RESEARCH ARTICLE

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The Impact of *interferon lambda 3* Polymorphism on Hepato-Cellular Carcinoma Progression in Hepatitis C Virus Patients: Treatment-Naïve and Experienced

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Abstract

Objectives: In this study, we investigated the association between the IFN-λ3 rs12979860 single nucleotide polymorphism (SNP) and the transition from late fibrosis to HCC in Egyptian HCV-chronically infected patients. **Methods:** The rs12979860 SNP was genotyped using real-time PCR in DNA from the whole blood of healthy subjects (n=60) and HCV patient s (n=342). We stratified the patients into (1) treatment-naïve patients (n=218) with advanced fibrosis (F2-F4, n=123) and HCC (n=95 Treatment-experienced patients (n=124) who received SOF-based therapy for 12 weeks and achieved SVR (SVR12). DAA-treated patients were divided into 2 groups: group I (n=63) included patients with advanced hepatic fibrosis (F2-F4) who did not develop HCC within a year after treatment, and group II (n=61) included patients who were free of focal hepatic lesions before starting DAA therapy but developed HCC within a year. **Results:** Our results demonstrated that treatment-naïve patients with the CT/TT genotypes and the T allele were more likely to have HCC (odds ratio 3.1, 95% CI 1.44-6.71, P = 0.003 and odds ratio 1.89, 95% CI 1.28-2.76, P = 0.001, respectively). Binary regression analysis defined 3 independent predictors associated with HCC development: age (odds ratio 1.039, 95% CI 1.004-1.076, P = 0.028), alanine aminotransferase (odds ratio 1.008, 95% CI 1.002-1.015, P = 0.010), and rs12979860 (odds ratio 3.65, 95% CI 1.484-8.969, P = 0.005). **Conclusions:** However, the rs12979860 SNP did not show any correlation with the progression of HCC post-treatment. Despite the debate on the contribution of IFN-λ3 rs12979860 to susceptibility to HCV-related HCC, our data confirm the role of this SNP in this context.

Keywords: Hepatitis C virus- Infectious disease- single nucleotide polymorphism- direct acting antivirals

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Introduction

Hepatitis C virus (HCV) infection is a global health problem with ~ 71 million infected cases, as estimated in 2018 (El-Serag, 2012). Egypt led the world regarding the numbers of infected patients (approximately 10-20% of the whole population in 2015); 90% of them were infected with the most difficult genotype to treat, namely, genotype 4 (GT4) (Omran and others 2018). Most patients develop chronic infection, which leads to successive phases of liver diseases: fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Axley et al., 2018).

Direct-acting antiviral agents (DAAs) are the current treatment for HCV with great success in clearing the virus and curing the infection. Despite the current progress in HCV therapy, which reaches a minimum of 90% sustained virological response (SVR) in the majority of treated patients, DAA treatment is still questioned. One of the major limitations of DAAs is the emergence of HCC following the completion of the treatment course, either the early onset of HCC (de novo)/or recurrence of HCC after surgery (Baumert et al., 2017; Conti et al., 2016; Reig et al., 2016).

HCC accounts for 80% of all primary liver cancers, and

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it is ranked as the fifth most common cancer and the third leading cause of cancer death in the world (de Bitencorte et al., 2021). HCC development is very complex with the contribution of several etiological and risk factors, including viral factors (e.g., HCV and hepatitis B virus (HBV)) and nonviral factors (e.g., alcohol and diabetes) (Makarova-Rusher et al., 2016). Considering that cirrhotic patients are more susceptible to HCC (approximately 80%-90% of HCC patients have liver cirrhosis), chronic HCV infection is one of the main causative agents for HCC incidence (10%-25% of all HCC cases are due to HCV) (Bertuccio et al., 2017).

