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Disheveled EGL-10 and pleckstrin domain-containing 5 rs1012068 T/G gene polymorphism among Egyptian chronic HCV-infected patients: disease progression and related complications

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Abstract

Hepatitis C virus (HCV) infection related complications including fibrosis, cirrhosis and hepatocellular carcinoma (HCC) are influenced by host genetic factors. Identification of emerging host genetic variations is of promising value. Disheveled EGL-10 and pleckstrin domain-containing 5 (DEPDC5) rs1012068 T/G gene polymorphism has been implicated in liver disease. This study aimed to assess DEPDC5 rs1012068 T/G gene polymorphism with disease progression and related complications among Egyptian patients with chronic HCV infection. Sixty chronic HCV-infected patients and 60 apparently healthy controls were recruited in this study. Patients were classified into 20 with liver fibrosis, 20 with liver cirrhosis and 20 with HCC; all recruited from Outpatients Clinic and Tropical Medicine Inpatient Department, Faculty of Medicine, Beni-Suef University Hospital. DEPDC5 rs1012068 T/G gene polymorphism was assayed by real time-polymerase chain reaction (RT-PCR) TaqMan allelic discrimination. DEPDC5 rs1012068 GG genotype and G allele variants showed statistically significant higher frequency among patients with liver fibrosis when compared to controls (OR (95% CI) 10.500 (2.086 - 52.851), *P*= 0.004 and 0.388 (0.155 - 0.971), *P*= 0.011), respectively. DEPDC5 rs1012068G allele variant showed statistically significant higher frequency among patients with liver fibrosis when compared to HCC patients (OR (95% CI) 3.316 (1.286 - 8.550), *P*= 0.012) and to both HCC and cirrhosis patients (OR (95% CI) 2.579 (1.187-5.645), *P*= 0.016). In conclusion, our results suggest that DEPDC5 rs1012068 G allele could be considered genetic risk allele for liver fibrosis and disease progression among Egyptian patients with chronic HCV infection.

Keywords DEPDC5 rs1012068 T/G, hepatitis C virus, real-time polymerase chain reaction (RT-PCR), liver fibrosis

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Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease.[1] Chronic hepatitis C virus infection influences the liver, causing hepatic inflammation and fibrosis and increases the risk of cirrhosis and hepatocellular

carcinoma (HCC).[2] Hepatitis C virus infection is a chronic health problem which can be fatal if left without treatment.[3] Seventy one million people with chronic HCV infection is the estimated prevalence globally, where approximately 2 million new infections occur yearly.[4] Egypt shows the highest prevalence rates of HCV in the world with about 10% chronic HCV infection among people aged 15 to 59 years.[5, 6]

Dishevelled EGL-10 and Pleckstrin domain-containing (DEPDC) proteins are globular protein domains of approximately 80 amino acids; all share a high degree of sequence and structure similarity with different combinations of domains generating seven members, (DEPDC1-DEPDC7) providing various functions where most of which are involved with signal transduction.[7] Dishevelled EGL-10 and Pleckstrin domain-containing 5 (DEPDC5) is a signaling molecule involved in the phosphoinositide-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway which has been demonstrated to block the effect of mammalian target of rapamycin (mTOR), a multi-functional protein involved in many cellular systems inflammation, cell growth and tumorigenesis.[8]

The gene encoding DEPDC5 is located on human chromosome 22q12.2-q12.3.[9] The DEPDC5 locus variants lead to amino acidic change; they are biologically more likely to result in a functional change of protein where rs1012068 intronic variant was reported to cause changes in DEPDC5 structure and/or function and affect the protein expression.[10] DEPDC5 rs1012068 T/G gene polymorphism has been indicated to be associated with progression of liver disease.[6]

This study aimed to assess the association between DEPDC5 rs1012068 T/G gene polymorphism with disease progression and related complications including liver fibrosis, cirrhosis, and HCC among Egyptian patients with chronic HCV infection.

