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ROLE OF ADIPONUTRIN GENE POLYMORPHISM IN DIAGNOSIS OF HCV RELATED HEPATOCELLULAR CARCINOMA (HCC).

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Abstract

This study evaluates the role of PNPLA3 gene polymorphism (rs738409) by PCR/ Taqmanassay in 75chronic HCV patients (50 with HCC and 25without) and 25 controls. Thetestedadi ponutrin single nucleotide polymorphism (SNP) showed that the C allele of PNPLA3 (rs738409) gene is common in all groups than G allele and higher in HCC (95%) than cirrhotic (80%) and controls (72%). Frequency of HCC was higher for patients with CC genotype (90%), than those with CG (10%) and GG genotype (0%). The study detected highly statistically significant difference between HCC and cirrhotic groups as regard CC genotype about 4.2 folds (p=0.04) and C allele (p=0.004).Also, CC genotype is more prominent in cirrhotic patients (68%) than controls group (52%) but difference not significant.

In Conclusion these results suggest that PNPLA3 (rs738409) polymorphism with CC genotype was significantly associated with liver cirrhosis and increased risk to develop, HCC in Egyptian patients with chronic hepatitis C infection.

Introduction

HCC is the most serious and dangerous complication of liver cirrhosis. It was are ported that HCC is the 5th most common cancer in men worldwide (Schutte *et al.,* 2009). Each year, HCC is diagnosed in more than half a million people worldwide (SEER Program *et al.,* 2010) and in adult men frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. In addition in adult women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death (Jemal *et al.,* 2011).

Egypt has rising morbidity and mortality rates from HCC, where the relative frequency of HCC in Egypt increased from 4.0% in 1993 to 7.3% in 2003 (Lehman and Wilson *et al.,* 2009).Chronic infection with HCV is a major risk factor for development of HCC (El-Serag *et al.,* 2002). There is estimation that about 20% of chronic viral infected cases would progress to cirrhosis and about 20% of tumors developed from cirrhotic livers (Thompson *et al.,* 2007).

Detailed analysis and characterization of molecular, genetic and epi-genetic events would

Key words: *Adiponutrin; Gene Polymorphism; chronic HCV; Hepatocellular Carcinoma.*

revolutionize early diagnosis of HCC (Marquardt *et al.,* 2012). Gene and protein expression profiling will allow better screening of different stages of HCC as well as establishment of criteria for targeted therapies (D'Alessandro *et al.,* 2013). CC genotype is more risky to develop cirrhosis than CG and GG genotypes by about 1.39 folds but without significant difference (OR=1.39, P=0.38).

Adiponutringene is expressed in the liver and the adipose tissue (Rom *et al.,* 2010). It encodes 481 amino acids protein of unclear physiological and biological function. Nevertheless, adiponutrin exhibits strong activity in lipolysis and triglyceride hydrolysis *in-vitro* (Lake *et al.,* 2005). A genome-wide association study reported that a variation in the adiponutrin gene is associated with high susceptibility to non-alcoholic fatty liver diseases (NAFLD) while susceptibility for HCC still not will established (Romeo *et al.,* 2008). Moreover, adiponutrin may influence fibrosis in patients with fatty liver (Rotman *et al.,* 2010 and Valenti *et al.,* 2011). The genetic susceptibility of adiponutrin for HCC development may be considered as a natural extension for the previously reported association between NAFLD and adiponutrin genetic variation (Czaja *et al.,* 1998).

The present study aimed to assess the role of PNPLA3gene polymorphism (rs738409) in diagnosis of currenthepatocellular carcinoma in chronic hepatitis C patients.

Subjects:

This cohort prospective study was conducted on 100 patients admitted to the Department of Hepatology, Gastroenterology and Infectious Diseases in Benha University Hospital in the period from April 2014 to December 2015. The patients were be divided to: Group I: included 50 HCV cirrhotic patients with HCC (34 males and 16 females), Group II: included 25 HCV cirrhotic patients without HCC (15 males and 10 females). All patients were anti- HCV and PCR for HCV positive, HCC was diagnosed by high Alpha feto-protein level> 400 ng/ml and Tri-phasic abdominal CT scan. The mean age of HCV cirrhotic patients with HCC was 57.32±8.51 years compared to 56.92±8.3 years for the HCV cirrhotic patients without HCC. Group III: included 25 healthy control subjects (13 males and 12 females).

The study protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Benha. A written informed consent was obtained from each participant before starting the study.

