RESEARCH ARTICLE

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Association of Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha Coding Variants with Hepatocellular Carcinoma Risk in the Moroccan Population: A Case-Control Study

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common primary malignancy. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) plays a crucial role in regulating the biogenesis of mitochondria. We aimed to assess the association between *PPARGC1A* polymorphisms and HCC risk in a Moroccan population. **Methods:** In this case-control study, 147 patients with HCC and 147 controls without pre-existing liver disease were matched for ethnicity. TaqMan SNP allelic discrimination assays were used for genotyping of *PPARGC1A* rs8192678 and rs12640088 polymorphisms. **Results:** The result revealed that individuals with the GA/AA genotypes for rs8192678 had a significantly higher risk of HCC compared to those with the GG genotype (OR=6.68; P<0.0001, and OR=9.78; P<0.0001, respectively). In particular, the A allele of rs8192678 was over-represented in HCC patients compared to controls (40% versus 12%, P<0.0001, respectively). With respect to *PPARGC1A* rs12640088 variant, two genetic models (codominant and dominant) were tested to explore any potential variations in the distribution of SNP A>C among HCC cases and control subjects group. Overall, no significant association between rs12640088 and HCC was found (P>0.05). Interestingly, a significantly higher level of aspartate aminotransferase was observed in HCC patients with GG-GA genotypes (280 IU/L) compared to those with GG genotype (164 IU/L) at rs8192678 (P=0.0019). **Conclusion:** Our results suggest that the *PPARGC1A* rs8192678 polymorphism is associated with an increased risk of HCC in Moroccan population and may serve as a prognostic marker for liver cancer.

Keywords: Susceptibility- PGC1- SNP- liver cancer- biomarker

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Introduction

With approximately 906,000 new cases and 830.000 deaths in 2020, hepatocellular carcinoma (HCC) is the sixth most common human primary malignancy and the third leading cause of cancer-related mortality worldwide (Ferlay et al., 2015; Kim and El-Serag, 2019; Sung et al., 2021). HCC is characterized by a masculine predilection with the fifth highest incidence in men versus the ninth rank in women (Sim H-W and Knox, 2018). Hepatitis B virus (HBV), hepatitis C virus (HCV), non-alcoholic steatohepatitis (NASH), alcoholic liver disease, aflatoxins, and smoking are the main risk factors for HCC. Additionally, there are a few other minor causes such as anabolic steroids, thorium dioxide, vinyl chloride, and certain genetic predispositions like geneticlike genetic haemochromatosis (El-Serag and Rudolph, 2007; Bosetti et al., 2014).

Direct and indirect mechanisms triggered by viral infections promote the development of HCC. Chronic viral infections of the liver cause inflammation and lesions, eventually progressing to cirrhosis, which is a precursor to HCC (Font-Burgada et al., 2015; Shin et al., 2016). However, only a subset of infected individuals progress to HCC during their lifetime, suggesting that other agents, such as environmental exposures (ethanol, tobacco, mycotoxins) as well as genetics factors, may interact and modulate HCC development (Walker et al., 2018). Despite therapeutic advances in the management of HCV and HBV infections, global HCC mortality ratesmortality rates

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continue to rise. In 2018 alone, there were 600.000 deaths attributed to HCC, and this number is projected to surpass one million by 2030 (Cronin et al., 2018; WHO., 2020).

The peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PPARGC1A*) gene is located on chromosome 4, specifically4, specifically on cytoband 4p15.1, spanning approximately 110 Kb. It is predominantly expressed in metabolically active tissues, such as the liver, kidneys, brain, and skeletal muscle. The protein encoded by the *PPARGC1A* gene, known as PGC-1a, serves as a transcriptional coactivator with a crucial role in regulating different metabolic pathways. These pathways include adaptive thermogenesis, fatty Kelly and Scarpulla, 2004acid oxidation, gluconeogenesis, lipogenesis (Soyal et al., 2006), and biogenesis of mitochondria, , all of which contribute to energy metabolism regulation(Kelly and Scarpulla, 2004).