The pathogenesis of HCV infection and the progression of hepatic diseases accompanied by infection vary greatly among individuals, indicating the contribution of host characteristics to this process, such as age, ethnicity, and genetic factors, in particular, those controlling the expression of cytokine-encoding genes (Matsuura and Tanaka, 2016). Inflammatory cytokines have a double-edged sword. While they restrict viral replication inside host cells, their excessive production can cause deleterious effects on tissue architectures, especially in soft tissues such as the liver.

Interferon lambda 3 (IFN-λ3) is a proinflammatory cytokine that plays a crucial role in combating HCV by regulating the transcription of interferon-stimulating genes. The IFN-λ3-encoding gene is located on chromosome 19q13 (de Bitencorte et al., 2021). Several studies, including ours, have found that the rs12979860 single nucleotide polymorphism (SNP) present upstream of IFN-λ3 (~3 kb) has high predictive power for clinical (e.g., the progression of hepatic fibrosis) and treatment (e.g., the response to pegylated-IFNα plus ribavirin therapy and DAA therapy) outcomes in the case of HCV infection (El-Awady et al., 2012; Ge et al., 2009; Marwa 2021; Rauch et al., 2010; Tanaka et al., 2009; Thomas et al., 2009).

Although the prevalence of chronic HCV is on the decline, mortality from HCV-related liver diseases is still on the rise. HCV patients who experience liver cirrhosis remain at risk of developing HCC even after they reach SVR. Although there have been advancements in therapeutic and diagnostic tools, HCC still has a poor prognosis. In the current study, we aimed to evaluate whether the IFN-λ3 rs12979860 SNP is a predisposing genetic factor to identify HCV patients at risk of HCC development.

Materials and Methods

Ethical statement

The institutional ethical review board (medical research ethics committee at National Research Center, Cairo, Egypt) approved all experiments according to Helsinki Declaration 1975 revised in 2008. Before blood samples were drawn, written informed consent was obtained from each subject.

Study subjects

This study was performed on Egyptian HCV-infected patients with chronic liver disease collected from the Cairo University Centre for Hepatic Fibrosis, Endemic Medicine Department, Faculty of Medicine, Cairo University, and Kafr El-Sheikh Cardiac and Liver Center between September 2016 and February 2019. All the study patients were screened positive for HCV (both the antibody and the antigen), whereas they tested negative for anti-schistosomal antibody, hepatitis B surface antigen (HBsAg), autoimmune markers, and diabetes mellitus. No history of alcoholics, drug addiction, or other etiologies leading to chronic liver diseases was reported. All HCC patients had a history of liver cirrhosis due to chronic HCV infection, and they did not have recurrent HCC, did not receive prior intervention for HCC, or did not have malignancies other than HCC. The study controls (n = 60) were healthy subjects who tested negative for HBsAg and HCV antibodies and had normal liver enzymes as well as normal abdominal ultrasound. The HCV patients were stratified into 2 groups: (1) treatment-naïve patients (n=218) who had not previously received any treatment for HCV or HCC, and they were categorized into 2 groups: group I (n=123) included patients with advanced fibrosis (i.e., F2-F4) (Castera and Pawlotsky 2005), and group II included patients with HCC (n=95). (2) Treatment-experienced patients (n=124) who received SOF-based therapy for 12 weeks (with no history of previous treatment) and achieved SVR (SVR12) posttreatment (negative HCV RNA at the end of the treatment). DAA-treated patients were divided into 2 groups: group I (n=63) included patients with advanced hepatic fibrosis (i.e., F2-F4) who did not develop HCC within one year after the cessation of the treatment, and group II (n=61) included patients who were free of focal hepatic lesions before starting the DAA treatment, as confirmed by transabdominal ultrasonography, but they developed HCC within a year post-DAA therapy. The degree of hepatic fibrosis of treatment-naïve patients was diagnosed using both the Knodell and Metavir histological liver biopsy scoring system and transient elastography (FibroScan) measurement, while the degree of fibrosis of DAA-treated patients was estimated by the fibrosis-4 index (FIB-4).