Subjects and Methods

Subjects

Egyptian patients infected with chronic HCV (n = 60) were recruited from those attending Outpatients Clinic and Tropical Medicine Inpatient Department, Faculty of Medicine, Beni-Suef University Hospital; and diagnosed on the basis of the presence of antibodies against HCV (anti HCV) and serum HCV RNA for more than six months. The patients were classified into three groups: Group I: patients with liver fibrosis (n = 20), Group II: patients with liver cirrhosis (n = 20) and Group III: patients with HCC (n = 20). Sixty normal healthy subjects volunteered to participate in this study as a control group. Control subjects were characterized by the absence of any known serological marker for HCV, HCV RNA or any evidence of liver disease. Patients with co-infection with hepatitis B virus (HBV), and/or human immunodeficiency virus (HIV), alcohol ingestion, autoimmune hepatitis, hemo-chromatosis and Wilson's disease were excluded from the study. The study protocol was reviewed and approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (January 2019). The study conforms to the Code of Ethics of the World Medical Association (Declaration of Helsinki).[11] Data confidentiality was preserved. An informed consent was obtained from each participant.

Methods

Data collection and Imaging

All participants were subjected to full history taking, general examination and imaging including fibrosis score (FIB), upper endoscopy and abdominal ultrasound. Signs for inclusion of patients were liver fibrosis: atrophic right lobe, hypertrophy of the caudate and lateral left lobes, liver surface nodularity, an expanded gallbladder fossa, and narrow hepatic veins. Liver cirrhosis included: irregular surface, increased angle of lower border > 45°, coarse texture and irregular hepatic veins. Hepatocellular carcinoma included hypo echoic foci, portal vein thrombosis. Signs of portal hypertension included: splenomegaly > 13cm, portosystemic collateral, portal vein > 12mm, dilated splenic and superior mesenteric vein.

Biochemical analysis

Routine laboratory investigations; including alanine transaminase (ALT), aspartate

transaminase (AST) and albumin were analyzed on an automated chemistry analyzer (Bechman CX5 automated chemistry analyzer, Ireland) by its own commercial kits, according to manufacturer's instructions. Quantitative detection of serum alpha-feto protein (AFP) was assayed by enzyme immunoassay (EIA) based on solid phase enzyme immunoassay sandwich principle using CanAg AFP EIA Kit (Fujirebio Diagnostics Inc., Sweden) according to manufacturer's instructions.

Genotyping of DEPDC5 rs1012068 T/G

Genomic DNA was isolated from ethylene-diaminetetraacetic acid (EDTA) anticoagulated whole blood using QIAamp DNA Mini Kit (cat. No. 51104, Qiagen,USA) according to manufacturer's instructions. DEPDC5 rs1012068 T/G gene polymorphism was assessed by real time- polymerase chain reaction (RT-PCR) TaqMan allelic discrimination assay, purchased from Clinilab-Aldrich Corporation (Spruce St, St Louis MO, USA). This assay was designed to utilize single nucleotide polymorphism (SNP) specific primers and two allele-specific TaqMan® minor groove binder (MGB) probes. For DEPDC5 rs1012068 T/G, probe AGGGAGAGGCTGCAATCAGGGGCTA was specific for allele 1 sequence and was labelled with the reporter dye VIC and the other TTAGAAGAATGTTACAGCCCTCCCT was specific for allele 2 sequence and was labelled with the reporter dye FAM. The reactions were performed in a total reaction volume of 25 µl using 20 ng of genomic DNA. The mixture contained 900 nM of each primer and 200 nM of each labeled probe and TaqMan universal PCR master mix. The PCR was performed by Step-One real-time PCR Applied Biosystem; according to the following cycling parameters: 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds for denaturation and 60°C for 1.5 minutes for annealing and extension.[12] Interpretation of data was done using allelic discrimination plot as a scatter plot of allele 1 (VIC dye) versus allele 2 (FAM dye) using Life Technologies real-time instrument software plots (Figure 1).

Statistical methods

Data were analyzed using IBM SPSS advanced statistics version 25 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation (SD) for normally distributed variables or median and interquartile range (IQR) for non-normally distributed variables as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). Binary logistic regression was used to assess the prediction of the genotype that increases the probability of the target disease (fibrosis, cirrhosis, and HCC compared with controls) with consideration of TT genotype as a reference category. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. A *P*-value < 0.05 was considered significant.[13]

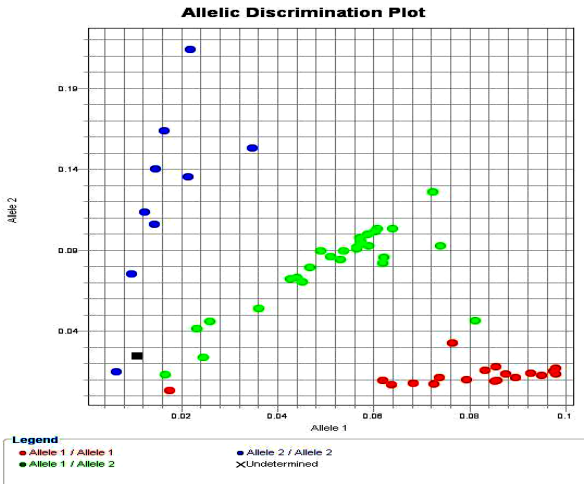

Figure 1. Allelic discrimination plot showing TT, GT and GG genotypes of DEPDC5 rs1012068 T/G gene.

