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Nascency of Physiopathological Activation by The Effect of Genomic Single Nucleotide Exchange Factor in The *PNPLA3* rs738409 Genotype of Patients with Hepatocellular Carcinoma

Anıl Delik[1]*, Yakup Ülger[2]

[1] Cukurova University, Faculty of Science and Literature, Department of Biology, Adana, Türkiye
[2] Cukurova University, Faculty of Medicine, Department of Gastroenterology, Adana, Türkiye
*anildelik@gmail.com
* Orcid No: 0000-0002-6443-9392

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Abstract

Background Patatin-like Phospholipase Domain-Containing 3 (*PNPLA3*) rs738409 is a genetic variant that is associated with an increased risk of developing hepatocellular carcinoma (HCC) in patients with chronic liver disease. This functional mechanism may cause liver cancer by altering protein function without affecting gene expression. Our aim in this study is to investigate the potential effect of *PNPLA3* polymorphism on HCC development and to report its results. Material and Methodology A case-control study was designed involving 224 diagnosed and pathologically confirmed patients with HCC. Four groups were formed as ([HBV] n = 110, [HCV] n = 38, [other etiologies] n = 76) and 62 healthy controls. *PNPLA3* genotyping in patients diagnosed with HCC was concluded by DNA isolation from blood samples. *PNPLA3* rs738409 variant was genotyped in RT PCR device with Taq Man allelic separation test designed by the manufacturers according to protocols. The C nucleotide and G nucleotide were detected in VIC; FAM hydrolysis probes were used for genotyping and binding. SPSS program was used for statistical analysis. Results The *PNPLA3* genotypes were determined for the groups of HBV-related HCC, HCV-related HCC, other etiologies-related HCC, and control. The HBV-related HCC group had CC (n = 58), CG (n = 36), and GG (n = 16) genotypes. The HCV-related HCC group had CC (n = 22), CG (n = 9), and GG (n = 7) genotypes. The other etiologies-related HCC group had CC (n = 35), CG (n = 26), and GG (n = 15) genotypes. The control group had CC (n = 36), CG (n = 13), and GG (n = 13) genotypes. Conclusions *PNPLA3* rs738409 is an inherited risk factor for HCC development in chronic liver disease. Our study found that the GG genotype can directly activate liver cancer in other etiology groups. According to our findings, we think that *PNPLA3* polymorphism can be used as a biomarker in the development of HCC due to other etiologies group.

Keywords: Adiponutrin, Chronic Liver Disease, Cirrhosis, Genetic, Genotyping

1. Introduction

Hepatocellular carcinoma (HCC) is the most lethal cancer of the liver and ranks among the top causes of cancer-related deaths worldwide. [1]. According to the data in 2015, it was reported that there were 900,000 liver carcinogenesis cases worldwide [2,3]. Liver cancer, with a death / incidence rate of 0.95, constitutes one of the deadliest cancers in general. The etiology of HCC is well defined by studies [4], with the most important risk factors being chronic hepatitis B virus (HBV), nonalcoholic fatty liver disease (NAFLD), chronic hepatitis C virus (HCV), metabolic syndromes and alcohol intake. The association between HCC genetic susceptibility and higher risk of HCC in HBV and HCV-infected patients in several studies conducted in Asia, supports the role of genetic susceptibility in liver cancer [5,6]. In addition to environmental factors, the functions of genetic and epigenetic mechanisms are inevitable in the development of liver cancer, which has very high mortality rates [7] and can provide important information in explaining the variations observed in individuals susceptible to HCC development among high-risk populations. The link between various genome studies performed in HCC patients and HCC-*PNPLA3* is noted in studies [8].

