNPLA3 mutant genotypes compared to their wild-type and heterozygous counterparts. The analysis indicates that the DEPDC5 gene significantly influences AFP levels, with the wild type associated with lower levels (OR = 5.52, p = 0.002). Regarding HCC classification, the wild type is notably correlated with lower HCC stages (I and II) compared to the mutant genotype (OR = 2.71, p < 0.001).

In logistic multivariate regression for DEPDC5, significant associations were found solely between HCC grade and AST levels. The wild-type (CC) genotype of the PNPLA3 gene was connected to lower AFP levels (OR = 2.57, p = 0.026). However, there was no significant relationship between PNPLA3 and the HCC stage (p = 0.558), suggesting that PNPLA3 has a minor impact on HCC categorization.

Table 6: Logistic regression of clinical characteristics and SNPs with HCC occurrence

Variable	DEPDC5			PNPLA3		
	OR	95% CI (Lower-Upper)	*p*-value	OR	95% CI (Lower-Upper)	*p*-value
Univariate logistic regression analysis						
Gender, male vs. female	0.40	0.15-1.07	0.068	2.07	0.84-5.07	0.112
Age (years), low vs. high	0.80	0.32-2.02	0.640	1.09	0.49-2.43	0.838
AFP (ng/ml), low vs. high	5.52	1.86-16.34	0.002*	2.57	1.12-5.9	0.026
HCC classification: (I+II) vs. (III +IV)	2.71	1.61-4.58	<0.001*	0.89	0.59-1.33	0.558
ALT (IU/L), low vs. high	5.52	1.86-16.34	0.002*	0.66	0.29-1.47	0.306
AST (IU/L)**,** low vs. high	4.95	1.67-14.65	0.004*	1.05	0.47-2.34	0.909
DBIL (mg/dL)**,** low vs. high	2.70	1-7.25	0.049*	1.33	0.59-2.97	0.493
TBIL (mg/dL) low vs. high	2.34	0.9-6.13	0.083	1.63	0.73-3.66	0.237
ALB (g/dL), low vs. high	0.24	0.09-0.68	0.007*	1.09	0.49-2.43	0.838
HB (g/dL), low vs. high	0.69	0.27-1.73	0.428	0.74	0.32-1.67	0.464
Platelets (10^3/μL)**,** low vs. high	0.18	0.06-0.54	0.002*	0.78	0.35-1.74	0.539

Logistic regression models were used to calculate odds ratios (95% confidence interval) and corresponding p-values for each SNP site and HCC occurrence.

3. Discussion

This study found that biochemical markers such as AFP, ALT, AST, DBIL, and TBIL were significantly higher in HCC patients compared to the control group. These findings align with recent research [17,18], which reported that these markers are closely associated with HCC progression. Elevated levels of these markers indicate liver dysfunction and more advanced disease stages. Dhanasekaran and his colleagues [19] emphasized the importance of ALT and AST in evaluating liver function and the progression of HCC. Their research showed that these markers were significantly elevated in the advanced stages of HCC, supporting our findings of higher ALT and AST levels in HCC patients.

In contrast, our study showed a marked reduction in ALB levels among patients, reinforcing the notion that lower ALB levels indicate deteriorating health in HCC patients. This is consistent with [20], those who reported that reduced albumin levels were associated with more aggressive tumor characteristics and elevated AFP levels.

Sterling and his colleagues [21] demonstrated that AFP levels rise significantly with the progression of HCC, making AFP a sensitive biomarker for assessing disease stages. This is in line with our findings, which revealed that higher AFP levels were correlated with more advanced stages of HCC in patients with DEPDC5 mutations. The clear relationship between elevated AFP levels and the severity of liver cancer has been well documented. Similarly, Trevisani and his colleagues [22] highlighted the importance of AFP as a key biomarker for identifying patients requiring more intensive medical care, echoing our observation of higher AFP levels in individuals with DEPDC5 mutations.

Despite its limitations, AFP remains the most used biomarker for HCC. In recent decades, several novel HCC biomarkers have been identified [5,23]. Our study found that AFP levels were significantly higher in patients with the mutant DEPDC5 genotype (GG) compared to those with the wild type (TT), consistent with previous research linking elevated AFP levels to increased HCC risk and progression [24].

Recent evidence suggests that two common genetic polymorphisms in the DEPDC5 gene, rs1012068, and rs5998152, may be linked to an increased risk of HCC, particularly in individuals chronically infected with HCV or HBV. However, these findings have not been consistently replicated due to variations in sample sizes or differences in HCC etiology [25], reported that mutations in the DEPDC5 gene are associated with a higher risk of HCC, with patients carrying the GG mutation showing elevated AFP levels and faster disease progression, indicating DEPDC5's role in liver cancer development.

Our findings are in line with [6, 26], which focused on the relationship between DEPDC5 mutations and advanced HCC stages. Their study confirmed that individuals with DEPDC5 mutations were more likely to develop advanced-stage HCC (stages III and IV), consistent with our observation that DEPDC5 mutations are linked to more severe disease progression. Furthermore, Wang and his colleagues [27] explored DEPDC5's role in regulating autophagy, a critical process for cell survival and cancer progression. Their results indicated that DEPDC5 mutations disrupt autophagy, contributing to uncontrolled cancer cell growth. This supports our conclusion that DEPDC5 mutations are associated with more aggressive HCC. Our study suggests that the DEPDC5 gene plays a significant role in increasing HCC risk. The mutant GG genotype was strongly associated with a higher likelihood of developing HCC (OR=15.98, CI=5.53-46.13, $p<0.001$), supporting findings by [8], those who identified a strong link between DEPDC5 mutations and liver cancer severity. However, some earlier studies have shown conflicting results, with DEPDC5 showing limited or no statistical significance in certain populations [7]. This suggests that genetic variations may have different effects

depending on population characteristics or environmental factors.

Some studies have suggested a weak link between PNPLA3 and elevated AFP levels. While these studies acknowledge a relationship between PNPLA3 and AFP, they indicate that this relationship may be complex and influenced by multiple factors, such as disease progression. [26, 28], However, our results confirm that this link is statistically significant. Specifically, PNPLA3 mutations significantly affect AFP levels, and HCC patients with the GG genotype had higher AFP levels [29].

The potential association between the PNPLA3 (rs738409) polymorphism and HCC risk in patients with HCV-related cirrhosis remains debated. In our case-control study, the link between this variant and HCC risk was either minimal or absent. Yang and his colleagues [30] found that PNPLA3 mutations increase the risk of liver diseases like cirrhosis and HCC, especially in individuals with obesity or non-alcoholic fatty liver disease (NAFLD). However, this contrasts with our findings, which showed no significant connection between PNPLA3 and HCC risk. In our study, there were no significant differences in the genotype distribution of PNPLA3 between HCC patients and controls (p=0.705), aligning with previous research that found no strong association between PNPLA3 mutations and HCC risk [7, 30], These findings suggest that DEPDC5 may play a more significant role in HCC levels and disease progression than PNPLA3.

Conclusion

The findings indicate that the DEPDC5 gene significantly influences AFP levels and HCC classification, highlighting a strong association with HCC risk and progression. In contrast, the PNPLA3 gene shows a more limited effect, with only modest relevance to AFP levels and no clear connection to HCC classification. This suggests that DEPDC5 may play a more critical role in HCC development compared to PNPLA3.

Conflict of Interest: All authors declare that they have no conflicts of interest.

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