Methods:

Blood sampling:

Venous blood sample (2 ml) was obtained from each subject in sterile vaccutainer tube containing EDTA, mixed well and aliquoted into 2 sterile eppendorff tubes to be stored at -80°C for further study of gene polymorphism.

(A) Genomic DNA extraction:

DNA was extracted from 200µl blood sample; using *Purelink® Genomic DNA mini kit according to the manufacturer's instructions.

(B) Real time PCR for detection of PNPLA3 (rs738409):

Detection of PNPLA3 (rs738409) gene polymorphism was done by 5' Nuclease Taqman SNP Genotyping Assay Technology. In 20 µl reaction, genomic PCR amplification was done

**Catalog No. K1820-01 (Invitrogen, Life Technologies).*

Using *Taqman 5' allele discrimination assay for each SNP. This genotyping assays were a human single nucleotide polymorphism (SNP) assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples.

Each Taqman assay supplied for either SNP was 40X. PNPLA3 rs738409 contained sequence specific primers for both alleles (C and G) of the SNP and 2 Taqman probes; one probe labeled with VIC dyedetects the C allele and the other labeled with FAM detects the G allele.

This 40X SNP genotyping assay was diluted before real time PCR. Mixing was done to 20X working stock with 1X TE buffer. Then it was aliquoted and stored at -20^{o}C.

Amplification was done in Step one **Real Time PCR System. The following thermal cycling conditions were run during which the amount of DNA in each sample was quantified.

Statistical analysis

The clinical data were expressed as Mean and standard deviation (± SD) for quantitative data, frequency and distribution for qualitative data. Quantitative data were compared using independent Student's t-test. On the other hand, qualitative data were compared using chi square test (X2-value) and fisher exact test (FET). Logistic regression: OR (Odds Ratio) of regression: It is the expected (B) in regression; it was done to quantify how much is the predicted outcome among individuals with the independent variables compared with the cirrhotic and control groups.

The statistical package for the social sciences software (SPSS) version 16 was used. A Pvalue<0.05 was considered statistically significant while > 0.05 statistically not significant and P value <0.01 was considered highly significant in all analyses.

** (Applied Biosystem, Foster City, California, USA).*
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Results

The demographic and laboratory characteristics of the HCV patients with or without HCC and control subjects are shown in table-1. There were no statistically significant difference between HCC and cirrhotic groups as regard mean age and sex distribution, while there was statistically significant difference between studied groups as regard almost all studied parameters, despite that there was no statistically significant difference between HCC and cirrhotic groups except neganding ESR (p= 0.001), PT(p=0.002) and AFP(p= 0.001).

The SNP under investigation on the (PNPLA3) gene polymorphism (rs738409) was found in all studied groups. The distribution of different genotypes and alleles frequencies in studied groups is summarized in table-2. The C allele of PNPLA3 (rs738409) gene is common in all groups than G allele and higher in HCC (95%), than cirrhotic (80%), than control group (72%) and frequency of HCC was higher for patients with CC genotype (90%), than those with CG genotype (10%) and GG genotype (0%).

The Comparison between studied groups as regard PNPLA3 (rs738409) genotypes is showed in tables-3,4 and 5. There was highly statistically significant difference between HCC and cirrhotic groups as regard CC genotype (p=0.04) and C allele (p=0.004), while there was no significant difference between

both as regard GG genotype (p= 0.20). Also, there was highly statistically significant difference between HCC and control groups as regard CC genotype (p=0.001), but no statistically significant difference (p=0. 386) between cirrhotic and control groups as regard CC genotype.

This street compared the patients characteristics related to HCC development in table-6.Age more than 50 years, smoking, history of bleeding, Lower limb edema, presence of ascites, low platelet count, elevated serum creatinine, blood urea, AST, ALT and P.T, Also, presence of (CC) genotype and C allele of PNPLA3 (rs738409) gene had significant association with HCC development in univariate analysis. Stepwise multivariate analysis revealed that elevated AFP, presence of (CC) genotype and C Allele of PNPLA3 (rs738409) gene were significant independent predictors of HCC.

Table-1: Demographic and laboratory characteristics of all subjects.