Protein 3 (adiponutrin), which contains the patatin-like phospholipase domain, is a protein of 481 amino acids

that is most highly expressed in hepatocytes [9]. This SNP triggers increased hepatocellular triglyceride accumulation and is associated with the development of HCC [10]. *PNPLA3* increases hepatocellular lipid accumulation to higher levels by affecting the enzymatic hydrolysis of triglycerides. Due to the chemical structure of methionine, its long side chain greatly reduces the catalytic activity of the enzyme despite the functional catalytic couple [11]. The damaged *PNPLA3* protein accumulates on hepatic lipid droplets and paves the way for the development of HCC due to its decreased lipolytic activity [12]. In a genetic study conducted in a population of 2503 patients with HCC development on a cirrhotic basis, it was revealed that the *PNPLA3* variant is a very important risk factor. [13]. In studies conducted, the emergence of HCC proliferation in the development of HCV-related cirrhosis is associated with the *PNPLA3* variant [14]. Therefore, we hypothesize that the *PNPLA3* genetic variation (rs738409: C>G) can be a pre-diagnosis non-invasive biomarker for liver cancer.

2. Materials and Methods

2.1. Study Design

A total of 224 patients with HCC who applied to the Department of Gastroenterology and 62 controls were included in this study. Ethics committee approval was obtained for the study and informed consent forms were obtained from all patients. HCV, HBV infective, other etiologies (such as, alcohol intake, NAFLD, metabolic disease) and control group. Control group was formed from the population without liver disease, over 18 years old, no smoking, no alcohol, normal liver enzyme levels. Ultrasonography and liver function tests of healthy controls were determined as normal values. Diagnosis of HCC patients was made using ultrasonography, computed tomography, and tumor biopsy. The category of other etiologies included autoimmune liver disease, steatohepatitis (alcoholic, NASH), cryptogenic cirrhosis, Budd Chiari syndrome, Wilson's disease and hereditary tyrosinemia.

2.2. Determination of *PNPLA3* Single Nucleotide Polymorphism (SNP)

Primers specially designed for this study were used for *PNPLA3* rs738409 (to provide amplification). One of the probes is attached to the C nucleotide and the other to the G nucleotide. RT-PCR endpoint analysis was used to detect mutant variants.

2.3. Genotyping

For our study, genomic DNA was obtained and isolation from whole blood was provided using a DNA isolation kit (DNA kit, Germany, Hidden, QIAGEN). *PNPLA3* rs738409 variant was genotyped in RT PCR device with Taq Man allelic separation test designed by the manufacturers according to protocols. The amplification primers used in our study were synthesized by Thermo Fischer using standard phosphoramide chemistry and fluorescent dye (victoria green fluorescent [VIC]), and 6-carboxyfluorescein (fluorescein amidites [FAM]) labeled probes were purified by reverse-phase high-performance liquid chromatography (HPLC). The primer sequences used for the *PNPLA3* rs738409 polymorphism were designed as Forward primer: 5′-GAGGGTGTATGTTAGTTCCCCGT-3′, Reverse primer: 5′-AGCACACTTCAGAGGCCCC-3′. The C nucleotide and G nucleotide were detected in VIC; FAM hydrolysis probes were used for genotyping and binding. One of the probes binds to the C nucleotide, while the other binds to the G nucleotide. Endpoint analysis uses this difference to distinguish between wild-type and mutant types.

3.4. Statistical analysis

Statistical Package Software for Social Science, version 21 SPSS program was used for statistical analysis. Kolmogorov-Smirnovv's test was used for normal distribution. Chi-square test, Student's t-test, Mann-Whitney U test, ANOVA and Kruskal-Wallis tests were used to analyze the data obtained in our study.

3. Results and Discussion

3.1. Demographic, laboratory data and genetic polymorphism

The main draft of the study is the relationship and comparison between *PNPLA3* genotype and HCC (HBV, HCV and other etiologies). Patients with HCC (n = 224) a mean age of 62.4 years old, with 78.6% of male; the main etiologies of underling liver disease were HBV (n = 110), HCV (n = 38), other etiologies (n = 76) and 88.8% of the patients had cirrhosis. HBV related (CC = 58, 53.3%, CG = 36, 32.7%, GG = 16, 14%), HCV related (CC = 22, 57.9%, CG = 9, 23.7%, GG = 7, 18.4%), Others etiologies (CC = 35, 46.1%, CG = 26, 34.2%, GG = 15, 19.7%), control (CC = 36, 58%, CG = 13, 21.3%, GG = 13, 21.3%) respectively, (p = 0.49) (Table 1).

