Genetic Polymorphism of Uridine-Diphosphoglucuronosyltransferase 1A7 Gene in Chronic Viral Hepatitis and Hepatocellular Carcinoma in Egyptian Patients

Thesis

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common cause of primary liver neoplasm. The incidence of HCC worldwide varies according to the prevalence of hepatitis B and C infections. UDP-glucuronosyltransferases 1A7 (UGT1A7) gene polymorphisms including the W208R SNP (codon 208: TGG→CGG) have been shown to encode enzymes with lower carcinogen detoxification activity and are associated with certain cancers. ***Objectives:*** The present study aims to elucidate the role of UGT1A7 SNP (622 T→C) in the pathogenesis of HCC, and if this polymorphism is associated with elevated bilirubin level.

Subjects and Methods: Genomic DNA from the blood of 22 patients with HCC, 25 patients with chronic viral hepatitis B and/ or C, and 16 apparently healthy controls was analyzed for UGT1A7 polymorphism using PCR – RFLP. ***Results:*** A statistically significant increase was found in the frequency of risky genotypes (TC, CC) in HCC group as compared to the protective genotype (TT) (P 0.01). UGT1A7 T allele and C allele were designated as H (high activity) & L (low activity) alleles respectively. A statistically significant increase was found in frequency of L allele in HCC group (54%) as compared to control group (25%) p = 0.03, on the other hand the H allele showed statistically significant decrease in HCC group (46%) compared to control group (75%) p = 0.03. Increase in total bilirubin level in cases harboring UGT 1A7 Polymorphism compared to wild genotype (p< 0.01) was observed, thus demonstrating its role in the pathogenesis of hyperbilirubineamia. ***Conclusions:*** The UGT1A7 polymorphism was associated with elevated bilirubin level and may play a role in the pathogenesis of HCC.

Key words: UGT1A7, HCC, viral hepatitis, bilirubin.

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List of Abbreviations

AFB_1	Aflatoxin B_1
APC	Adenomatous Polyposis Coli
CDK	Cyclin dependent kinases
CGH	Comparative genomic hybridization
CKI	CDK inhibitors
CNS-II	Crigler–Najjar syndrome type II
CNVs	Copy number variants
DE	Dimensional electrophoresis
DMEs	Drug-metabolizing enzymes
ESI	Electrospray ionization
FAP	Familiar adenomatous polyposis
FISH	Fluorescence in situ hybridization
G1	Gap1
GP3	Glypican3
GS	Gilbert's syndrome
HAs	Heterocyclic amines
HBV	Hepatitis B virus
HCA	Heterocyclic amines
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
IDU	injection drug users
IGF-II	Insulin-like growth factor II
IFN	Interferon
IRES	Internal Ribosomal Entry Site
IRS-1	Insulin receptor substrate 1
LC/MS/MS	Liquid chromatography/tandem mass spectrometry
LD	Linkage disequilibrium
Lef	Lymphocyte enhancer-binding factor
LHB	Large envelope protein
MALDI TOF-MS	Matrix assisted laser desorption/ionization time-of-flight mass spectrometry
MHC	Major histocompatibility complex
MRP	Multidrug resistance protein
MS	Mass spectrometry
NK	Natural killer
Nt	Nucleotide

NTR	Nontranslated regions
PAH	Polycyclic aromatic hydrocarbons
PIVKA-II	Protein induced by vitamin K-absence or Antagonist-II
Rb	Retinoblastoma
SCCA-1	Squamous Cell Carcinoma Antigen -1
SELDI TOF-MS	Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry
SNPs	Single-nucleotide polymorphisms
TCF	Transcription factor
TGF-β	Transforming growth factor–beta
TIMP	Tissue inhibitor of metalloproteinases
TRAP	Telomeric repeat amplification protocol
UDPGA	Uridine diphosphoglucuronic acid
UGTs	Uridine 5'-diphosphate (UDP)-glucuronosyltransferases
UPLC-MS	Ultra-performance liquid chromatography-tandem mass spectrometry

