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Association of UDP-Glucoronyl Transferase 1-A7 Polymorphism at Codon 208 with Hepatocellular Carcinoma and Liver Cirrhosis

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Abstract:

Introduction: Universally, hepatocellular carcinoma (HCC) is one of the foremost broad malignancies. UDP-glucuronosyltransferase (UGT) enzymes are found in a wide range of living things, including humans and microbes. Uridinediphosphoglucuronic acid's transfer (UDPGlcA) glucuronic acid group to a particular substrate's functional group is catalyzed by membrane-bound conjugating enzymes called UGTs. The several UDP-glucuronosyltransferases that are encoded by this complicated locus include this gene.

Aim of the study: Determine whether hepatocellular cancer and liver cirrhosis are associated with the UDP-GLUCORONYL TRANSFERASE1-A7 polymorphism at codon 208.

Subjects and Methods: Seventy patients were drawn from the Kasr El Ainy tropical unit at Cairo University. A full blood sample was used to obtain genomic DNA. Melting curve analysis in real-time PCR was used to identify the genetic variations in UGT1A7. finding 208 codon mutations was done by screening. Fluorescence resonance energy transfer (FRET) probes and primers flanking the polymorphisms intrigued in UGT1A7's exon 1 were created using the issued nucleotide sequence (GenBank U39570).

Results: The findings demonstrated that HCC cases had higher levels of codon 208 (heterozygous (1,3) and homozygous (3,3) genotypes as compared to wild type (1, 1). On the other hand, homozygous and heterozygous liver cirrhosis patients have the same frequency as wild type. When compared to heterozygous genotype, the frequency of wild genotype is higher in the control group.

Conclusion: Polymorphism at codon 208 in UGT1A7 has been linked to hepatocellular carcinoma (HCC) and is therefore regarded as a risk factor for both HCC and liver cirrhosis.

Keywords: Hepatocellular Carcinoma; Liver Cirrhosis; Codon 208; UGT1A7.

1. Introduction

Over 500,000 persons worldwide receive a diagnosis of hepatocellular carcinoma each year, with about 20,000 of those instances occurring in the United States [1]. For men, liver cancer is fifth in incidence, whereas for women, it ranks seventh. The majority of the disease burden (85%) is carried by poor nations, with the greatest incidence rates recorded in areas where hepatitis B virus (HBV) infection is endemic: Sub-Saharan Africa and Southeast Asia [2]. Hepatocellular carcinoma usually manifests after the age of forty and peaks around seventy years of age. Men are two to four times as likely as women to develop liver cancer.

Enzymes called UDP-glucuronosyltransferases, or UGTs, are present in a wide range of living organisms, including bacteria and humans [3]. The glucuronic acid group's transfer of uridine-diphosphate-glucuronic acid (UDPGlcA) to the functional group of a particular substrate is catalyzed by membrane-bound conjugating enzymes or UGTs. This gene is a component of a multigene complex that codes for many UDP-glucuronosyltransferases. In humans, the UGT1A locus gene products are expressed differently depending on the tissue. It has been observed that the human oesophagus, stomach, and colon are extrahepatic organs where the UGT1A7, UGT1A8, and UGT1A10 genes are expressed [4, 5].

Numerous substances that are significant to toxicology and medicine are metabolized by UGT1A7. The variant UGT1A73 allele (Lys129Lys131Arg208) is produced by the three missense variants at codons 129/131 and 208. UGT1A7 has been linked to the glucose uptake of carcinogens, including heterocyclic amines produced from food and polycyclic aromatic hydrocarbons [5, 6]. The UGT1A73 allele encodes a protein with lower enzymatic activity against benzopyrene metabolites than the UGT1A71 allele [7]. Research on disease susceptibility has revealed that the UGT1A73 allele is a risk gene for the occurrence of HCC, and oro-laryngeal cancer [8-10], colorectal cancer [10, 11], and pancreatic cancer [12].