Laboratory results were examined according to the etiology distribution and parameters with significant differences were specified; Mean and standard deviation for total cholesterol HCC (143 ± 47), HBV related (154.3 ± 43), HCV related (109.8 ± 28), other etiologies (141.4 ± 58) (p = 0.008). Glucose for HCC (119 ± 64), HBV related (110 ± 40), HCV related (162 ± 114), other etiologies (107 ± 41) respectively (p = 0.006). All other results and p values are shown in Table 2. AFP <400 ng/ml overall HCC (n = 132, 69.5 %), HBV related (n = 74, 70.5%), HCV related (n = 23, 67.6%), other etiologies (n = 35. 68.6%), AFP > 400 ng/ml overall

HCC (n = 58, 30.5 %), HBV related (n = 31, 29.5%), HCV related (n = 11, 32.4%), other etiologies (n = 16. 31.4%), respectively (p = 0.94). Cirrhosis according to *PNPLA3* genotypes in HCC, with cirrhosis patients CC genotype (n = 90, 54.5%), CG genotype (n = 52, 31.5%), GG genotype (n = 23, 13.9%), without cirrhosis patients CC genotype (n = 7. 35%), CG genotype (n = 7, 35%), GG genotype (n = 6, 30%), (p = 0.11) (Table 3). The G allele of rs738409 was significantly associated with increased HCC susceptibility (odds ratio [OR] = 1.25, 95% confidence interval [CI] = 1.04–1.50, p = 0.018). GG genotype of rs738409 was also associated with higher HCC risk (OR = 1.59, 95% CI = 1.06–2.39, p = 0.024). CG genotype of rs738409 showed a trend toward increased HCC risk (OR = 1.016, 95% CI = 0.99–1.03, p = 0.11). GG genotype of rs738409 was associated with elevated HCC susceptibility (OR = 1.018, 95% CI = 1.00–1.03, p = 0.03). The CG+GG genotypes of rs738409 were significantly linked to HCC risk (OR = 1.021, 95% CI = 1.00–1.03, p = 0.01). CC+GG genotypes of rs738409 were associated with increased HCC susceptibility (OR = 1.003, 95% CI = 0.98–1.01, p = 0.03). *PNPLA3* rs738409 variant appears to play a role in HCC susceptibility. These findings contribute to our understanding of genetic factors influencing liver cancer risk (Table 4).

Table 1. Patatin like phospholipase domain contaning 3 (*PNPLA3*) genotype rates in hepatocellular carcinoma (HCC) patients according to etiological distribution

Characteristics	**CC Genotype**	**CG Genotype**	**GG Genotype**	***P* value**
HBV, *(n,%)*	58 (53.3)	36 (32.7)	16 (14)	
HCV *(n,%)*	22 (57.9)	9 (23.7)	7 (18.4)	
Other Etiology *(n,%)*	35 (46.1)	26 (34.2)	15 (19.7)	0.49
Control *(n,%)*	36 (58)	13 (21)	13 (21)	
Overall *(n,%)*	151 (52.8)	84 (29.3)	51 (17.9)	

HBV: Hepatitis B, HCV: Hepatitis C

Table 2. Inıtial presentation clinical outcome 224 patients with hepatocellular carcinoma (HCC) according to etiologies.