PPARGC1A interacts with about twenty nuclear factors, including PPARγ, HNF4, NRF1/2, and estrogen-related receptor-α (ERRα) , among others (Mastropasqua et al., 2018). In hepatocytes, it stimulates the transcription of HNF-4a and forkhead box O1 (FOXO1), thus regulating the expression of essential gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), pyruvate dehydrogenase kinase isoenzyme 4 (PDHK4), and glucose-6-phosphatase (G6Pase) (Finck and Kelly, 2006). Dysfunction or deregulations of this transcriptional coactivator ,coactivator, , often characterized by overexpression, has been associated with the onset and progression of obesity and related metabolic disorders (Ramos-Lopez et al., 2018).

A recent study suggested that *PPARGC1A* acted as a tumor suppressor in HCC (Hsu et al., 2016). However, there is still a lack of studies investigating the role of single-nucleotide polymorphisms (SNPs) within the *PPARGC1A* gene in HCC susceptibility.

In this study, we sought to investigate the potential relationship between two specific *PPARGC1A* gene polymorphisms, namely rs-8192678 G > A (Gly482Ser) located at position + 1564 in exon 8 and rs12640088 A>C in the intron (Vimaleswaran et al., 2005) and the development of hepatocellular carcinoma in the Moroccan population. These SNPs have previously been found to be associated with several human diseases, such as type 2 diabetes and obesity, both of which are known as predisposing factors for HCC (Barroso et al., 2006; Villegas et al., 2014).

Materials and Methods

Patients and Controls

This case-control study enrolled a total of 294 consenting participants, including both patients and controls, between January 2004 and August 2021. The study was conducted at two neighbouring healthcare facilities located in Casablanca, namely the Department of Medicine B at the Ibn Rochd University Hospital and the Department of Medicine "A" Ibn-Sina at the University Hospital in Rabat, as well as the Medical Centre for Biology of the Institut Pasteur of Morocco. The study protocol was approved by the Ethics Committees of the Faculty of Medicine of Casablanca (2004) and the Ibn Rochd University Hospital Center (2018//426) and was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Out of the total participants, there were 147 controls and 147 patients diagnosed with HCC. Each participant completed a structured questionnaire that covered demographics, medical history, lifestyle, and other relevant characteristics. Peripheral blood samples were collected from the patients using tubes containing EDTA. The HCC cases and controls were matched based on ethnicity.

The recruitment process for HCC patients has been described previously in studies by Ezzikouri et al., (2007, 2008) and Ezzikouri et al., (2018). Briefly, the diagnosis of HCC was established through a CT or MRI scan showing features consistent with HCC, abnormal levels of serum alpha-fetoprotein, and, when possible, histological examination using hematoxylin-eosin staining, following the guidelines of the American Association for the Study of the Liver (AASLD) for HCC (Jordi Bruix and Morris Sherman, 2011). The liver histology of the patients was assessed non-invasively using FibroTest-ActiTest, which combines various biomarkers such as α-2-macroglobulin, GGT, apolipoprotein A1, haptoglobin, total bilirubin, age, and sex (Bio predictive, Paris, France).

Controls were enrolled during the same period as the patients were required to have normal blood cell counts, normal levels of aminotransferase (ALT and AST), and negative serological markers for hepatitis. They also had no pre-existing liver disease. Participants with confirmed co-infection with human immunodeficiency virus or the presence of autoimmune liver disease were excluded from this study.

Serological, Biochemical, and Molecular Virology Analyses

Serological markers were approved for HBsAg, anti-HCV (Axsym/Architect, Abbott Diagnostics, Wiesbaden Delkenheim, Germany), and anti-HIV (Genscreen Ag/Ab HIV Ultra, Biorad, Marnes La Coquette, France). Levels of plasma HCV-RNA were measured by qPCR using COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics, Germany). HCV RNA level below the detection threshold (15 IU/mL) was registered as negative for HCV RNA. ALT, AST, gamma-glutamyl transferase (GGT), bilirubin, total cholesterol, high-density lipoprotein (HDL), low- density lipoprotein (LDL), and triglycerides were measured using Architect ci4100 (Abbott Diagnostics, Wiesbaden Delkenheim, Germany).

