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Study of the effect of ACYP2 single nucleotide polymorphisms rs843711 and rs843706 in Egyptian patients with hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a multifactorial disease with both genetic and environmental factors contributing to its pathogenesis. ACYP2 is a gene that is related to cell differentiation, apoptosis and prevention of malignant tumors. The ACYP2 gene also affects telomere length. The aim of this study was to evaluate the association between ACYP2 single nucleotide polymorphisms (SNPs) (rs843711), and (rs843706) and incidence of HCC in Egyptian HCC patients. The study included 30 patients with HCC and 30 normal controls. Detection of ACYP2 gene SNPs rs843711, and rs843706 in all study participants was done using real time polymerase chain reaction (RT-PCR). The results showed that all participants including HCC patients and controls carried the heterozygous CA (100%) of the rs843706 SNP (p> 0.05). As for the rs843711, 3.3% of HCC patients had the homozygous TT genotype, 46.7% had the heterozygous CT genotype and 50% had the wild CC genotype, while in the control group, 60% had the heterozygous CT genotype and 40% had the wild CC genotype with no significant difference between both groups (p>0.05). We concluded that there was no association between SNPs ACYP2 rs843706 and rs843711 and occurrence of HCC.

Keywords: ACYP2 Single Nucleotide Polymorphism, Hepatocellular Carcinoma.

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Introduction

Hepatocellular carcinoma (HCC) is considered as the 6th most common cancer worldwide and the 3rd most common cause of cancer related mortality.[1] In Egypt, it is the most common cause of death from cancer. HCC is multifactorial with many risk factors including hepatitis C, hepatitis B, aflatoxins, non-alcoholic steatohepatitis, alcohol intake and iron overload.[2,3]

HCC pathogenesis is a multistep and complex process. Lesions that cause hepatitis and result in hepatic damage with the subsequent occurrence of cirrhosis can promote liver carcinogenesis. Moreover, several genetic and epigenetic changes have been associated with the molecular pathogenesis of HCC (e.g., mutations affecting p53 tumor suppressor gene and polymorphism of ACYP2 impacting telomere length) [4]

Telomere is a short structure that is located at the end of chromosomes. The main function of telomere is to maintain the integrity of chromosome and regulate the cell cycle. Mutations affecting telomere-related genes may result in subsequent loss or gain of function hence participating as a risk factor for many diseases. Abnormal telomere length has been found to be linked to increased risk of several cancers including HCC.[5]

The ACYP2 gene encodes small cytosolic acylphosphatase enzyme that is responsible for multiple membrane proteins and is involved in the regulation of intracellular Ca^{2+} homeostasis. The ACYP2 gene is also related to cell differentiation, apoptosis, and prevention of malignant tumors. Polymorphisms in ACYP2 gene may be linked to tumorigenesis. ACYP2 is an important telomere length related gene. Polymorphisms of ACYP2 gene have been associated with shorter telomere length, escape from apoptosis and promotion of carcinogenesis.[6] Studies have shown that polymorphisms of ACYP2 were associated with lung cancer,[7] colorectal cancer,[8] breast cancer [9], gastric cancer,[10] liver cancer.[11] The aim of the current study was to assess the association between ACYP2 single nucleotide gene polymorphisms (rs843711), and (rs843706) and increased incidence of HCC in Egyptians.

Subjects and Methods

The study included 60 adult subjects, 30 HCC patients and 30 normal controls. HCC patients enrolled in the study were recruited from The Department of Tropical Medicine, Ain Shams University Hospitals. HCC Patients were diagnosed based on contrast enhanced helical computed tomography or dynamic magnetic resonance irradiation.[12] Patients with previous history of other cancers, patients previously treated from HCC, patients with active liver disease and poorly compliant patients were excluded from the study.

The control group included age and sex-matched subjects with normal Aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen (BUN), creatinine levels, normal prothrombin time (PT) and International normalized ratio (INR). They were with normal blood pressure, blood glucose level and no history of any disease.