Characteristics	**Overall HCC (*n*= 224)**	**HBV (*n*=110)**	**HCV (*n*= 38)**	**Other Etiologys (*n*=76)**	***P* value**
Age *(years),mean ± SD*	62.4 ± 11	61 ± 10.5	67.1 ± 7.5	62 ± 1.9	0.01
Gender					0.31
Female (*n,%*)	48 (21.4)	19 (17.3)	9 (23.7)	20 (26.3)	
Male (*n,%*)	176 (78.6)	91 (87.7)	29 (76.3)	56 (73.7)	
HBG (*kg/m^2), mean ± SD*	12.1 ± 2.1	12.2 ± 1.9	11.4 ± 1.8	12.2 ± 2.6	0.3
TC (*mg/dl), mean ± SD*	143 ± 47	154.3 ± 43	109.8 ± 28	141.4 ± 58	0.008
LDL (*mg/dL), mean±SD*	87.1 ± 41	94.2. ± 40	64.3 ± 23.5	89.3 ± 51.5	0.06
TG (*mg/dL), mean ± SD*	102 ± 58.5	110.7 ± 64	83.5 ± 64	95.6 ± 55.1	0.28
HDL *(mg/dL),mean ±SD*	35.2 ± 19.6	38..2 ± 24	28.7 ± 9.8	33.8 ± 11.5	0.28
HCT *mean ± SD*	34.9 ± 6.1	35.12 ± 6.7	33.74 ± 4.5	35.5 ± 6	0.41
PLT *x1000/m^3, mean ± SD*	145.4 ± 95	138.7 ± 79	132.5 ± 86	167.4 ± 123	0.13
Ferritin (*U/L), mean ± SD*	376 ± 458	388 ± 480	353 ± 363	365 ± 484	0.93
Glucose*mean ± SD*	119 ± 64	110 ± 40	162 ± 114	107 ± 41	0.006
Creatinin, *mean ± SD*	0.96 ± 0.4	0.95 ± 0.46	1.1 ± 0.6	0.81 ± 0.3	0.45
HbA1c, *mean ± SD*	6.25 ± 1.4	6 ± 1.1	6.8 ± 2.2	N.A.	0.39
TP, *mean ± SD*	6.9 ± 1.1	7 ± 0.7	7.2 ± 0.5	6.1 ± 2.3	0.13
Albumin *gr/dl, mean ± SD*	3.03 ± 0.6	3.01 ± 0.6	3.02 ± 0.6	3.07 ± 0.5	0.87
PT, *mean ± SD*	16.1 ± 5.5	16.1 ± 6	16.4 ± 4.6	16 ± 4.8	0.93
Smoking					0.10
Yes (*n,%*)	94 (47.7)	57 (51.8)	12 (33.3)	25 (47.2)	
No (*n,%*)	104 (52.3)	53 (48.2)	23 (63.7)	28 (52.8)	
INR, *mean ± SD*	1.33 ± 0.2	1.32 ± 0.2	1.35 ± 0.2	1.34 ± 0.3	0.80
CRP, *mean ± SD*	27.8 ± 40	30.9 ± 43	17.4 ± 24	28.4 ± 41.1	0.30

AFP (n=190)					0.94
<400 *ng/ml, (n.%)*	132 (69.5)	74 (70.5)	23 (67.6)	35 (68.6)	
>400 *ng/ml*, (*n,%*)	58 (30.5)	31 (29.5)	11 (32.4)	16 (31.4)	
MELD, *mean ± SD*	12.65 ± 4.9	12.86 ± 5.4	12.64 ± 4.5	12.14 ± 4.2	0.80
Tümör diameter	6.22 ± 4	5.6 ± 3.2	7 ± 5.9	6.7 ± 3	0.13
Cirhosis					0.24
Yes, (*n,%*)	167 (88.8)	89 (86.4)	32 (97)	46 (88.5)	
No, (*n,%*)	21 (11.2)	14 (13.6)	1 (3)	6 (11.5)	
Vascular ınvasion					0.96
Yes, (*n,%*)	62 (34.4)	33 (34)	11 (33.3)	18 (36)	
No, (*n,%*)	118 (65.6)	64 (66)	22 (67.7)	32 (64)	