Extraction of peripheral blood DNA

Genomic DNA was extracted from blood samples collected in EDTA-coated tubes using a Qiagen DNA extraction kit (Qiagen, Santa Clarita, CA). DNA was quantified using a NanoDrop™ Spectrophotometer (Thermo Scientific), and DNA samples were stored at -20°C until use.

Genotyping of the IFN-λ3 polymorphism

IFN-λ3 rs12979860 was genotyped by real-time PCR using the TaqMan MGB™ probe for allelic discrimination assay (Applied Biosystems, USA) and following the manufacturer's protocol. The assay mix contained 1 μL DNA, 1.25 μL of a 20X primer/probe, and 12.5 μL of 2X TaqMan® genotyping PCR master mix, and the final volume was completed to 25 μL with nuclease-free water (Invitrogen/Life Technologies, USA). The assay was performed on a Rotor-Gene real-time PCR system (Qiagen, Santa Clarita, CA) following the following thermal cycle profile: 95°C for 10 min and 40 cycles of

95°C for 15 s and 60°C for 1 min.

Statistical analysis

The patients' data (clinical and demographic) were compared between different groups using either the parametric unpaired Student's t test (data are presented as the mean ± standard deviation) or the nonparametric Mann–Whitney test (data are presented as the median ± range) based on the normality of the data. Chi square (χ^2) or Fisher's exact tests were used to analyze the distribution of different genotypes within groups (data are presented as frequencies and percentages together with odds ratios and 95% confidence intervals [CIs]). Stepwise binary logistic regression analysis was implemented to determine the predictors for HCC development. Different statistical comparisons were analyzed by Prism software version 5 (GraphPad, La Jolla, CA, USA), except the regression analysis, which was performed by SPSS version 16 (SPSS, Chicago, IL, USA). $P \leq 0.05$ was selected as the cut off for statistical significance between groups.

Results

HCV-infected patients' baseline data. Table 1 summarizes the patients' clinical and demographic data. The study included 218 treatment-naïve HCV patients: 123 patients with advanced stages of liver fibrosis (i.e., F2-F4) and 95 patients with HCC. The 2 groups of treatment-naïve patients had similar female/male ratios, hemoglobin (HB) levels, and total bilirubin levels (Table 1; $P > 0.05$). While HCC patients had higher mean age, alanine aminotransferase (ALT), aspartate transaminase (AST), and alpha-fetoprotein (AFP) levels, their albumin and platelet counts were lower than those of the F2-F4 group (Table 1; $P < 0.01$ for all parameters). Table 1 also shows the baseline features of 124 DAA-treated HCV patients with advanced fibrosis who achieved SVR12: 61 patients developed HCC within a year after the completion of the treatment course, and 63 patients did not develop HCC posttreatment. There were no statistically significant differences found in sex, ALT, or AST (Table 1; $P > 0.05$ for all parameters). However, the mean age, total bilirubin, and AFP were higher in the HCC posttreatment group, while albumin, HB, and platelet count were lower (Table 1; $P < 0.04$ for all parameters).

Distribution of the IFN-λ3 rs12979860 genotypes in treatment-naïve chronic HCV patients. Our data showed a differential distribution of the IFN-λ3 rs12979860 genotypes (CC, CT, TT) among healthy controls and treatment-naïve HCV patients with late fibrosis (i.e., F2-F4) and HCC (Table 2). CC was the most predominant genotype in the healthy controls (46.7%); however, the percentage of CC carriers decreased as the pathological state of the liver became more severe (26.9% for F2-F4 patients and 10.5% for HCC patients, Table 2). In contrast, the CT and TT genotypes were more predominant in

Table 1. The Clinical and Demographic Characteristics of HCV-Chronically Infected Patients