Laboratory Methods

A venous blood sample (10 ml) was collected under aseptic precautions from each participating subject. Of these, an aliquot blood sample (3 mL) was delivered into EDTA vacutainer and stored at -20°C to be used for DNA extraction and detection of ACYP2 rs 843711 and rs 843706 gene polymorphisms by the real-time polymerase chain reaction. The remaining blood sample (7 mL) was distributed between 1) Gel separating tube for assay of AST, ALT, total bilirubin, direct bilirubin, and albumin, 2) EDTA tube for complete blood count (CBC). Complete blood count was done by an automated blood counter (Sysmex XN-1000, Sysmex Corporation, Japan), according to the manufacturer's instructions. While AST, ALT, total bilirubin, direct bilirubin, and albumin were done by an automated blood chemistry analyzer (Cobas c6000, Roche diagnostics, Switzerland), according to the manufacturer's instructions.

Assay of ACYP2 polymorphisms by the RT PCR

Extraction of genomic DNA from whole blood was done using commercial kits (The GeneJET™ Whole Blood Genomic DNA Purification Mini Kit, Catalogue no K0721, Thermo Fisher Scientific Inc., USA), according to the manufacturer's instructions. Detection of ACYP2 polymorphisms (rs 843711) and (rs 843706) was performed by commercial kits (TaqMan real time PCR kit, Catalogue no 4351374, Thermo Fisher Scientific Inc., USA), according to the manufacturer's instructions.

Genotyping for ACYP2 polymorphisms (rs 843711) and (rs 843706) were performed on a real time PCR instrument (DT-lite Real Time PCR System, DNA technology, Russia), according to the manufacturer's instructions. The PCR reaction mix is demonstrated in Table 1 and the thermal cycling program is described in Table 2. The probe sequence was as follows: for (rs843706): ATGAACACAATAGTTTCCAGTCTTC[A/C] GCATCAGATAAGACCAAGTTATTCA while for (rs843711): TTAGAGCTCAGGGAACCAGTGCAAA[C/T] ACTGACACTCTGACTATTGAGTTGT.

Statistical Methods

The Statistical Package for the Social Sciences (SPSS) version 23 was used for statistical analysis. Quantitative variables are presented as mean and standard deviation for parametric data, while non-parametric variables presented as median and inter-quartile range (IQR). Qualitative data are presented as number and percentages. The Chi-square test was used to compare qualitative parameters. Quantitative data were compared using independent t-test for parametric data and Mann-Whitney test for non-parametric data. A *p*-value <0.05 was considered significant.

Table 1. The PCR Reaction mixture.

Component	Volume (µL) per reaction
TaqMan Genotyping Master Mix (2X)	10 µL
TaqMan SNP Genotyping Assay (40X)	0.5 µL
Extracted ctDNA	2 µL
DNase -free Water	7.5 µL
Total Volume per Well	20 µL

Table 2. Thermal profile of real time PCR.

Step	Temperature	Duration	Cycles
Initial heating step	95°C	10 minutes	1 cycle
Denaturation	95°C	15 seconds	40
Annealing/Extension	55 °C	60 seconds	40

Results

Demographic, clinical data and risk factors in the HCC group and the control group are shown in Table 3. They revealed no significant difference in the age and sex between the two groups (p> 0.05). HCC patients were 22 males and 8 females with mean age of 53.33 ± 3.83. Controls were 22 males and 8 females with mean age of 51.5 ± 4.26. The presence of comorbidities diabetes mellitus and hypertension was significantly higher in the HCC group (p=0.001 and p=0.005, respectively). Both platelet count and total leucocytic count (TLC) were significantly lower in the HCC group than the control group (p<0.001 and p=0.002, respectively). However, serum levels of AST, ALT, total bilirubin, and direct bilirubin were significantly higher in HCC group than the control group (p<0.001, p=0.037, p<0.001 and p<0.001, respectively). Serum albumin levels were significantly lower in the HCC group than the control group (p<0.001) (Table 3).