HGM, hemoglubin, LDL: Light dansisite lipoprotein, TG. Trigiliserit, HDL: High dansisite lipoprotein, GGT: Gama glutamil transpherase, TC: Total colesterol, T.B: Total bilirubin, PLT: platelet, CRP: Creaktive protein, AFP: alfa feto protein, MELD: Model for end stage Liver disease, SD: Standart devialation, PT; protrombin, N.A.: not available

Table 3. Distrubitıon of *PNPLA3* polymorphism (CC, CG, GG) in hepatocelluler carcinoma (HCC)

Characteristics	**CC Genotype (*n*=115)**	**CG Genotype (*n*=71)**	**GG Genotype (*n*=37)**	***P* value**
Age *(years),mean ± SD*	62.1 ± 11	63.1 ± 9.1	61.9 ± 12.4	0.82
Gender				0.44
Female (*n,%*)	26 (54.2)	17 (35.4)	5 (10.4)	
Male (*n,%*)	88 (51.2)	53 (30.8)	31 (18)	
HBG (*kg/m^2), mean ± SD*	12.3 ± 2.1	11.8 ± 2.3	12.5 ± 1.9	0.46
TC (*mg/dl*), *mean ± SD*	151.4 ± 49	137.5 ± 49	129.1 ± 37	0.30
LDL (*mg/dL*), *mean±SD*	94.5 ± 46	80.9 ± 39	79.6 ± 33.5	0.39
TG (*mg/dL*), *mean ± SD*	101.4 ± 50	114.1 ± 75	80.2 ± 31	0.25
HDL *(mg/dL),mean ±SD*	35.4 ± 26	35.6 ± 15	34.5 ± 9.5	0.98
HCT *mean ± SD*	35.8 ± 5.8	33.4 ± 6.9	35.7 ± 5.3	0.04
PLT *x1000/m^3*, *mean ± SD*	147.7 ± 94	151.2 ± 97	124 ± 97	0.43
Ferritin (*U/L*), *mean ± SD*	356.5 ± 473	411 ± 446	374 ± 461	0.84
Glucose*mean ± SD*	116.8 ± 45	109.5 ± 38.5	151.7 ± 129	0.11
Creatinin, *mean ± SD*	0.79 ± 0.18	0.95 ± 0.25	1.21 ± 0.8	0.03
HbA1c, *mean ± SD*	6.03 ± 0.65	6.9 ± 1.7	4.47 ± 0.18	0.06
TP, *mean ± SD*	6.92 ± 0.71	6.91 ± 1.72	4.47 ± 0.18	0.99
Albumin *gr/dl*, *mean ± SD*	3.08 ± 0.64	3.04 ± 0.71	2.88 ± 0.50	0.39
PT, *mean ± SD*	15.59 ± 4.53	17.14 ± 7.3	15.4 ± 2.7	0.19
Smoking				0.61
Yes (*n,%*)	53 (57.6)	25 (27.2)	14 (15.2)	
No (*n,%*)	49 (48)	34 (33.3)	19 (18.6)	
INR, *mean ± SD*	1.3 ± 0.3	1.36 ± 0.2	1.31 ± 0.2	0.53
CRP, *mean ± SD*	23.9 ± 33	33.4 ± 51	25.7 ± 34	0.43
AFP (n=190)				0.014
<400 *ng/ml, (n.%)*	72 (55.8)	42 (32.6)	15 (11.6)	
>400 *ng/ml*, (*n,%*)	28 (49.1)	16 (28.1)	13(22.8)	
MELD, *mean ± SD*	12.54 ± 5.2	12.6 ± 45	12 ± 4.7	0.89
Tümör diameter, *mean ± SD*	6.57 ± 4.24	5.73 ± 3.75	6.06 ± 4.09	0.45
Cirhosis				0.11
Yes, (*n,%*)	90 (54.5)	52 (31.5)	23 (13.9)	
No, (*n,%*)	7 (35)	7 (35)	6 (30)	