INTRODUCTION AND AIM OF WORK

Hepatocellular carcinoma (HCC) is the most common cause of primary liver neoplasms and the fourth most frequent type of cancer worldwide following lung, breast and bowel cancers with an increasing incidence, causing one million deaths per year ***(Oscar et al.,2007).*** The incidence of HCC worldwide varies according to the prevalence of hepatitis B and C infections which are by far the major risk factors. Areas such as Asia and sub-Saharan Africa with high rates of infectious hepatitis have incidences as high as 120 cases per 100,000 ***(American Cancer Society., 2007).***

The burden of HCC has been increased in Egypt with a doubling in the incidence rate in the past 10 years. In Egypt hospital-based studies have reported an overall increase in the relative frequency of all liver related cancers (>95% as HCC), from approximately 4% in 1993 to 7.3% in 2003 ***(el-Zayadi et al., 2005).*** This has been attributed to the same previously mentioned biological factors in addition to the endemic infections in the community such as schistosomiasis. Recent investigations in Egypt have shown the increasing importance of HCV infection in the etiology of liver cancer, this has been estimated to account for 40–50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15%, respectively) ***(Elizabeth et al., 2008; Anwar et al., 2008; and Iyer et al., 2009).***

The increased incidence of HCC in hemochromatosis and α-1-antitrypsin deficiency as well as the detection of chromosomal aberrations indicates a role of genetic events in HCC development. Therefore, in view of the multifactorial cause of HCC, the identification of modifiers of carcinogenetic initiation is required to define and assess the risk of HCC development ***(Vogel et al., 2001).***

Modifier genes encoding proteins that participate in cellular detoxification and defense are attractive candidates that can modify the impact of environmental factors capable of inducing neoplastic transformation ***(Wang et al., 2004).***

The human UDP-glucuronosyltransferases (UGTs) represent an enzyme super family that is capable of catalyzing the glucuronidation and detoxification of diverse compounds, including therapeutic drugs, endogenous metabolites (*e.g.*, bilirubin and steroid hormones), and known human carcinogens, such as heterocyclic and polycyclic hydrocarbons and heterocyclic amines ***(Strasburg and Manns., 2000; Wang et al., 2004; and Urawa et al., 2006).***

UGT1A7 gene polymorphisms including the W208R SNP (codon 208: TGG→CGG) have been shown to encode enzymes with lower carcinogen detoxification activity and are associated with certain cancers e.g: orolaryngeal , HCC, colorectal cancer and pancreatic cancers (***Zheng et al., 2001; and Borentain et al., 2007).***

A well-known defect of glucuronidation in humans is illustrated by the group of individuals with Gilbert's syndrome and is associated with genetic polymorphism in the UGT1A gene ***(Mercke et al., 2006).***

Aim of the Work

The present study aims to elucidate the role of UGT1A7 SNP (622 T→C) in the pathogenesis of HCC, and if this polymorphism is associated with elevated bilirubin level.

CHAPTER 1
Hepatitis B and C Viruses

Viral hepatitis is liver inflammation due to a viral infection. It is a major public health problem in many countries all over the world and especially in Middle East, Asia, East-Europe, and Africa ***(Little and Rubin., 1987; Liang et al., 2008; and Liaw and Chu., 2009).***

Hepatitis B virus (HBV)

Hepatitis B virus is the most serious cause of viral hepatitis and a potentially life-threatening liver disease, affecting about 2 billion people worldwide with about 350 million complicated with chronic infection ***(WHO Fact Sheet No. 204., 2008).***

HBV structure

Human hepatitis B virus is a member of the hepadnavirus family; it is a DNA enveloped virus, which is remarkably stable to organic solvents, and also heat- and pH-resistant ***(Gripon et al., 2002).***

The virus was first discovered as "Australia antigen", later renamed HBsAg (for hepatitis B surface antigen) ***(Ahn et al., 2003)***. HBeAg (hepatitis B e antigen) was identified several years later as a marker for patients at high risk for transmission of the disease ***(Shuping et al., 2005),*** also hepatitis B patients sera contain circulating antibodies against HBcAg (hepatitis B core antigen), and develop antibodies against HBeAg and HBsAg (anti-HBe and anti-HBs) at later stages of infection ***(Beck and Nassal., 2007)***