The aim of this study was to find the association between the UDP-GLUCORONYL TRANSFERASE1-A7 polymorphism at codon 208 with hepatocellular carcinoma and liver cirrhosis.

2. Subjects and Methods

2.1.Subjects

Seventy patients recruited from the tropical unit of Kasr El Ainy, Cairo University were incorporated into this study and classified as follows:

- **Group 1:** Thirty HCC patients, with a mean age of 57 ± 9.0 years, had the following tests: Taking the patient's history, examining them to rule out bilharzia is, smoking, and gender, To diagnose primary HCC based on the combination of focal lesions found by any imaging technique and/or alpha-fetoprotein (AFP) level > 250 ng/ml for HCC, serum samples were taken and tested for hepatitis B surface antigen (HBsAg) (negative), Anti-HCV (positive), AFP level, AST, ALT, Albumin, Bilirubin Total, Bilirubin direct, and U/S or CT.
- **Group 2:** Twenty patients, with a mean age of 51 ± 10.9 years, were diagnosed with cirrhosis. The diagnosis was made either by histology or by combining the clinical picture (fatigue, edema, ascites) with laboratory tests. After serum samples were taken out, tests were performed on them for hepatitis B surface antigen (HBsAg) (negative), anti-HCV (positive), albumin, AST, ALT, and total and direct bilirubin, as well as imaging utilizing U/S or C.T. and endoscopic symptoms.
- **Group 3:** Twenty healthy controls (volunteers) with normal liver functions and no clinical or laboratory signs of hepatocellular carcinoma or cirrhosis had a mean age of 51.8 ± 8.6 years. They also had normal liver functions and no cirrhosis or focal lesions on imaging.

2.2.Methods

Sample Collection

3 ml serum samples for determination of HBsAg, Anti HCV, AFP level, and Liver functions (AST, ALT, Albumin, Bilirubin total and direct). Subjects had their 2 mm of venous blood taken into sterile EDTA vacutainer tubes in an aseptic manner. Samples were kept at -20°C until extraction.

DNA extraction and Real time PCR

A sample of whole blood was used to extract genomic DNA. Using real-time PCR (polymerase chain reaction), the genetic polymorphisms in UGT1A7 were identified as follows: DNA was isolated from whole blood using the high pure PCR template preparation kit.

2.3.Statistical Method

Microsoft Windows, SPSS 15.0 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 was utilized for data management and analysis. The mean + SD was the depiction of the statistical data. The means of the three groups were compared using the Post Hoc test and one-way analysis of variance (ANOVA), whereas the means of the two groups were compared using the Student's t-test. To express non-parametric quantitative data, the median (25th–75th quartiles) was utilized, and medians were compared using the Mann-Whitney and Kruskall-Wallis tests. Pearson's correlation coefficient (r) for parametric data and Spearman's correlation coefficient (r) for non-parametric data were utilized to find out the relation between quantitative variables. Frequency and percentage were used to represent qualitative data. Affiliation between qualitative data was done using the Chi-square test. Risk estimate was done by odds ratio. *P*- value was considered significant at 0.05.

3. Results

Seventy cases were used in this investigation. Twenty patients with liver cirrhosis had an average age of 51 ± 11 years, 30 cases with HCC had an average age of 57 ± 9.2 years, and 20 cases served as controls, with an average age of 51.8 ± 8.6 years. A p =0.003 indicates a strong correlation between codon 208 and liver cirrhosis and HCC. cirrhosis (**Table 1**).

Table 1. UGT1A7 genotype & allele frequency in HCC, liver cirrhosis & control groups.

Variables	HCC (N=30)	Liver cirrhosis (N=20)	Control (N=20)	*P*-value
Codon 208 (WR) Heterozygous	5 (25%)	4 (20%)	16 (53.3%)	0.003*
(RR) Homozygous	0 (0%)	6 (30%)	6 (20%)	
(WW) Wild	15 (75%)	10 (50%)	8 (26.6%)	

* significant.