Vascular ınvasion				0.70
Yes, (*n,%*)	31 (51.7)	21 (35)	8 (13.3)	
No, (*n,%*)	62 (53)	35 (29.9)	20 (17.1)	

LDL: Light Dansisite lipoprotein, TG. Trigiliserit, HDL: High dansisite lipoprotein,TC: Total colesterol, T.B Total bilirubin, GGT: Gama glutamil transpherase, TC: Total colesterol, PLT: platelet, CRP: Creactive protein, AFP: alfa feto protein, MELD: Model for end stage Liver disease, SD: Standart devialation,HGM, hemoglubin, PT; protrombin

Table 4. The *PNPLA3* genotypes and allele frequencies in HCC and control group

***PNPLA3* alleles**	**HCC n=76 (%)**	**Control n=62 (%)**	**P value**	**OR (%95 CI)**
Allel				
C	96 (63,1)	83 (66,9)		1.00 (reference)
G	56 (36,9)	41 (33,1)	0.018	1.25 (0.04-1,50)
Codominant				
CC	35 (46)	34 (54,8)		1.00 (reference)
CG	26 (34,2)	15 (24,2)	0.11	1.016 (0.99-1.03)
GG	15 (19,8)	13 (21)	0.024	1.59 (1.06-2,39)
Dominat				
GG	15 (19,7)	13 (21)	0.03	1.018 (1.00-1.03)
CC+CG	61 (80,3)	49 (79)		1.00 (reference)
Recessive				
CC	35 (46)	34 (54,8)		1.00 (reference)
CG+GG	41 (54)	28 (45,1)	0.01	1.021 (1.00-1.03)
Overdominant				
CC+GG	50 (65,8)	47 (75,8)		1.00 (reference)
CG	26 (34,2)	15 (24,2)	0.03	1.003 (0.98-1.01)

HCC: hepatocellular carcinoma

There are not enough studies to define the effective role of *PNPLA3* in the development of HCC in cancer development. However, we can think of *PNPLA3*'s genetic susceptibility for HCC development as a natural extension of the relationship between NAFLD and *PNPLA3* genetic variation. Similarly, this is common in patients with other HCC risk factors, including fatty liver disease, HCV infection [15], diabetes mellitus, obesity [16] and alcohol use [17]. Alcohol lubrication and NAFLD create a similar pathological condition [18,19]. The rs738409 (G) variant of the *PNPLA3* gene is a major genetic factor that contributes to steatosis and fibrosis progression in various liver diseases, according to independent studies [20-23].

The prognosis of HCC patients with the GG genotype of the *PNPLA3* variant was worse than those with the CC or CG genotype. The GG genotype has a distinct prognostic impact on HCC survival compared with the main survival predictors. Patients with the *PNPLA3* GG genotype may have a higher risk of vascular invasion,