HBV Genome

HBV is a circular, double-stranded DNA virus of approximately 3200 nucleotides, it contains four overlapping open reading frames that encode the surface (viral envelope (PreS1, PreS2, S)), core (nucleocapsid(core, precore)), X (a nonstructural protein that operates as a multifunctional regulator modulating gene transcription, cell responses to genotoxic stress, protein degradation, apoptosis, and several signaling pathways), and polymerase proteins ***(Gunther et al., 1999; Bartholomeusz et al., 2001; and Jammeh et al., 2008).***

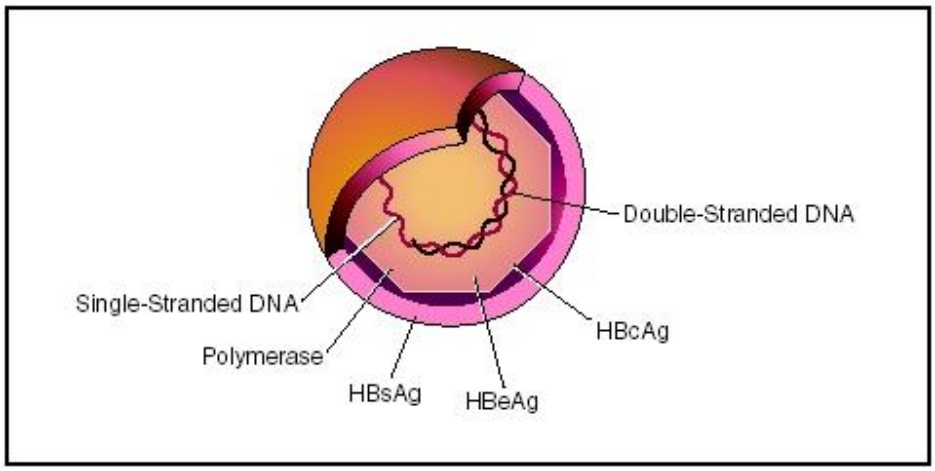

Figure (1): Structure of HBV ***(Giner Galvan et al., 2007)***.

After the virus enters a hepatocyte, the viral genome is delivered to the nucleus, and the relaxed circular DNA is converted to covalently- closed- circular DNA (cccDNA), the (cccDNA) serves as a template for the transcription of viral RNA. The Hepatitis B virus replication cycle includes reverse transcription of RNA intermediates to prime DNA synthesis and translation of the hepatitis B proteins, including HBsAg and HBeAg. Thus, cccDNA plays a key part in the maintenance of chronic hepatitis B infection ***(Liaw and Chu., 2009).***

The entire nucleotide sequences of HBV genomes have been classified into 8 genotypes (A-H) based on an inter group divergence of more than 8% in the complete nucleotide sequence. Apart from genotypes E and C, the genotypes have subtypes with sequence difference in at least 4% ***(Liaw and Chu., 2009).***

The most common core promoter mutations involve a two-nucleotide substitution: A-T at nucleotide (nt) 1762 and G-A at nt 1764 ***(Kurosaki et al., 1996; and Kao et al., 2003),*** it was shown that the likelihood of presence of T1762/A1764 mutations in the core promoter parallels the progression of liver disease, and that this mutation is found most frequently in HBV genotype D followed by genotypes C than B patients and are seen least frequently in genotype A ***(Chang et al., 2004).***

These core promoter mutations enhanced replication capacity and reduced virion secretion, which may increase viral load in the liver, thus triggering liver damage either directly or indirectly through the immune response ***(Baumert et al., 1996; Sugauchi et al., 2003; and Shuping et al., 2005),*** if such massive liver damage occurs during acute infection, fulminant hepatitis may develop, however, if it occurs during chronic infection, it increases hepatocyte turnover, induces fibrosis, and enhances the chance of hepatocellular transformation and malignancy***(Jammeh et al., 2008).***

Mutations have further been detected in the pre-S regions of the HBV genome. PreS deletions were shown to be more frequent in patients with HBV genotype C .A pre-S defective HBV variant has been associated with fulminant hepatitis B; which results from a defect or impairment in virion secretion it has been also isolated from patients with fibrosing cholestatic hepatitis ***(Glebe et al., 2005; and Engelke et al., 2006)***. Furthermore, a recent study demonstrates evidence that patients with progressive liver disease have a higher frequency of pre-S deletion. ***(Choi et al., 2009).***