Genomic DNA Isolation and PPARGC1A Polymorphisms Genotyping

Genomic DNA was isolated from the peripheral blood mononuclear cells as described previously. Genotyping for *PPARGC1A rs8192678* and rs12640088 was performed using the TaqMan SNP genotyping allelic discrimination method (Thermo Fisher Scientific, Foster City, CA, USA) using a Light Cycler® 480 Real-Time PCR System (Roche Diagnostics, Manheim, Germany). Genomic DNA was amplified in a 10 µL reaction volume containing 1X

SensiFAST Genotyping Lo-Rox Mix (Bioline, London, UK), SNP Genotyping Assay, genomic DNA (20 ng), and completed with DNase-free water. SNP genotyping was done in duplicate. The results were found to be 100% concordant.

Statistical Analysis

Categorical variables are represented as frequencies (%), while quantitative variables were presented as either median with interquartile range or mean ± standard deviation. The appropriate statistical tests, such as the Mann–Whitney U test and Student's t-test, were used as deemed appropriate for the analysis. The Hardy–Weinberg equilibrium (HWE) was estimated using the SNP-HWE program. Genotype frequencies were compared under dominant, co-dominant, and recessive models of inheritance using the Chi-2 test. The results were expressed as p-values, odds ratio (OR), and 95% confidence interval (CI). The ORs were adjusted for biologically relevant covariates and potential confounders associated with the risk of liver disease progression, including age and gender. Haplotype construction was done using SNPStats software (Xavier Sole´ et al., 2006).

All statistical procedures were performed using R software for Windows and GraphPad PRISM (GraphPad Software, San Diego, CA, USA). P-value<0.05 was considered to indicate a significant difference. All statistical tests were two-sided.

Results

Clinical and Demographical Features

A total of 294 individuals, comprising 147 controls and 147 HCC cases, were enrolled in this case-control study. The demographic, biochemical, and viral characteristics of the study population are summarized in Table 1. Notably, the HCC subjects were found to be older than the controls (P<0.001), with a mean age of 63, whereas the controls had a mean age of 55.8 years. Consistent with expectations, HCC patients exhibited higher serum levels of aminotransferases compared to controls (p<0.0001). In addition, the creatinine level was significantly higher in the HCC group than in the control group (P<0.0001). With regard to viral infections, hepatitis C virus was identified as the main risk factor for HCC development (60.5%), followed by hepatitis B virus infection (14.2%). Among