poor tumor differentiation, lymph node involvement, or other clinical factors that affect HCC prognosis, although this hypothesis lacks definitive evidence. Hassan et al. proposed that cirrhosis symptoms, such as bleeding, fluid accumulation in the abdomen, and low platelet count, make the clinical management of HCC more challenging and often force oncologists to lower the optimal dose of intensive chemotherapy or restrict surgical removal [24]. This could reduce the patients' survival by impairing their response to treatment and narrowing their effective treatment options. It is hard to assess the relationship between *PNPLA3* genotypes and the response to different treatment types separately, because of the variety of treatment methods used. In our study, the relationship between HCC *PNPLA3* was examined and CC genotype was found as 52,8%, CG genotype 29.3%, GG genotype 17.9% in all HCC patients and *PNPLA3* relation was found to be more significant in other etiology group patients including alcoholic, NAFLD group. CC genotype is 46.1%, CG genotype is 34.2%, GG genotype is 19.7%, respectively in other etiology group. The survival curve of the *PNPLA3* other etiologies group shows that patients with the GG genotype have a lower survival rate (p = 0.65). Our study is consistent with the results of Hassan et al. The relationship between *PNPLA3* and HCC was first reported to increase the prevalence of liver cancer in patients with rs738409 (GG) homozygous HCV-associated liver disease [25]. This research involved a small subset of patients in a case-control study [26]. Chronic inflammation caused by the complex interplay of oxidative stress, liver fat accumulation, and insulin resistance is a hallmark of liver diseases related to HCV. This inflammation creates a pro-oncogenic microenvironment that favors tumor growth. These traits are intrinsically connected, and they mutually enhance each other in the process of alcoholic carcinogenesis [27]. In addition, it has been reported that some HCV-dependent protein is directly linked to tumorigenesis and accelerates carcinogenic processes [28]. This report suggests that *PNPLA3* has a weaker effect on HCC risk in patients with HCV-associated cirrhosis compared with alcoholics. In our study, CC genotype 57.9%, CG genotype 23.7%, GG genotype 18.4% were found in the relationship between *PNPLA3*-HCV. It was determined as the lowest group as the GG genotype ratio. In our study, it was determined that the *PNPLA3* gene rs738409 C> G polymorphism increased the risk of HCC development due to other etiology (NASH) and the GG genotype increased OR:1.59 (1.00-1.04) times more risk and was statistically significant (p= 0.024) (Table 4). Studies have shown that *PNPLA3* polymorphism is an independent factor associated with serum AFP. Moritou et al. investigated the patient characteristics related to the *PNPLA3* 148 M genotype [29]. In our study, serum AFP level was the factor that we found significantly related to *PNPLA3* genotype (p=0.014). Although it was stated in some studies that AFP level could be a significant biomarker for the diagnosis of HCC patients, Caviglia et al. reported that they could not reach this result stated in their study. According to our result, we think that the effective use of AFP level with other serum biomarkers may create a useful result for the diagnosis of liver cancer. In a case-control study involving 200 patient populations, it was reported that the combination of AFP level and exosomal hnRNPH1 mRNA markers increased the diagnostic value for liver cancer by 0.891 (95% CI = 0.873-0.939, p = 0.005). The sensitivity of the study was 87.5% and the specificity was 84.8% [13,24]. In our study, the AUC value was determined as 0.51 without combining AFP with other biomarkers (95% CI = 0.407-0.618, p = 0.82). The *PNPLA3* variant causes an increase in triglyceride accumulation in liver cells, and together it causes non-activation of the protein hydrolase enzyme and activation of hepatic stellate cells, triggering the development of liver cancer [30].

4. Conclusion

In conclusion, limitations of our study include that it was conducted in a single center, did not include patients from different geographical regions and ethnic groups, and long-term follow-up data are incomplete, making it difficult to assess the long-term effects of the *PNPLA3* rs738409 genotype. Despite these limitations, our study contains important genetic data that may be needed in the diagnosis of HCC. A strong diagnostic marker can be created by integrating the *PNPLA3* polymorphism CG and GG genotype with other serum non-invasive biomarkers in patients with chronic liver HCC due to NAFLD and alcohol etiology.

Author Contributions

Concept – A.D., Y.Ü.; Design – A.D., Y.Ü.; Supervision – A.D., Y.Ü.; Resources – A.D., Y.Ü.; Materials – A.D., Y.Ü.; Data Collection and/or Processing – A.D., Y.Ü; Analysis and/or Interpretation – A.D.; Literature Search – A.D.; Writing Manuscript – A.D.; Critical Review – Y.Ü..; Other – Y.Ü.

Ethics

Ethics Committee approval was received at the Local Ethics Committee, Cukurova University Balcalı Hospital Faculty of Medicine Ethics Committee, dated 08.03.2024, code of ethics committee: 33, meeting number: 142

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