Results

Sixty chronic HCV-infected patients were included in this study; 31 males (51.7%) and 29 females (48.3%) with mean age of 52.5±12

years. Sixty apparently healthy volunteers were included as control group; 30 males (50%) and 30 females (50%) with mean age of 55.3 ±7.3 years. The control subjects were age and sex matched with patients (*P* = 0.328 and 0.494 respectively).

By evaluating the different studied laboratory parameters, HCV patients showed statistically significantly higher concentration than controls as regard ALT and AST (*P* <0.001). However, albumin concentration was significantly lower in HCV patients than controls (*P* <0.001) (Table 1).

Table 1. Laboratory findings in HCV-infected patients and controls.

Variable	Patients (n=60) mean ± SD	Controls (n=60) mean ± SD	*P*-value
ALT (IU/L)	45.20 ± 29.93	24.95± 14.59	<0.001
AST (IU/L)	46.37±21.20	25.85±13.50	<0.001
Albumin (g/dl)	2.91 ± 0.97	3.93 ± 0.39	<0.001

ALT: alanine transaminase, AST: aspartate transaminase, intergroup comparison was done using Independent t-test, *P* <0.05 is significant.

AFP concentration was statistically significantly higher among HCC patients with median (IQR) of 92.50 ng/ml (26.75-229.25) when compared to control subjects and patients with benign conditions (fibrosis and cirrhosis) with median (IQR) of 6.90 ng/ml (5.00-9.07) (*P* <0.001).

The frequency of DEPDC5 rs1012068 genotypes and alleles in controls was compared to all patients (Table 2), fibrosis patients (Table 3), cirrhosis patients (Table 4) and HCC patients (Table 5). Statistical significance was found among fibrosis group where DEPDC5 rs1012068 GG genotype and G allele variants showed higher frequency when compared to controls (*P*= 0.004 and 0.011, respectively) (Table 3). Other comparisons between groups did not reach statistical significance (*P* >0.05).

Table 2. DEPDC5 rs1012068 T/G genotypes and alleles between all patients and controls.

rs1012068 Genotypes, and Alleles	All patients (n=60) Frequency n (%)	Controls (n=60) Frequency n (%)	*P*-value	OR	95% CI for OR	
					Lower	Upper
GT TT	25 (41.7%) 25 (41.7%)	30 (50%) 27 (45%)	NS	0.900	0.421	1.925
GG TT	10 (16.6%) 25 (41.7%)	3 (5%) 27 (45%)	NS	3.600	0.888	14.602
TT GG and GT	25 (41.7%) 35 (58.3%)	27 (45%) 33 (55%)	NS	0.873	0.315	2.420
T G	75 (62.5%) 45 (37.5%)	84 (70%) 36 (30%)	NS	0.714	0.331	1.544

Intergroup genotype distribution was done using Binary logistic regression, *P* >0.05 is not significant (NS).

Table 3. DEPDC5 rs1012068 T/G genotypes and alleles between fibrosis patients and controls.

rs1012068 Genotypes, and Alleles,	Fibrosis patients (n=20) Frequency n (%)	Controls (n=60) Frequency n (%)	*P*-value	OR	95% CI for OR	
					Lower	Upper
GT TT	7 (35%) 6 (30%)	30 (50%) 27 (45%)	NS	1.050	0.314	3.514
GG TT	7 (35%) 6 (30%)	3 (5%) 27 (45%)	0.004	10.50	2.086	52.851
TT GG and GT	6 (30%) 14 (70%)	27 (45%) 33 (55%)	NS	0.524	0.143	1.923
T G	19 (47.5%) 21 (52.5%)	84 (70%) 36 (30%)	0.011	0.388	0.155	0.971

Intergroup genotype distribution was done using Binary logistic regression, *P* >0.05 is not significant (NS).