Variables	Group I (No.50)	Group II (No.25)	Group III (No.25)	T	P values
	Mean±SD	Mean±SD	Mean±SD		
Age (years) Mean ± SD	57.32±8.51	56.92±8.3	29.04±7.22	0.19 4.24 12.68	p1=0.85 p2=0.001 p3=0.001
Sex (No,%) Male Female	 34(68%) 16(32%)	 15(60%) 10(40%)	 13(52%) 12(48%)	0.47 1.82 0.33	p1=0.49 p2=0.18 p3=0.57
PLT(c/mm^3)	87.54±45.02	80.16±24.09	274.96±38.0	1.12 17.86 24.31	p1=0. 27 p2=0.001 p3=0.001
ESR(ml/hour)	59.33±24.37	22.2±25.13	9.32±4.4	6.1 10.14 2.52	p1=0.001 p2=0.001 p3=0.015
ALT(IU/dl)	51.02±23.84	60.12±46.69	28.24±4.55	1.12 4.72 3.4	p1=0.27 p2=0.001 p3=0.001
AST(IU/dl)	62.06±26.97	67.56±38.03	29.76±6.15	0.72 5.89 4.91	p1=0.47 p2=0.001 p3=0.001
Total bilirubin (mg/dl)	4.16±6.86	3.37±1.9	1.09±0.21	0.032 3.2 5.99	P1=0.97 p2=0.002 p3=0.001
Direct bilirubin (mg/dl)	2.16±2.38	1.95±1.3	0.40±0.11	0.41 3.7 5.93	p1=0.68 p2=0.001 p3=0.001
S. albumin (g/dl)	2.72±0.64	2.6±0.43	4.6±0.33	0.79 13.81 18.57	p1=0.43 p2=0.001 p3=0.001
PT. (sec)	16.6±7.46	10.59±7.79	1.02±0.05	3.24 10.19 6.01	p1=0.002 p2=0.001 p3=0.001
AFP(ng/dl)	526.16±1588.8	23.67±19.15	1.70±1.58	1.58 1.61 5.6	p1=0.001 p2=0.001 p3=0.001

Group I: HCC group.
Group II: cirrhotic group.
Group III: control group.
T: student test.
P1: P value between group I and group II.
P2: P value between group I and group III.
P3: P value between group Π and group III.

Table-2: PNPLA3 I148M (adiponutrin) genotypes and alleles frequencies in studied groups.

Genotypes and alleles	Group I (No.50) No,%	Group II (No.25) No,%	Group III (No.25) No,%
Adiponutrinsnp			
CC	45 (90%)	17 (68%)	13 (52%)
CG	5 (10%)	6 (24%)	10 (40%)
GG	0 (0%)	2 (8%)	2 (8%)
Dominant genotypes			
CC + CG	50 (100%)	23 (92%)	23 (92%)
GG	0 (0%)	2 (8%)	2 (8%)
Recessive genotype			
CG +GG	5 (10%)	8 (32%)	12 (48%)
CC	45 (90%)	17 (68%)	13 (52%)
Allele model C	95 (95%)	40 (80%)	36 (72%)
G	5 (5%)	10 (20%)	14 (28%)

Table-3: Comparison between HCC and cirrhotic groups as regard PNPLA3 I148M genotypes.

Genotypes and alleles	Group I (No.50) No,%	Group II (No.25) No,%	FET	P value	OR(95%CI)
CC	45	17	-	-	R
CG	5	6	2.96	.085	0.45(0.24-0.86)
GG	0	2	2.1	0.148	0.27(0.18-0.41)
Dominant genotype CC + CG	50(100%)	23(92%)	1.61	0.205	1.087(0.97-.22)
GG	0(0.0)	2(8%)			
Ressive genotype CG +GG	5(10%)	8(32%)	4.2	0.04	4.24(1.22-4.77)
CC	45(90%)	17(68%)			
Allele model C	95(95%)	40(80%)	X^2=8.33	0.004	4.75(1.53-4.78)*
G	5(5%)	10(20%)			0.211(0.078-0.65)**

X^2 test =chi square test. ^=Fischer exact test.
* G genotype is the reference. ** C genotype is the reference.

Table-4: Comparison between HCC and control groups as regard PNPLA3 I148M genotypes.

Genotypes and alleles	Group I (No.50) NO,%	Group III (No.25) NO,%	FET	P value	OR (95%CI)
CC	45	13	-	-	R
CG	5	10	10.61	0.001	0.31 (0.17-0.55)
GG	0	2	2.83	0.09	0.22 (0.14-0.36)
Dominant genotype CC + CG	50 (100%)	23 (92%)	1.61	0.205	1.087 (0.97-1.22)
GG	0 (0.0)	2 (8%)			
Recessive genotype CG +GG	5 (10%)	12 (48%)	11.65	0.001	*3.15 (1.78-5.56)
CC	45 (90%)	13 (52%)			
Allele model C	95 (95%)	36 (72%)	X^2=15.94	0.001	*7.39 (2.48-21.99)
G	5 (5%)	14 (28%)			**0.135(0.05-0.40)

X^2 test =chi square test. * G genotype is the reference.
^=Fischer exact test. **C genotype is the reference.