	Treatment-naïve			Treatment-experienced		
	Late fibrosis	HCC		Late fibrosis	HCC	
Characteristics			P value			P value
Number	123	95		63	61	
Female/ Male	29/94	24/71	0.8	Dec-51	Dec-49	**1**
Age (years)	52.7 ± 0.82	55.6 ± 0.78	**0. 01**	47.2 ± 0.69	59.8 ± 1.18	**0.0001**
Bilirubin total (mg/dL)	3.3 ± 0.44	2.8 ± 0.24	**0.3**	0.9 ± 0.03	1.18 ± 0.13	**0.04**
Albumin (g/dL)	3.4 ± 0.07	3± 0.08	**0.0005**	4.1 ± 0.04	3.4 ± 0.1	**0.0001**
HB (g/dL)	11.12 ± 0.22	11.02 ± 0.27	0.7	13.29 ± 0.16	12.13 ± 0.34	**0.009**
ALT (U/L), median (range)	37 (10-155)	48 (11-485)	**0.002**	48.0 (16-97)	45.0 (25-50)	0.3
AST (U/L), median (range)	63.5 (12-198)	72 (15-414)	**0.02**	41(26-100)	38 (30-50)	0.2
AFP, median (range)	12 (0.6-154)	486 (0.8-60,000)	**0.0001**	5.2 (0.1-102)	17.2 (1.4-8548)	**0.0078**
Platelet count (*1000)	153.4 ± 6.6	81.30 ± 9.3	**0.0001**	170.8 ± 4.5	143.3 ± 12.2	**0.01**

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; HB, hemoglobin; ALT, alanine aminotransferase; AST, aspartate transaminase; AFP, alpha fetoprotein; P-values were calculated by Chi-square test (data are expressed as frequency and percentage), Student's t test (data are expressed as the mean and standard deviation), and Mann-Whitney test (data are expressed as the median and range); Statistically significant differences are shown in bold.

Table 2. Distribution of the IFN-λ3 rs12979860 Genotypes among Healthy Subjects and Treatment- naïve HCV Chronically Infected Patients with Late Stages of Fibrosis and HCC

Genotype	Controls (n=60) n (%)	Late fibrosi (n=123) n (%)	HCC (n=95) n (%)	P value Late fibrosis vs HCC
CC	28 (46.7%)	33 (26.9%)	10 (10.5%)	0.001
CT	25 (41.6%)	71 (57.7%)	56 (59%)	
TT	7 (11.7%)	19 (15.4%)	29 (30.5%)	

n means sample size; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; P-values were calculated by Chi-square test, and data are presented as frequency and percentage.

Table 3. Distribution of the IFN-λ3 rs12979860 Genotypes and Alleles among Treatment-naïve HCV-Chronically Infected Patients with Late Stages of Fibrosis and HCC.

Genotypes	Late fibrosis (n=123) n (%)	HCC (n=95) n (%)	OR (95% CI)	P value
CC (F)	33 (26.9%)	10 (10.5%)	1.00 (Ref)†	-
CT/TT (Unf)	90 (73.1%)	85 (89.5%)	3.1 (1.44-6.71)	0.003
Alleles				
C (F)	137 (55.7%)	76 (40%)	1.00 (Ref)†	-
T (Unf)	109 (44.3%)	114 (60%)	1.89 (1.28-2.76)	0.001

n means sample size; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; F, favorable; Unf, unfavorable; OR, odds ratio; CI, confidence interval; " †" denotes a reference category; Fisher's exact test was followed to calculate the P-values, and data are demonstrated as frequency and percentage.

Table 4. Binary Logistic Regression Analysis of the Predictors for HCC Development in Treatment-naïve Patients

	Regression coefficient	Standard error	OR	95% CI	P value
Genotypes (Unf vs F)	1.294	0.459	3.648	1.484-8.969	0.005
Age	0.039	0.018	1.039	1.004-1.076	0.028
ALT	0.008	0.003	1.008	1.002-1.015	0.01

Unf, unfavourable; F, favourable; ALT, alanine aminotransferase; OR, odds ratio; CI, confidence interval.

Table 5. Distribution of the IFN-λ3 rs12979860 Genotypes among DAA-treated HCV-chronically Infected Patients (HCC vs no HCC development after treatment).