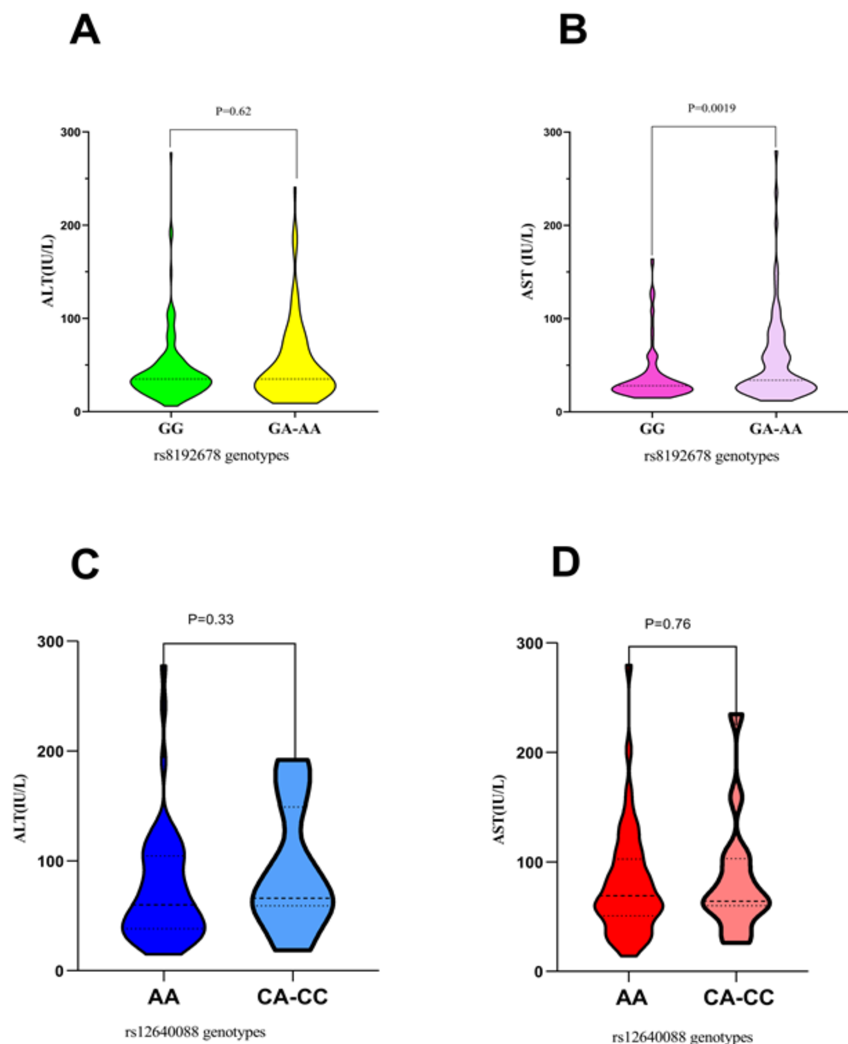

Figure 1. Association of rs819678 and rs12640088 with Liver Enzymes. (A) ALT levels according to rs819678 genotypes. (B) AST levels according to rs819678 genotypes (C) ALT levels according to rs12640088 genotypes. (D) AST levels according to rs12640088 genotypes. Data are expressed as median.

Table 1. Clinical and Demographic Characteristics of the Control Group and Patients with HCC

	Controls (N =147)	HCC (N =147)	P-value
Mean age±SD, yr.	55.78 ±12.88	63 ± 11.42	<0.001
Sex (%)			
Male	57 (38.78)	91 (61.9)	
Female	90 (61.22)	56 (38.1)	<0.001
AFP >20ng* (%)	NA	31 (79.48)	
Mean alanine aminotransferase ± SD, IU/L	35.76 ± 17.04	74.85 ± 54.42	<0.0001
Mean aspartate aminotransferase ± SD, IU/L	28.86 ± 13.80	77.38 ± 46.77	<0.0001
Mean creatinine ± SD, µmol/L	65.85 ± 15.08	90.53 ± 46.72	<0.0001
Cirrhosis (%) *	NA	66 (77.78)	_
Anti HCV+ (%)	NA	89 (60.54)	_
HBs Ag+ (%)	NA	21 (14.28)	_
Anti-HBc+ (%)	NA	16 (10.88)	_
NIDD (%)	NA	16 (10.88)	_
Cryptogeny (%)	NA	30 (20.40)	_
Median HCV viral (range), IU/L (log)	NA	5.95	_
HCV genotype (%)			
1	NA	61 (83.56)	_
2	NA	12 (16.44)	_
HBV genotype (%)			
D	NA	18 (85.72)	_
A	NA	3 (14.28)	_
Tumor diameter (cm) (%)			
<2	NA	27.72	_
2-4	NA	43.24	_
4.1-10	NA	18.91	_
Vascular invasion (%)	NA	5 (23.80)	_

HCC hepatocellular carcinoma, NA non applicable, AFP alpha fetoprotein, HBV hepatitis B virus, HCV hepatitis C virus, NIDD non insulin dependent diabetes mellitus. * Data are missing

the HCV genotypes, genotype 1 was fond in 83.5% of HCC, while genotype 2 was found in 16.43% of patients. For HBV, genotype D was observed in 86.36% of cases, while genotype A was observed in 13.63%.

Frequency of PPARGC1A Genotypes

In this case-control study conducted in a Moroccan population, 147 HCC patients and 147 controls were genotyped for *PPARGC1A* SNPs *rs8192678* and rs12640088 to evaluate their association with hepatocellular carcinoma. The minor allele frequency (MAF) for *rs8192678* in in the control group was fond to be 0.12, which is lower compared to the frequencies reported in Caucasian and Asian populations (Tai et al., 2016). On the other hand, the MAF for rs12640088 was 0.11, which aligns with the frequencies reported in Caucasian and Asian populations (Tai et al., 2016) (Table 2). This result suggests that Moroccan population exhibits allele frequencies that are relatively similar to those observed in Caucasian and Asian populations.