Table 3. Demographic and Clinical data of the HCC patients and the control groups.

		HCC group	Control group	*p*-value
Age	Mean±SD	53.33 ± 3.83	51.5 ± 4.26	NS
Gender	Females	8 (26.7%)	8 (26.7%)	NS
	Males	22 (73.3%)	22 (73.3%)	
Diabetes mellitus	No	20 (66.7%)	30 (100.0%)	0.001
	Yes	10 (33.3%)	0 (0.0%)	
Hypertension	No	23 (76.7%)	30 (100.0%)	0.005
	Yes	7 (23.3%)	0 (0.0%)	
Platelet	Median (IQR)	105 (67 – 136)	234 (223 – 269)	<0.001
TLC	Median (IQR)	4 (3 – 6)	5.45 (4.7 – 6.6)	0.002
Alanine aminotransferase	Median (IQR)	23.5 (19 – 33)	20 (15 – 26)	0.037
Aspartate aminotransferase	Median (IQR)	33 (26 - 48)	22 (19 – 27)	<0.001
Total Bilirubin	Median (IQR)	1.65 (0.7 – 2.4)	0.5 (0.5 – 0.9)	<0.001
Direct Bilirubin	Median (IQR)	0.6 (0.3 – 1)	0.2 (0.1 – 0.2)	<0.001
Albumin	Mean±SD	3.38 ± 0.72	4.51 ± 0.44	<0.001

p > 0.05 is not significant (NS).

The ACYP2 rs843706 genotyped frequencies are illustrated in Table 4. All subjects in both groups had the heterozygous CA genotype (100%). No statistically significant difference was found between both groups (p > 0.05). The ACYP2 rs843711 genotyped frequencies are illustrated in Table 5 and Figure 1. In the HCC group, one patient (3.3%) had the homozygous TT genotype, 14 patients (46.7%) had the heterozygous CT genotype, and 15 patients (50%) had the wild CC genotype. While in the control group, 18 subjects (60%) had the heterozygous CT genotype, and 12 subjects (40%) had the wild CC genotype. There was no statistically significant difference between both groups (p>0.05). The C allele was present in 73.3% of HCC patients group and in 70% of the control group. The T allele was present in 26.7% of the HCC group and in 30% of the control group. Allele frequency showed no statistical difference between both groups (p>0.05).

Regarding the dominant model, the CC was present in 50% of HCC patients group and in 40% of the control group. While the CT + TT was present in 50% of the HCC group and in 60% of the control group with no statistically significant difference between the two groups (p>0.05). Regarding the recessive model, the CC + CT was present in 96.7% of HCC patients group and in 100.0% of the control group, while the TT was present in 1% of the HCC group and in 0% of the control group with no statistically significant difference between the two groups (p>0.05).

Different clinical and laboratory data were compared between the CC genotype and both CT+TT genotypes (Table 6). There was no statistical difference observed in platelet counts, TLC and levels of ALT, AST, total bilirubin, direct bilirubin, and albumin.

Table 4. The frequency of rs843706 genotype in HCC patients and the control groups.

	HCC group	Control group	*p*-value
rs843706 genotype			NS
Heterozygous CA	30 (100.0%)	30 (100.0%)	

p > 0.05 is not significant (NS).

Table 5. The frequency of rs843711 genotype in the HCC patients and normal control groups.

	HCC group	Control group	*p*-value
rs843711 genotype			
Wild CC	15 (50.0%)	12 (40.0%)	NS
Heterozygous CT	14 (46.7%)	18 (60.0%)	
Homozygous TT	1 (3.3%)	0 (0.0%)	
Gene alleles			
C	44 (73.3%)	42 (70.0%)	NS
T	16 (26.7%)	18 (30.0%)	
Dominant model			
CC	15 (50.0%)	12 (40.0%)	NS
CT + TT	15 (50.0%)	18 (60.0%)	
Recessive model			
CC + CT	29 (96.7%)	30 (100.0%)	NS
TT	1 (3.3%)	0 (0.0%)	

p > 0.05 is not significant (NS).