Genotypes	Late fibrosis (n=63) n (%)	HCC (n=61) n (%)	P value
CC	25 (52.2%)	15 (56.5%)	0.14
CT	30 (43.3%)	30 (35.5%)	
TT	8 (4.5%)	13 (8.2%)	

n, means sample size; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; P-values were calculated by Chi-square test, and data are shown as frequency and percentage.

HCC patients (Table 2; 59% and 30.5%, respectively) than in F2-F4 patients (Table 2; 57.7% and 15.4%;, respectively, $P < 0.001$) and healthy controls (41.6% and 11.7%, respectively). When comparing the prevalence of the favorable genotype and allele among different groups of treatment-naïve HCV patients (considering CC as a reference genotype), the HCC patients had higher frequencies of the unfavorable CT/TT combined genotypes (Table 3; odds ratio 3.1, 95% CI 1.44-6.71, $P = 0.003$) and T allele (Table 3; odds ratio 1.89, 95% CI 1.28-2.76, $P = 0.001$) than did the F2-F4 patients. Collectively, these data show that IFN-λ3 rs12979860 is a possible prognostic biomarker for the transition from advanced fibrosis to HCC.

Binary logistic regression analysis to determine the risk factors for HCC development. Binary regression analysis was used to determine the cofounding factors that can predict the transition from late fibrosis to HCC. The regression model was built with 3 significant, independent factors associated with the risk of HCC development: age (Table 4; regression coefficient 0.039, odds ratio 1.039, 95% CI 1.004-1.076, $P = 0.028$), ALT (Table 4; regression coefficient 0.008, odds ratio 1.008, 95% CI 1.002-1.015, $P = 0.01$), and the rs12979860 unfavorable CT/TT genotypes (Table 4; regression coefficient 1.294, odds ratio 3.65, 95% CI 1.484-8.969, $P = 0.005$). The results confirm that rs12979860 has a significant predictive value for the progression of fibrosis to HCC.

Distribution of the IFN-λ3 rs12979860 genotypes in treatment-experienced chronic HCV patients with or without HCC posttreatment. Next, we proposed that the IFN-λ3 rs12979860 SNP might similarly contribute to the progression of HCC post-DAA treatment. To test this hypothesis, we investigated the distribution of the rs12979860 variants among 2 groups of SVR patients: patients with F2-F4 who did not develop HCC within a year after the completion of SOF-based treatment and patients who developed HCC within the specified time period. Our data showed a comparable distribution of the three different genotypes of rs12979860 (Table 5; $P = 0.14$) among the 2 groups of HCV patients: no HCC posttreatment group (Table 5, CC 52.2%, CT 43.3%, and TT 4.5%) and HCC posttreatment group (Table 5; CC 56.5%, CT 35.5% and TT 8.2%). Overall, IFN-λ3 rs12979860 is not correlated with the risk of HCC development after the clearance of HCV infection by DAA.

Discussion

Understanding the interaction between HCV and the host immunity that leads to HCC development is important, particularly defining the role of immunogenetic risk factors in this context. Our previous studies presented SNPs in key innate immunity genes, including IFN-λ3 rs12979860, as predisposing factors for progressive liver fibrosis accompanied by HCV infection (Ibrahim et al., 2016). In the current study, and in support of our earlier work, we showed a positive correlation between IFN-λ3 rs12979860 and the progression of HCC development from late stages of fibrosis in treatment-naïve patients.

However, rs12979860 lost such predictive power for HCC development accelerated by DAA treatment.