Association Between PPARGC1A Polymorphisms and the Risk of HCC

The impact of the two SNPs on HCC risk is presented in Table 2. Taking the GG genotype as the reference, individuals carrying the GA/AA ("A-carrier") genotypes at the *rs8192678* polymorphism had a significantly increased risk of HCC (adjusted odds ratio (OR) =6.68, 95% confidence interval (CI): 3.68–12.13; P<0.0001, and OR=9.78; 95% CI: 3.54–27.07; P<0.0001), respectively. The A allele was fond to be strongly overrepresented in HCC patients (40%) compared to controls (12 %) (P<0.0001).

Under the dominant model for *rs8192678* (AA-GA), a significant difference was observed between controls and HCC cases, with a 7.3-fold higher risk of developing HCC in those with the A-carrier genotypes compared to individuals with the GG genotype (P<0.0001). Likewise, we observed a significant difference between cases and control subjects under the recessive model (P=5e-04) (Table 2).

For *PPARGC1A* rs12640088 variant, two genetic models (Codominant and dominant) were tested to explore any potential variations in the distribution of SNP rs12640088 A>C amongst HCC cases and control group. Overall, no significant association between rs12640088

Table 2. Genotyping and Allelic Distribution of *PPARGC1A* Polymorphisms in HCC Patients and Controls, adjusted for Age and Sex

PPARGC1A	Controls n=147 (%)	HCC n=147 (%)	OR (CI 95%)	P-value
rs8192678				
G/G	117 (79.6)	51 (34.7)	1	
G/A	24 (16.3)	73 (49.7)	6.68 (3.68–12.13)	<0.0001
A/A	6 (4.1)	23 (15.7)	9.78 (3.54–27.07)	<0.0001
Dominant model				
GG	117 (79.6)	51 (34.7)	1	
AA – GA	30 (20.4)	96 (65.3)	7.26 (4.16–12.65)	<0.0001
Recessive model				
GA – GG	141 (95.9)	124 (84.3)	1	
AA	6 (4.1)	23 (15.7)	4.96 (1.84–13.34)	5.00E-04
Allele frequency				
G	0.88 ± 0.02	0.60 ± 0.02	1	
A	0.12 ± 0.02	0.40 ± 0.02	4.87 (3.17–7.42)	<0.0001
rs12640088				
A/A	115 (78.2)	117 (79.6)	1	
A/C	32 (21.8)	29 (19.7)	0.85 (0.47–1.55)	0.37
C/C	0 (0)	1 (0.7)	2.94 (0.12–73.19)	1
Dominant model				
AA	115 (78.2)	117 (79.6)	1	
CA – CC	32 (21.8)	30 (20.4)	0.89 (0.49–1.62)	0.71
Allele frequency				
A	0.89 ± 0.01	0.89 ± 0.01	1	
C	0.11 ± 0.01	0.11 ± 0.01	0.96 (0.58–1.59)	0.89

Table 3. Haplotype Analysis of *PPARGC1A* Gene Polymorphisms and Risk of HCC Adjusted for Age and Sex

rs8192678	rs12640088	Controls (%)	HCC (%)	OR (95% CI)	P-value
G	A	78.2	53.2	1	-
A	A	10.9	36.2	4.30 (2.59–7.14)	<0.0001
G	C	9.5	6.31	0.79 (0.34–1.85)	0.59
A	C	13.9	4.23	5.59 (0.91–34.23)	0.064

and HCC was found (P>0.05) (Table 2). Moreover, allelic frequencies between controls and HCC patients were not fond to be different (P>0.05).

Impact of rs8192678 and rs12640088 SNPs on liver enzymes

Next, we investigated the impact of *rs8192678* and rs12640088 variants on liver enzymes levels among HCC patients (Figure 1). For *rs8192678*, no significant difference was observed regarding ALT levels across different genotypes (P=0.62) (Figure 1A). In contrast, for *rs8192678* SNP, patients with GA/AA genotypes exhibited higher levels of AST (280 IU/L) comparing to those with GG genotype (164 IU/L) (P=0.0019) (Figure 1B). Regarding the rs12640088, no significant differences were observed in ALT and AST levels according to genotypes (P>0.05) (Figure 1C-1D).