In codon 208, the wild genotype (WW) was higher in the control group than in liver cirrhosis and HCC groups. The mutant heterozygous genotype (WR) was higher in the HCC group than both liver cirrhosis and control group, the homozygous (RR) was absent in the control group (**Figure 1**).

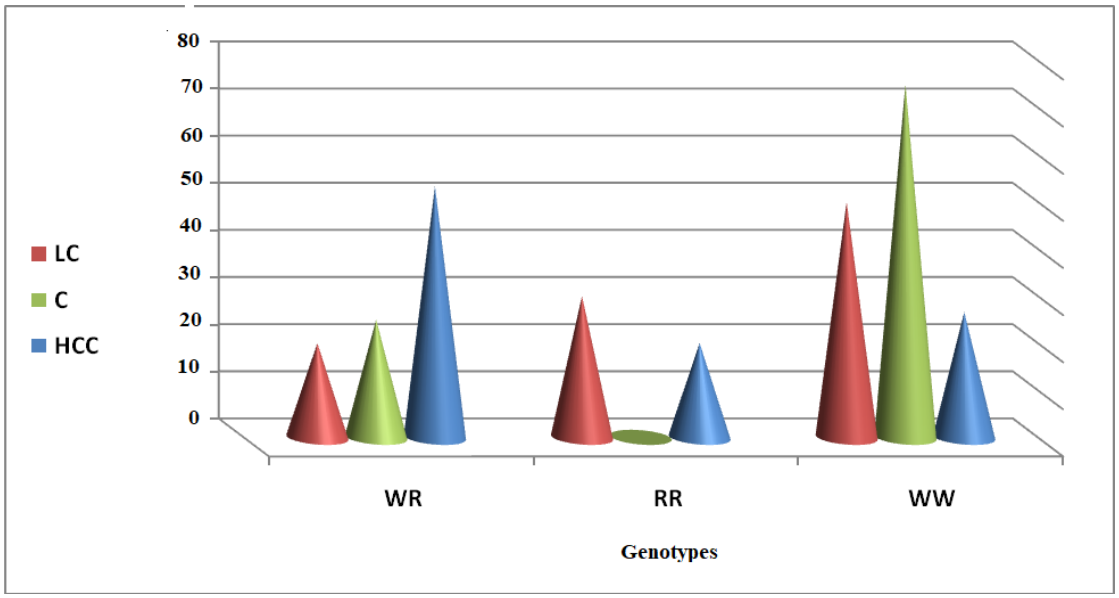

Figure 1: Comparison between WW, RR, WR genotypes in HCC, liver cirrhosis and control.

Risk estimation for homozygous (R R) and heterozygous (WR) versus wild type (WW) IN HCC and liver cirrhosis cases was not significant (OR =2.75, 95 % CI =0.834-9.066), (*p* =0.084). Risk estimation for homozygous (R R) &heterozygous (W R) versus wild type (W W) in HCC and liver cirrhosis versus control cases was significant (OR =5.333, 95 % CI=1.663-17.1), (*p* = 0.032) (**Tables 2-4**).

Table 2: Risk estimation of codon 208 genotypes in HCC and liver cirrhosis groups.

Group	**HCC**	**L¢**	**OR**	**95 % CI**	***P*-VALUE**
Homozygous (KK) and heterozygous (NR/KK)	22 (73.3%)	10 (50%)	2.75	(0.834-9.066)	0.084
Wild (NR)	8 (26.6%)	10 (50%)			

Table 3: Risk estimation of codon 208 genotypes in HCC and control groups.

Group	HCC	Control	OR	95 % CI	*P*-VALUE
Homozygous (KK) and heterozygous (NR/KK)	32 (64%)	5 (25%)	5.333	(1.663-17.1)	0.032*
Wild (NR)	18 (36%)	15 (75%)			

* significant.