IFN-λ3, a type III interferon, plays essential antiviral and antitumor activities (Simili et al., 2019). However, the exact mechanisms underlying these functions are still not completely unraveled. To trigger its antiviral effect, IFN-λ3 induces IFN-stimulated genes, which ultimately inhibit viral replication in infected cells (de Veer et al.,2001; Stark, 2007). rs12979860 is the most significant SNP of the IFN-λ3 gene (located 3 kb upstream of the gene in an intronic region). It has a causal association with different aspects of HCV disease. It is highly correlated with the clearance of HCV either spontaneously (Thomas et al., 2009) or treatment-induced (peg IFN (Ge et al., 2009) and DAAs (Marwa, 2021)). Moreover, we and others have shown significant effects of IFN-λ3 rs12979860 variants on fibrosis progression in HCV-infected patients (Abe et al., 2010; Bochud et al., 2012; El-Awady et al., 2012). However, the relationship between IFN-λ3 rs12979860 and the incidence of HCC did not reach conclusive and consistent results. A recent study conducted on the Italian population reported that rs12979860-C allele carriers among HCV-infected patients with liver cirrhosis are less likely to develop end-stage liver diseases, including HCC (Fabris et al., 2011). In agreement with this study, another finding reported a protective role for the C-allele against the development of HCV-related HCC and the recurrence of HCV following liver transplantation (Eurich et al., 2012). Similarly, the current data suggest that the IFN-λ3 rs12979860-CC genotype seems to diminish the overall risk of HCC development, and it is a promising marker for the transition from the late stages of liver fibrosis to HCC. In contrast to our finding, several studies concluded that IFN-λ3 rs12979860 genetic variation has no effect on the development of HBV or HCV-related HCC (Akkiz et al., 2014; Zekri et al., 2014).

SOF is a nucleotide analog that has been used since 2013 to treat HCV infection (Lin et al., 2009; Silva et al., 2013; Zhang, 2016). SOF specifically targets HCV NS5B RNA polymerase and thereby interferes with virus replication. Over the past few years, SOF has shown great efficacy against different HCV genotypes (Lin et al., 2009; Silva et al., 2013; Zhang, 2016), and due to its potency, SOF is shared by different therapeutic protocols.

Despite the spike in the SVR achieved by DAAs (more than 90% in most cases), DAAs are still nucleotide analogs that can cause several complications in treated patients. A major reported side effect of DAA treatment is the high incidence of HCC posttreatment, either the early occurrence (de novo) or recurrence of pre-existing HCC (Conti et al., 2016; Reig et al., 2016; Romano et al., 2018). Cirrhotic patients are the most susceptible category to HCC development (Goto and Kato, 2015; Lee et al., 2014); 1•05% of HCV patients who achieve SVR remain at risk of HCC development if they suffer from advanced liver diseases (Huang et al., 2017). A plausible explanation for the latter is the suppression of host cell immunity following HCV clearance by DAA treatment. The decline in the levels of inflammatory cytokines, associated with the immune suppression status, might lead to a reduction in immunosurveillance and in turn the proliferation of invisible dysplastic nodules spread out across the damaged liver (Chu et al., 2017), which all end in the development of HCC (Chu et al., 2017; Rinaldi et al., 2020). SNP studies have linked polymorphisms in key immunity-related genes to the effectiveness of HCV treatments and predisposition to associated liver diseases, mainly HCC development. However, the role of these genetic variables in HCC occurrence in the case of DAA therapy is still unclear. Considering that SVR patients approaching cirrhosis are the most susceptible category to HCC, the patients in this study were selected from SVR patients who suffer from advanced liver fibrosis. In contrast to Simili et al (Simili et al., 2019), who characterized a protective role for the CC genotype against the development of HCC posttreatment, we did not find a preference in genotype distribution between SVR patients either with or without HCC posttreatment. Our study comes in line with the Ahmed et al., (2021) study, which could not find a differential distribution of rs12979860 genotypes between HCC and non-HCC patients.

In conclusion, this study elaborates on the role of IFN-λ3 rs12979860 genotyping as a prognostic tool to predict HCV-infected patients who have a higher susceptibility to HCC progression. However, our results showing the negative impact of rs12979860 on DAA-related hepatocarcinogenesis should be interpreted with caution because of the small sample size included in the study. Therefore, studies with a larger number of patients are warranted to validate our conclusion.