Haplotype analysis

Haplotype analysis is often more effective than single locus analysis in detecting associations with a disease. In this regard, four possible haplotypes, G-A, A-A, A-C, and G-C were represented. Using the GA haplotype as the reference, it was fond that the A-A haplotype (OR= 4.30, 95% CI= 2.59-7.14, P<0.0001) was associated with an increased risk of hepatocarcinogenesis. However, the A-C and G-C haplotypes were not fond to be associated with the risk of liver cancer development (P>0.05) (Table 3).

Discussion

HCC is the most common primary malignancy of the liver worldwide. It arises from a combination of various risk factors, including genetic and epigenetic alterations such as TERT, TP53, and CTNNB1 promoters (Zucman-Rossi et al., 2015), exposure to certain chemicals, as well as inborn and acquired metabolic diseases. Additionally, physical mechanisms and certain

oncogenic signalling pathways have been implicated in the regulation of HCC growth and progression (Warburg effect). Previous studies have highlighted the involvement of pathways such as PI3K/AKT, JNK1, HIF-1α, and c-MYC in the regulation of aerobic glycolysis, also known as the Warburg effect, which contributes to HCC proliferation (Nie et al., 2015). Notably, cancer cells exhibit increased glucose utilization, leading to limited oxygen consumption and production of lactic acid, even in the presence of oxygen (Barroso et al., 2006; El-Serag and Rudolph, 2007; Bosetti et al., 2014; Chen et al., 2018; Cronin et al., 2018; ElFihry et al., 2020; Rosa Maria Pascale et al., 2020). However, the molecular mechanism underlying the Warburg effect and its role in hepatocarcinogenesis remain poorly understood.

In addition to its critical roles in mitochondrial regulation, *PPARGC1A* has been found to monitor lipid and glucose metabolism. Genetic variations in this gene have previously been associated with type 2 diabetes, obesity, and other related diseases (Mirzaei et al., 2012). Furthermore, *PPARGC1A* has been implicated in processes such as tumor metastasis, apoptosis, autophagy, and tumorigenesis (Mastropasqua et al., 2018).

Only a couple of studies have described the changes in *PPARGC1A* expression in human HCC tissues. Lin et al., reported that *PPARGC1A* mRNA expression was significantly increased in 16 HCC tissues compared to non-tumor tissues (Yu-Cheng Lin et al., 2013). Similarly, Schaefer et al., found higher *PPARGC1A* protein expression in 20 HCC tissues, whereas no expression was detected in non-tumor patients (Schaefer et al., 2005). However, a third study by Yu et al., showed that *PPARGC1A* protein expression was decreased in 20 HCC patient tissues compared to non-tumor livers (Yu et al., 2006). Huang et al., demonstrated in their study that *PPARGC1A* (as well as MRPL54, ZC3H13, IFIT5) was downregulated and should be considered as an onco-suppressor in HCC (Huang et al., 2020). Additionally, Liu et al., suggested that *PPARGC1A* might be a potential therapeutic target for HCC (Liu et al., 2017). Consequently, the results regarding *PPARGC1A* expression in HCC are controversial, and the role of this gene remains unclear in human HCC. In other tumor types, such as lung cancer and invasive breast cancer, *PPARGC1A* has been reported to be upregulated, promoting invasion and metastasis, which is associated with a worse prognosis (LeBleu et al., 2014; Li et al., 2017). Conversely, other studies found that *PPARGC1A* was downregulated and acted as a tumor suppressor in prostate cancer and melanoma. They demonstrated that *PPARGC1A* prevented cell proliferation, migration, and metastasis, and showed a positive correlation with better prognosis in these cancer types (Luo et al., 2016; Torrano et al., 2016).