Table-5: Comparison between cirrhotic and control groups as regard PNPLA3 I148M genotypes.

Genotypes and alleles	Group II (No=25) NO,%	Group III (No.25) NO,%	FET	P value	OR (95%CI)
CC CG GG	17 6 2	13 10 2	- $X^{2=}$1.53 ^0	- 0.216 1	R 0.69 (0.4-1.21) 0.87 (0.3-2.51)
Dominant Genotype CC + CG GG	23 (92%) 2 (8%)	23 (92%) 2 (8%)	0.0	1.0	1.0 (0.36-2.78)
Recessive Genotype CG +GG CC	8 (32%) 17 (68%)	12 (48%) 13 (52%)	0.75	0.386	1.39 (0.80-2.39)
Allele model C G	40 (80%) 10 (20%)	36 (72%) 14 (28%)	X^2=0.493	0.482	*1.56 (0.615-3.94) **0.64 (0.25-1.62)

X^2 test =chi square test. ^=Fischer exact test.
* G genotype is the reference. ** C genotype is the reference**.**

Table-6: Univariate and multivariate analysis for the significant factors related to HCC (in comparison with cirrhosis and controls).

Variables	Univariate analysis OR (95%CI)	P	Multivariate R(95%CI)	Analysis P
Age > 50 years	5.09 (2.09-12.4)	<0.001		
Smoking	2.85 (1.01-8.2)	0.046		
History of hypertension	2.19 (0.61-7.8)	0.22		
History of bleeding	11.9 (4.4-32.1)	<0.001		
Jaundice	1.4 (0.63-3.06)	0.42		
LL edema	3.08 (1.33-7.1)	0.008		
Ascites (moderate, severe)	2.28 (1.02-5.1)	0.044		
PLTs > 9000(c/mm^3)	0.37 (0.16-0.83)	0.016		
Creat> 1.2(mg/dl)	2.9 (1.18-7.06)	0.017		
Urea > 50(mg/dl)	3.4 (1.3-9.2)	0.011		
ALT > 50(IU/ml)	2.8 (1.2-6.4)	0.014		
AST > 60(IU/ml)	4.0 (1.6-9.7)	0.002		
T. bilirubin > 4(mg/dl)	1.77 (0.68-4.5)	0.23		
D. bilirubin > 2(mg/dl)	2.06 (0.83-5.1)	0.11		
Albumin< 2.5(g/dl)	2.2 (0.96-5.2)	0.059		
PT > 13(sec)	11.0 (3.9-30.6)	<0.001		
AFP > 25(ng/ml)	11.7(4.5-30.3)	<0.001	6.8(2.28-42.9)	0.002
Genotype (CC)	6.0 (2.03-17.7)	0.001	4.45(2.04-25.4)	0.006
Allele C	6.02.2-16.5	<0.001	4.81(2.1-23.9)	0.004

Discussion

HCC incidence is clearly rising comprised by the prevalence of major risk factors mainly hepatitis B virus HBV and hepatitis C virus HCV (Sene *et al.,* 2015). In Egypt, the incidence of HCC has doubled in the last 10 years and it is the second most incident and lethal cancer in men (Iyer *et al.,* 2010). It was reported that about 7.2% of chronic liver disease patients may develop HCC (El-Zayadi *et al.,* 2005). The prognosis of HCC remains poor and most patients have a 5 years survival rate of less than 5% mainly because of the late diagnosis (Chen *et al.,* 2010).

Early HCC diagnosis is feasible in only 30–60% of cases, which are optimal candidates for resection, liver transplantation or percutaneous ablation (Bruix *et al.,* 2011). Although it is obvious that development of new diagnostic modalities will significantly

increase the detection rate of HCC, there is still a need for other early detection methods. Following the genome wide association studies (GWAS) and gradually apply within success in the medical fields in last year's. Increasing number of studies have reported that genetic alterations might be used to predict the risk of various cancers (Liu *et al.,* 2013 and Hosen *et al.,* 2014). Without doubt, these findings will likely contribute to the primary prevention of cancers. Some (SNPs) might even be regarded as prognostic factors for the success (or failure) of certain chemotherapy strategies (Kim *et al.,* 2015). However, only a few genetic variants have been reproducibly demonstrated to be linked to hepato-carcinogenesis (Nahon *et al.,* 2012).