Author Contribution Statement

All the authors have contributed to and agreed on the content of the manuscript. Ibrahim MK and AbdElrahman M designed the experiments and analyzed the data. Ibrahim MK and AbdElrahman M wrote and revised the manuscript. Ibrahim MK performed the majority of the molecular biology experiments. Amal Z Barakat and Bader El Din NG performed some of the molecular biology experiments and revised the manuscript. Tawfik S, Khairy A, Zayed N, Yosry A, and elbatae H performed the clinical work.

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Ethical approval and Consent to participate

All experiments were approved by the medical research ethics committee of the National Research Centre in Cairo, Egypt, in accordance with the Helsinki Declaration of 1975, as revised in 2008. Before collecting blood samples, each patient signed a written informed consent form.Reg No for etical approval certificate 16198.

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This work was not approved by any scientific Body and was not part of any approved student thesis.

Ethical statement

The institutional ethical review board (medical research ethics committee at National Research Center, Cairo, Egypt) approved all experiments according to Helsinki Declaration 1975 revised in 2008. Before blood samples were drawn, written informed consent was obtained from each subject.

Availability of supporting data

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

References

Abe H, Ochi H, Maekawa T, et al (2010). Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol*, **53**, 439-43.

Akkiz H, Kuran S, Akgollu E, et al (2014). The role of interleukin 28B gene polymorphism in Turkish patients with hepatocellular carcinoma. *Ann Hepatol*, **13**, 788-95.

Axley P, Ahmed Z, Ravi S, Singal AK (2018). Hepatitis C Virus and Hepatocellular Carcinoma: A Narrative Review. *J Clin Transl Hepatol*, **6**, 79-84.

Baumert TF, Juhling F, Ono A, Hoshida Y (2017). Hepatitis C-related hepatocellular carcinoma in the era of new generation antivirals. *BMC Med*, **15**, 52.

Bertuccio P, Turati F, Carioli G, et al (2017). Global trends and predictions in hepatocellular carcinoma mortality. *J Hepatol*, **67**, 302-9.

Bochud PY, Bibert S, Kutalik Z, et al (2012). IL28B alleles associated with poor hepatitis C virus (HCV) clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. *Hepatology*, **55**, 384-94.

Castera L, Pawlotsky JM (2005). Noninvasive diagnosis of liver fibrosis in patients with chronic hepatitis C. *Med Gen Med*, **7**, 39.

Chu PS, Nakamoto N, Taniki N, et al (2017). On-treatment decrease of NKG2D correlates to early emergence of clinically evident hepatocellular carcinoma after interferon-free therapy for chronic hepatitis C. *PLoS One*, **12**, e0179096.

Conti F, Buonfiglioli F, Scuteri A, et al (2016). Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol*, **65**, 727-33.

de Bitencorte JT, Rech TF, Lunge VR, et al (2021). Association of interferon lambda-4 rs12979860 polymorphism with hepatocellular carcinoma in patients with chronic hepatitis C infection. *World J Hepatol*, **13**, 109-19.

de Veer MJ, Holko M, Frevel M, et al (2001). Functional classification of interferon-stimulated genes identified using microarrays. *J Leukoc Biol*, **69**, 912-20.

El-Awady MK, Mostafa L, Tabll AA,et al (2012). Association of IL28B SNP With Progression of Egyptian HCV Genotype 4 Patients to End Stage Liver Disease. *Hepat Mon*, **12**, 271-7.

El-Serag HB (2012). Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology*, **142**, 1264-73.

Eurich D, Boas-Knoop S, Bahra M, Neuhaus R, et al (2012). Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and posttransplant antiviral therapy. *Transplantation*, **93**, 644-9.

Fabris C, Falleti E, Cussigh A, et al (2011). IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol*, **54**, 716-22.

Ge D, Fellay J, Thompson AJ, et al (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*, **461**, 399-401.

Goto K, Kato N (2015). MICA SNPs and the NKG2D system in virus-induced HCC. *J Gastroenterol*, **50**, 261-72.