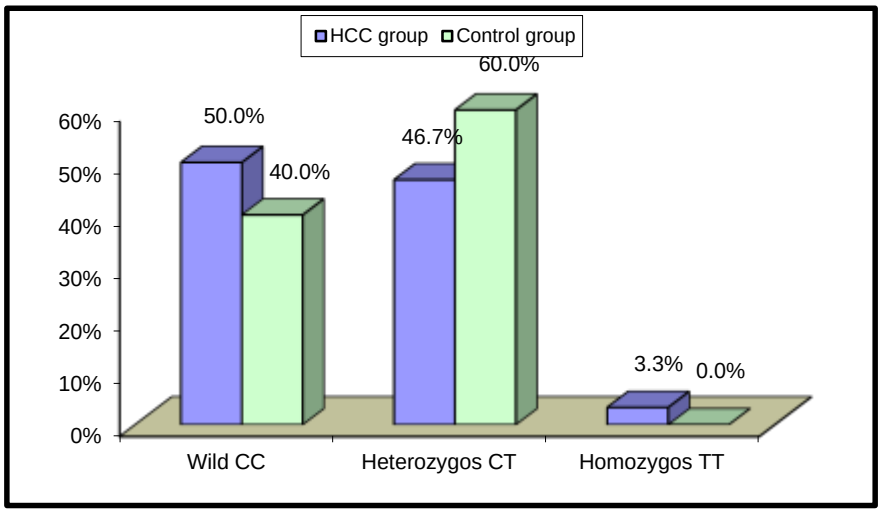

Figure 1. Bar chart showing comparison of genotyping findings between HCC group and the control group.

Table 6. Comparison of demographic, clinical characteristics, and laboratory data in rs843711 genotypes of the HCC patients.

		rs843711 genotype		*p*-value
		CC	CT+TT	
Age	Mean±SD	53.47 ± 4.45	53.20 ± 3.23	NS
Gender	Females	4 (26.7%)	4 (26.7%)	NS
	Males	11 (73.3%)	11 (73.3%)	
Diabetes mellitus	No	11 (73.3%)	9 (60.0%)	NS
	Yes	4 (26.7%)	6 (40.0%)	
Hypertension	No	11 (73.3%)	12 (80.0%)	NS
	Yes	4 (26.7%)	3 (20.0%)	
Platelet	Median (IQR)	101 (45 – 135)	115 (69 – 147)	NS
TLC	Median (IQR)	4.6 (2.9 – 6.5)	3.8 (3 – 4.5)	NS
Alanine aminotransferase	Median (IQR)	24 (18 – 44)	23 (19 – 30)	NS
Aspartate aminotransferase	Median (IQR)	36 (26 – 53)	33 (25 – 40)	NS
Total Bilirubin	Median (IQR)	1.5 (0.7 – 2.3)	1.8 (0.7 – 2.4)	NS
Direct Bilirubin	Median (IQR)	0.6 (0.2 – 1)	0.7 (0.3 – 1)	NS
Albumin	Mean±SD	3.43 ± 0.70	3.33 ± 0.76	NS

$p > 0.05$ is not significant (NS).

Discussion

Hepatocellular carcinoma represents 70%-90% of primary liver cancers worldwide. HCC is considered to be a multifactorial disease that is regulated by both genetic and environmental factors.[13] The present study aimed to investigate the association between ACYP2 SNPs (rs843711), and (rs843706) and incidence of HCC in Egyptians to determine its role in hepatocarcinogenesis. Our study included 30 HCC patients and 30 apparently healthy subjects (all were negative for both HCV and HBV) served as controls.

The present study showed male predominance among HCC patients 22 of 30 (73.3%) with mean age of 53 years. Zhang et al., 2017[14] also reported male predominance among Chinese HCC patients (77.4%) with mean age of 54 years as well Rashed et al., 2020,[15] stated that the higher risk of HCC in males can be associated to higher rate of men exposure to liver carcinogens and the higher estrogen level in females which partially plays a role in suppression of inflammation.