No enough data are available concerning the association of adiponutrin polymorphism with HCC in HCV Egyptian patients. Therefore, the current study aimed to evaluate the role of Adiponutrin gene polymorphism PNPLA3 (rs738409) in diagnosis of hepatocellular carcinoma in chronic hepatitis C Egyptian patients.

In this study, the mean age of patients with HCC was (57±8.5) year (ranging from 40-75 year) which was older than cirrhotic and control groups but without significant difference among HCC and cirrhotic groups (p value >0.05). This result agreed with (Abdel-Razek *et al.,* 2012) who reported in a study included 215 patients with HCC that the mean age of HCC patients was 58 year. Also, Atta *et al.,* (2008) reported in another Egyptian study including 41 HCC patients that, the mean age of HCC patients was 57± 8.4 year.

On the other hand (Tanaka *et al.,* 2008) reported that the age of HCC incidence was higher in Japan (70–79 year). This difference may be partially attributed to the difference in the risk factors distribution among Japanese patients with HCC, which was highly variable, depending on geographic region, race or ethnic group.

In the current study, HCC is presented more frequently in males than females with male to female ratio (2.12:1). This male predominance came in agreement with Keng *et al.,* (2013) who reported that the universal estimated male/female ratio of HCC is (2.5:1). Also Atta *et al.,* (2008) and Chuang *et al.,* (2009) concluded that male to female ratio in HCC was 3.6:1 and 2.4:1 respectively. On the other hand El-Shahat *et al.,* (2012) reported a non-significant difference in sex distribution between HCC patients.

High male to female liver cancer incidence rate ratios in some countries may reflect increased prevalence of known risk factors among men (Nafeh *et al.,* 2000) including higher susceptibility of males to environmental carcinogenic factors (El-Zayadi *et al.,* 2005 and Atta *et al.,* 2008) or endogenous (androgens) male hormones (Poustchi *et al.,*2010). Also, it has been reported that DNA synthetic activities are higher in male than in female cirrhotic and this might be one of the possible explanations for the gender discrepancy in HCC (Tangkijvanich *et al.,* 2004).

In the present study, there was no statistically significant difference was found between HCC group patients and cirrhotic patients regarding laboratory investigations except for ESR, PT and AFP. Prothrombin time

was significantly higher (p value <0.05) in HCC group than in cirrhotic group. These results were consistent with Hsu CY *et al.,* (2013) who stated that the liver profile tests (albumin and prothrombin time) were more frequently abnormal in HCC than in chronic hepatitis and cirrhosis. In the current study, AFP was markedly elevated in HCC group than in cirrhotic and control groups with highly statistically significant difference (P=0.001). These results were in agreement with Metwaly *et al.,* (2016) who mentioned that marked elevation of AFP level was observed in patients with HCC in comparison with healthy control subjects, patients with CHC and patients with LC. Similarly, Atta *et al.,* (2008) reported that mean value of AFP was higher in HCC group than hepatitis C and control group, and Baig *et al.,* (2009) found that, AFP is a significant marker and an indicator for hepatocellular carcinoma.

The rs738409 (SNP) of the patatin-like phospholipase domain-containing 3 (PNPLA3, adiponutrin) gene is associated with hepatic steatosis, inflammation, fibrosis and carcinogenesis in NAFLD (Romeo *et al.,* 2008, Kitamoto *et al.,* 2013 and Donati *et al.,* 2016). SuchPNPLA3 gene polymorphism has also been reported to be strongly associated with alcoholic liver disease (ALD) (Tian *et al.,* 2010) and chronic hepatitis C/B (Trépo *et al.,* 2011).

The present study investigated possible associations of a PNPLA3 gene polymorphism with development of HCC in Egyptian patients with CHC. For PNPLA3 polymorphism (rs738409 C>G) genotypes, 75 patients had CC, 21 had CG, and 4 had GG. The C allele frequency of PNPLA3 gene is common in all groups than G allele and higher in HCC (95%), than cirrhotic (80%), than control group (72%) and frequency of HCC was higher for patients with CC genotype (90%), than those with CG genotype (10%) and GG genotype (0%), although there was no statistically significant difference. Therefore, subsequent comparisons were made between patients with CC and those with CG or GG genotype.