Recently, Zhang et al., showed that another *PPARGC1A*-associated polymorphism (rs2970847 C>T) was associated with HCC risk (Zhang et al., 2018). However, there is limited data available on the association of two other SNPs located in *PPARGC1A* gene (*rs8192678* and rs12640088) with the development of HCC in patients chronically infected with hepatitis viruses outside of China. Therefore, in this case-control study, we aimed to evaluate the association of these SNPs with the development of HCC. In a previous study, we observed that *PPARGC1A rs8192678* and rs12640088 SNPs were not associated with the risk of liver disease progression in chronic hepatitis C. Nevertheless, different investigations conducted in various geo-epidemiological settings have observed a significant association between *PPARGC1A rs8192678* and several human diseases such as type 2 diabetes mellitus, obesity and hypertension (Kunej et al., 2004; Louise Ambye et al., 2004; Barroso et al., 2006; Sun et al., 2006; Zhang et al., 2007; Lagou et al., 2008; Lai et al., 2008). However, some reports about T2DM and/or insulin resistance have not confirmed these observations (Stumvoll et al., 2004; Wang et al., 2005; Nelson et al., 2007).

Regarding liver disease, there are conflicting findings on the association between *PPARGC1A* variants and non-alcoholic fatty liver disease (NAFLD). Yoneda et al., (2008) found that *PPARGC1A* variants promote susceptibility to NAFLD, while Hui et al. (2008) concluded that no such association exists, specifically for *rs8192678*. (Hui et al., 2008). In addition, Vimaleswaran et al., reported that the rs12640088 protein is associated with body fat and T2D in South Asians (Vimaleswaran et al., 2006). There is still a gap regarding the association between rs12640088 and HCC development. In our study, we also found a difference in liver enzyme levels, specifically aspartate transaminase (AST), between HCC subjects carrying different genotypes of *rs8192678*. Patients carrying the risk GA and AA genotypes had significantly higher levels of AST compared to non-carriers, which is consistent with previous findings by Lin et al. (2013), who observed higher AST and ALT levels in carriers of the risk A allele at *rs8192678* (Yu-Cheng Lin et al., 2013).

In our work, we observed that the A (Ser) allele was a risk allele, since there was a significant difference between HCC patients and controls ($P< 0.0001$). We can therefore conclude that there is an association between the Gly482Ser polymorphism and the development of HCC.

Overall, the role of *PPARGC1A* in HCC remains unclear due to conflicting results from different studies. While some studies suggest an upregulation of *PPARGC1A* in HCC tissues, others indicate downregulation and a tumor suppressor role. Additionally, the association of specific *PPARGC1A* SNPs with HCC development and liver disease progression is still being explored. Therefore, further research is needed to elucidate the precise role of *PPARGC1A* in HCC and its potential as a therapeutic target.

In conclusion, we found that PPRAGC1A *rs8192678* G>A polymorphism is associated with the development of hepatocarcinogenesis and may affect liver functions. However, we did not observe a significant association between the rs12640088 A>C polymorphism and HCC. It is important to note that the current study's limitation is the relatively small size of the cohort examined. Further should focus on enrolling larger cohorts to improve the internal and external validity of the results. Additionally,

further analyses of potential additional polymorphisms in PPRAGC1A gene will provide more insights into their role in liver cancer development. Overall, comprehensive studies elucidating the mechanistic function and involving large cohorts, especially among North African patients with NAFLD, are warranted to definitively refute or confirm the effect of the PPRAGC1A *rs8192678* G>A and rs12640088 A>C variants on the risk of development of HCC.

Author Contribution Statement

SE, SB, and PP conceived and designed the study. IT, RE, CZ, SE, and KA performed the experiments, data analysis and interpretation. MT, AB and WB helped in the collection of samples and clinical data interpretation. IT, SE, and PP wrote, did review and editing of manuscript. SE and HF contributed in supervision. All authors read and approved the final version of the manuscript.

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Research involving human and animal participants

This article contained study with human participants.

Animal and Human Rights Statement

The study was approved by the Ethics Committee of the Faculty of Medicine of Casablanca in accordance with the ethical guidelines of the Declaration of Helsinki. For study participation, written informed consent for genetic testing was obtained from all subjects.

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Conflict of interest

All authors declare that they have no conflict of interest.

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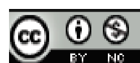

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