Table 4: Haplotype frequency in HCC and liver cirrhosis versus control.

Haplotype	Cases (Liver Cirrhosis & HCC)	Ccontrol	*P- value*
***1*1**	2/50 (4%)	6/20(30%)	0.000
***1*2**	13/50 (26%)	6/20(30%)	
***2*2**	3/50 (6%)	3/20(15%)	
***2*3**	8/50 (16%)	----------	
***3*3**	6/50 (12%)	----------	
***3*4**	4/50 (8%)	----------	
***4*4**	2/50(4%)	----------	
***1*3**	----------	1/20(5%)	
***1*4**	----------	3/20(15%)	
***1*3 /*2*4**	12/50 (24%)	1/20(5%)	

1 allele is higher in control 23/40 (57.5%) than in cases 29/100 (29%)- 2 allele is slightly higher in control 12/40 (30%) than in cases 27/100 (27%)- 3 allele is higher in cases 36/100 (36%) than control 2/40 (5%)- 4 allele is nearly the same in cases 8/100 (8%) & control 3/40 (7.5%) (**Table 5**).

Table 5: Haplotype allele frequency.

	Cases N =50	Control N =20	*P*- value
***1**	29 (29%)	23 (57.5%)	0.001*
***2**	27 (27%)	12 (30%)	
***3**	36 (36%)	2 (5%)	
***4**	8 (8%)	3 (7.5%)	

* significant.

4. Discussion

Hepatocellular carcinoma (HCC) positions among the foremost prevalent cancers around the world, HCC is the fifth most predominant malignancy in men and the ninth most common in women, with over 500,000 new cases found each year. The human UDP-glucuronosyltransferases (UGTs) enzyme superfamily metabolizes tobacco-specific nitrosamines and benzo(a)pyrene, as well as endogenous compounds including bilirubin and steroid hormones, using the glucuronidation reaction [13].

Gly115Ser, Asn129Lys, Arg131Lys, Glu139Asp, and Trp208Arg are the sites at which the nine alleles (UGT1A7*1–UGT1A7*9) of the UGT1A7 gene differ [5, 12]. At codons 129/131 and 208, three non-synonymous SNPs result in the variant allele UGT1A7*3, two of which are closely related (129 and 131). The population of *3/*3 (17%) usually carries this allele. The related Asn129Lys /Arg131Lys polymorphisms comprise the UGT1A7*2 allele, whereas the UGT1A7*4 allele only carries the Trp208Arg mutation. In comparison to the UGT1A7*3 allele in the general population, the additional genetic changes at codons 115 and 139 were shown to be less prevalent.

The objective of this study was to find the association between the UDP-Glucoronyl Transferase1-A7 polymorphism at codon 208 with hepatocellular carcinoma and liver cirrhosis. So, this study was conducted on 70 patients; 30 of them were suffering from HCC proved by U/S or C.T and Alpha-fetoprotein (AFP); 20 patients suffering from liver cirrhosis proved by histopathological examination, clinical lab

investigations and by imaging using U/S or C.T in addition to 20 individuals selected as a control group that proved by the absence of clinical, laboratory and imaging investigations.

The genetic polymorphisms in UGT1A7 were determined by real-time PCR (polymerase chain reaction). Screening for the mutations in codons 208. Fluorescence resonance energy transfer (FRET) probes and primers surrounding the polymorphisms of interest in exon 1 of UGT1A7 were employed.

The present study showed a significant association between liver cirrhosis, HCC and UGT1A7 polymorphism (codon 208). Tang et al (2007) found that the probability of developing liver cirrhosis was increased by the interaction between low-activity UGT1A7 genotypes and HBV (or HCV) infection [observed odds ratio (OR =54.59) more than the anticipated (OR =18.05). Advanced liver cirrhosis (Child-Pugh grades C and/or B) was also associated with a low/low genotype of UGT1A7 (OR =7.50, *p* =0.009) [15].