The present study also found a significant association between rs738409 and HCC risk using multiple different genetic models: allele model, C*vs.* G: OR = 4.75, 95% CI: 1.53-14.78; dominant model, CC+ CG *vs.* GG: OR = 1.087, 95% CI: 0.97-1.22 and recessive model, CC *vs.* CG + GG: OR = 4.24, 95% CI: 1.22-14.77. These results suggest that harboring two copies of the rs738409 C variant was associated with a high risk of HCC occurrence in comparison with cirrhotic group.

Also, there is a significant association between rs738409 polymorphism and HCC risk in comparison with control group using multiple different genetic models: allele model, C*vs.* G: OR = 7.39, 95% CI: 2.48-21.99; dominant model, CC+ CG *vs.* GG: OR = 1.087, 95% CI: CI: 0.97-1.22 and recessive model, CC *vs.* CG + GG: OR =3.15, 95% CI: 1.78-5.56**.**

This comes in contrast with Trepo *et al.,* (2014). Who demonstrated that the G allele of rs738409 is a risk factor for HCC Also, a meta-analysis of (Zhang *et al.,* 2015) found a significant association between rs738409 and HCC risk in comparison with non HCC group using multiple different genetic models: allele model, G *vs.* C: OR = 2.02,

95% CI: 1.77-2.30; dominant model, GG + CG *vs.* CC: OR = 2.03, 95% CI: 1.70-2.41; recessive model, GG *vs.* CG + CC: OR = 3.56, 95% CI: 2.70-4.69.These results suggest that harboring two copies of the rs738409 G variant was associated with a high risk of HCC occurrence.

Another meta-analyses (Zhan *et al.,* 2016) showed that the effect of rs738409 G allele on liver on cogenesis was higher in alcoholic liver disease (ALD) (OR=2.55), compared to chronic hepatitis C/B (OR= 1.32). This difference may be due to small sample size of the current study, different ethnicity, variations in individual susceptibility to HCC can also clearly be attributed to complex gene-gene and gene-environmental exposure interactions (El-Serag *et al.,* 2011) and cause of cirrhosis as the current study linked to HCV but the others tudies involved multiple causes especially alcoholic cirrhosis.

The current study also demonstrated that rs738409 polymorphism with CC genotype was associated with liver cirrhosis (OR=1.96). This comes in accordance with (Nakaoka *et al.,* 2015) who demonstrated that a PNPLA3 polymorphism was associated with the progression of fibrosis to cirrhosis.

In the present study, factors possibly associated with the development of HCC were assessed by univariate regression analysis compared with non HCC groups. These factors included age > 50 years, smoking, history of bleeding, lower limbo edema, ascites, low platelet count, elevated serum creatinine, blood urea, AST, ALT and PT, (CC) genotype and C allele of adiponutrin gene. While by multivariate binary logistic regression analysis for prediction of HCC, only AFP, CC genotype and C allele of adiponutrin gene were significant predictors of HCV-related HCC (OR ratio 6.8, p= 0.002, OR ratio 4.45, p= 0.006 and OR ratio 4.81, p= 0.004, respectively).

In Japanese studies, Moritou *et al.,* (2013), reported that a PNPLA3 polymorphism was significantly associated with serum AFP level and Sato *et al.,* (2013) reported that the median time between HCV infection and the development of HCC was significantly shorter for patients with the PNPLA3 GG genotype in HCV-related HCC. A significant association was reported between aPNPLA3 polymorphism and HCC in patients with CHC (Ezzikouri *et al.,* 2014).

On the other hand other studies did not find a significant association (Nischalke *et al.,* 2011, Rembeck *et al.,* 2012 and Takeuchi *et al.,* 2013). A meta-analysis performed by Trepo *et al.,* (2014), showed that a PNPLA3 polymorphism was strongly associated with HCC, although the association was stronger in patients with alcoholic liver disease (OR = 2.20; 95% CI: 1.802.67) than in patients with CHC (OR = 1.55; 95% CI: 1.03–2.34). The mechanism underlying the association between aPNPLA3 gene polymorphism with the progression of steatosis, fibrosis, and development of HCC has not been determined. It was reported that a PNPLA3 variant promotes the synthesis of hepatic lipid because of gain of function (Kumari *et al.,* 2012). Steatosis maintained by the PNPLA3 genotype may promote the progression of fibrosis and development of HCC (Valenti *et al.,* 2012).

The current study suggested that CC genotypes are importantly involved in the genesis of HCC in ceratin HCV patients.

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