The present study showed high prevalence of diabetes mellitus and hypertension among HCC patients. Mu et al., 2020[16] and Rahman et al., 2013[17] also reported similar results and stated that both diabetes and hypertension were among the components of metabolic syndrome that may lead to nonalcoholic steatohepatitis and consequently HCC. Moreover, insulin resistance in type 2 diabetic patients results in persistently increased insulin levels as well as increased levels of insulin-like growth factor-1 in most tissues including liver that may accelerate carcinogenesis.

The ACYP2 rs843706 polymorphism was investigated by RT-PCR. Our results revealed that all studied HCC cases and the control group were CA heterozygous genotype (100%) with no statistically significant difference between the two groups. Similarly, *Chen et al., 2017* [4] reported similar results and concluded that the CA heterozygous genotype was not associated with the incidence of HCC. On the other hand, Huang et al., 2020[18] reported that the rs843706 mutation was associated with a high incidence of HCC ($p < 0.05$) with the presence of the CC homozygous in 26.3% of HCC patients versus 31.5% in controls and the presence of AC+AA genotypes in 73.7% of HCC patients versus 68.5% in the controls. The negative result in our study may be due to the relatively small sample size of the population.

The ACYP2 rs843711 polymorphism was investigated by RT-PCR. Our results revealed that in HCC group 3.3 % had the homozygous TT genotype, 46.7% had the heterozygous CT genotype and 50% had the wild CC genotype. While in healthy control group, 60% had the heterozygous CT genotype and 40% had the wild CC genotype. No statistical difference was found between both groups. Similarly, Zhao et al., 2019[6] reported similar results and concluded that the rs843711 genotypes were not associated with the incidence of HCC. Meanwhile, our results disagreed with those of Huang et al., 2020[18] who reported an association between the TT genotype of rs843711 and incidence of HCC ($p < 0.05$). The homozygous TT genotype frequencies in HCC group were found in 30% and the heterozygous CT genotype was found in 46.2% and the wild CC genotype found in 26.8%. While in the healthy control group, 19% had the homozygous TT genotype, 49.4% had the heterozygous CT genotype and 31.6% had the wild CC.

Regarding the allelic frequency, our results revealed that *C* allele was higher in HCC group regarding rs843711. This result disagreed with that of Huang et al., 2020[18] who reported that the *T* allele was higher in the HCC group. Our results disagreed with data of the meta-analysis study conducted by Chang et al., 2022[19] which found that *ACYP2* rs843706, rs843711 mutations had a significant association with increasing the risk of HCC. Meanwhile, the meta-analysis conducted by Zhao et al., 2019[6] revealed no statistically significant difference in the relationship between *ACYP2* gene polymorphism and cirrhosis developed into liver cancer in selected 13 SNPs using real time PCR. The meta-analysis reported that neither rs843706 nor rs843711 polymorphisms influenced the risk of liver cancer. The variability and the discrepancy reported in different association studies might be the result of the

differences in several genetic, environmental factors, ethnic stratification, variation in study design and sample size and may be due to difficult technique used to detect the mutations.

To our knowledge, this is the first study on Egyptian patients for SNPs in ACYP2 gene. Our study has some limitations including the relatively small sample size. Furthermore, these findings need confirmation by sequencing either DNA from blood samples or DNA from tissue samples from the same patients to confirm the absence of polymorphism or their presence with low frequencies. In conclusion, this study did not find an association between ACYP2 rs843706 and rs843711 in HCC patients.

Author Contributions

DTG, RMS, SAE, MEAF performed the laboratory work, statistical analysis and analyzed the data. DT and RS drafted the paper. MEAF and AIH revised the paper critically. All authors contributed significantly to the study's conception, design, and final approval of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University, (Approval number FMASU MS 688/2021).

Informed consent

A written informed consent was obtained from each study subject participated in the study before being included in the study..

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