According to Wang et al. (2004), With odds ratios of 2.73 (95% confidence interval, 1.40-5.35) and 1.80 (95% confidence interval, 1.05-3.09), respectively, compared with the UGT1A7 H/H alleles, they reported that the proportions of UGT1A7 L/L and H/L alleles (genotypes) in patients with HCC (25% and 45%, respectively) were higher than those in patients without HCC (15% and 39%, respectively). The results of multivariate analyses demonstrated that the existence of cirrhosis, age >60 years, male sex, alpha-fetoprotein >20 microg/ml, and genotypes of IL-1 beta/-31T/T-511C/C as well as UGT1A7 L/L were linked with the presence of HCC (OR =2.33, 2.67, 4.20, 3.12, 3.09, and 2.90, respectively) [16].

As regards haplotype allele frequency was as follows: there was significant association between HCC and liver cirrhosis and haplotype allele frequency (p =0.001) (*1) 29/100 (29%) in cases while it was 23/40 (57.5%) for control group, (*2) 27/100 (27%) in cases while it was 12/40 (30%) for control group, (*3)36/100 (36%) in cases while it was 2/40 (5%) in control group, (*4) 8/100 (8%) in cases while it was 3/40 (7.5%) in control group.

Vogel and colleagues (2001) reported that the control population showed the presence of wild-type alleles in 29 (41%) compared with wild alleles in the control

group of our study 6/20 (30%). In patients with HCC: wild type allele was found in only (6.8%) 4/59 of patients compared with wild type in HCC in our study 2/50 (4%) which correspondent to our study. Also, in the control group UGT1A7 *1/*2 in 13 (19%) compared with (*1*2) genotype in our study 6/20 (30%), UGT1A7 *2/*2 in 9 (13%) compared with (*2*2) genotype in our study 3/20 (15%), UGT1A7 *3/*3 in 7 (10%) compared with (*3*3), which was only present in cases in our study 6/50 (12%) [9].

Tseng and colleagues found that UGT1A7*1/*1 for cases 64 (29.5%) and for control 134 (46.0%), (OR =0.49, 95% CI: 0.34-0.71). UGT1A7*1/*2 for cases 55 (25.3%) and for control 66 (22.7%), (OR =1.16, 95% CI: 0.77-1.75). UGT1A7*1/*3 for cases 53 (24.4%) and for control 54 (18.6%) (OR =1.42, 95% CI: 0.92-2.18). UGT1A7*2/ *2 for cases 13 (6.0%) and for control 12 (4.1%) (OR =1.48, 95% CI: 0.66-3.31). UGT1A7 *2/*3 for cases 25 (11.5%) and for control 19 (6.6%) (OR =1.86, 95% CI: 1.00-3.48). UGT1A7*3/*3 for cases 7 (3.2%) and for control 6 (2.1%) (OR =1.58, CI: 0.52-4.78). UGT1A7*1 for cases 172 (79.3%) and for control 254 (87.3%) (OR =0.56, CI: 0.35-0.90). UGT1A7*2 for cases 93 (42.9%) and for control 97 (33.3%), (OR =1.50, CI: 1.04-2.16). UGT1A7*3 for cases 85 (39.2%) and for control 79 (27.1%) (OR =1.73, CI: 1.19-2.52) UGT1A7*2 and *3 alleles were significantly related to HCC development (UGT1A7*2: OR =1.50, 95% CI: 1.04-2.16); UGT1A7*3: OR =1.73, 95% CI: 1.19-2.52), while the UGT1A7*1/*1 was associated with decreased risk of HCC occurrence (OR =0.49, 95%, CI: 0.34-0.71), which is matching our study [17].

Conclusion

The polymorphism at codon 208 in UGT1A7 is linked to hepatocellular carcinoma (HCC) and is therefore regarded as a risk factor for both liver cirrhosis and HCC.

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

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