Huang CF, Huang CY, Yeh ML,et al (2017). Genetics Variants and Serum Levels of MHC Class I Chain-related A in Predicting Hepatocellular Carcinoma Development in Chronic Hepatitis C Patients Post Antiviral Treatment. *E Bio Med*, **15**, 81-9.

Ibrahim MK, Salama H, Abd El Rahman M, et al (2016). Three Gene Signature for Predicting the Development of Hepatocellular Carcinoma in Chronically Infected Hepatitis C Virus Patients. *J Interferon Cytokine Res*, **36**, 698-705.

Lee MH, Lu SN, Yuan Y, et al (2014). Development and validation of a clinical scoring system for predicting risk of HCC in asymptomatic individuals seropositive for anti-HCV antibodies. *PLoS One*, **9**, e94760.

Lin TI, Lenz O, Fanning G, et al (2009). In vitro activity and preclinical profile of TMC435350, a potent hepatitis C virus protease inhibitor. *Antimicrob Agents Chemother*, **53**, 1377-85.

Makarova-Rusher OV, Altekruse SF, McNeel TS, et al (2016). Population attributable fractions of risk factors for hepatocellular carcinoma in the United States. *Cancer*, **122**, 1757-65.

Marwa K. Ibrahim MA, Noha G, et al (2021). The impact of genetic variations in sofosbuvir metabolizing enzymes and innate immunity mediators on treatment outcome in HCV-infected patients. Microbial Pathogenesis:Fothcoming.

Matsuura K, Tanaka Y (2016). Host genetic variants influencing the clinical course of hepatitis C virus infection. *J Med Virol*, **88**, 185-95.

Nancy Abdel Fattah Ahmed ASHA, Rizk Ahmed EB, El-Zayyadi IAE-H (2021). IL-28B single nucleotide polymorphism as a predictor of hepatocellular carcinoma after treatment of chronic hepatitis C patients with direct acting antivirals. *Egpt Liver J*, **11**.

Omran D, Alboraie M, Zayed RA, et al (2018). Towards hepatitis C virus elimination: Egyptian experience, achievements and limitations. *World J Gastroenterol*, **24**, 4330-40.

Rauch A, Kutalik Z, Descombes P, et al (2010). Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology*, **138**, 1338-45, 1345 e1-7.

Reig M, Marino Z, Perello C, et al (2016). Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol*, **65**, 719-26.

Rinaldi L, Nevola R, Franci G, et al (2020). Risk of Hepatocellular Carcinoma after HCV Clearance by Direct-Acting Antivirals Treatment Predictive Factors and Role of Epigenetics. *Cancers (Basel)*, **12**.

Romano A, Angeli P, Piovesan S, et al (2018). Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: A prospective population study. *J Hepatol*, **69**, 345-52.

Silva MO, Treitel M, Graham DJ, et al (2013). Antiviral activity of boceprevir monotherapy in treatment-naive subjects with chronic hepatitis C genotype 2/3. *J Hepatol*, **59**, 31-7.

Simili A, Mazzella G, Ravaioli F, et al (2019). Interleukin 28 Polymorphisms and Hepatocellular Carcinoma Development after Direct Acting Antiviral Therapy for Chronic Hepatitis

C. *J Gastrointestin Liver Dis*, **28**, 449-56.
Stark GR (2007). How cells respond to interferons revisited: from early history to current complexity. *Cytokine Growth Factor Rev*, **18**, 419-23.
Tanaka Y, Nishida N, Sugiyama M, et al (2009). Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet*, **41**, 1105-9.
Thomas DL, Thio CL, Martin MP, et al (2009). Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature*, **461**, 798-801.
Zekri AR, Salama H, Medhat E, et al (2014). IL28B rs12979860 gene polymorphism in Egyptian patients with chronic liver disease infected with HCV. *Asian Pac J Cancer Prev*, **15**, 7213-8.
Zhang X (2016). Direct anti-HCV agents. *Acta Pharm Sin B*, **6**, 26-31.