Table 4. DEPDC5 rs1012068 T/G genotypes and alleles between cirrhosis patients and controls.

rs1012068 Genotypes, and Alleles	Cirrhosis patients (n=20) Frequency n (%)	Controls (n=60) Frequency n (%)	*P*-value	OR	95% CI for OR	
					Lower	Upper
GT TT	10 (50%) 8 (40%)	30 (50%) 27 (45%)	NS	1.125	0.388	3.264
GG TT	2 (10%) 8 (40%)	3 (5%) 27 (45%)	NS	2.250	0.318	15.901
TT GG and GT	8 (40%) 12 (60%)	27 (45%) 33 (55%)	NS	0.815	0.232	2.860
T G	26 (65%) 14 (35%)	84 (70%) 36 (30%)	NS	0.796	0.312	2.033

Intergroup genotype distribution was done using Binary logistic regression, *P* >0.05 is not significant (NS).

Table 5. DEPDC5 rs1012068 T/G genotypes and alleles between HCC patients and controls.

rs1012068 Genotypes, and Alleles	HCC patients (n=20) Frequency n (%)	Controls (n=60) Frequency n (%)	*P*-value	OR	95% CI for OR	
					Lower	Upper
GT TT	8 (40%) 11 (55%)	30 (50%) 27 (45%)	NS	0.736	0.265	2.048
GG TT	1 (5%) 11 (55%)	3 (5%) 27 (45%)	NS	0.818	0.077	8.746
TT GG and GT	11 (55%) 9 (45%)	27 (45%) 33 (55%)	NS	1.494	0.430	5.192
T G	30 (75%) 10 (25%)	84 (70%) 36 (30%)	NS	1.286	0.480	3.442

Intergroup genotype distribution was done using Binary logistic regression, *P* >0.05 is not significant.

When comparing DEPDC5 rs1012068 T/G genotypes and alleles among liver fibrosis patients to other patient's groups, DEPDC5 rs1012068 G allele variant showed statistically significantly higher frequency among liver fibrosis patients when compared to HCC patients (*P*= 0.012) (Table 6), and to both HCC and cirrhosis patients (*P*= 0.016) (Table 7).

Table 6. DEPDC5 rs1012068 T/G genotypes and alleles between fibrosis and HCC patients

rs1012068 Genotypes, and Alleles	Fibrosis patients (n=20) Frequency n (%)	HCC patients (n=20) Frequency n (%)	*P* -value	OR	95% CI for OR	
					Lower	Upper
TT GG and GT	6 (30%) 14 (70%)	11 (55%) 9 (45%)	NS	2.852	0.777	10.467
T G	19 (47.5%) 21 (52.5%)	30 (75%) 10 (25%)	0.012	3.316	1.286	8.550

Intergroup genotype distribution was done using Binary logistic regression, *P*-value >0.05 is not significant (NS).

Table 7. DEPDC5 rs1012068 T/G genotypes and alleles between fibrosis and both HCC and cirrhosis patients.

Rs1012068 Genotypes, and Alleles	Fibrosis patients (n=20) Frequency n(%)	HCC and cirrhosis patients (n=40) Frequency n (%)	*P*-value	OR	95% CI for OR	
					Lower	Upper
TT GG and GT	6 (30%) 14 (70%)	19 (47.5%) 21 (52.5%)	NS	2.111	0.675	6.601
T G	19 (47.5%) 21 (52.5%)	56 (70%) 24 (30%)	0.016	2.579	1.187	5.645

Intergroup genotype distribution was done using Binary logistic regression, *P*-value >0.05 is not (NS) significant.

Discussion

Several studies aimed to identify promising prognostic genetic factors predicting clinical outcome and help in management of HCV-infected patients.[12] Our study was conducted to assess the association of DEPDC5 rs1012068 T/G gene polymorphism among chronic HCV-infected Egyptian patients with disease progression and complications including liver fibrosis, cirrhosis, and HCC.

In the current study, on comparing different studied groups, statistical significance was found in fibrosis group where DEPDC5 rs1012068 GG genotype and G allele variants showed higher frequency among patients with liver fibrosis when compared to controls (*P*= 0.004 and 0.011, respectively). Furthermore, DEPDC5 rs1012068 G allele variant showed statistically significant higher frequency among patients with liver fibrosis when compared to HCC patients (*P*= 0.012) and to both HCC and cirrhosis patients (*P*= 0.016). Our results came in agreement with a previous study which reported that DEPDC5 rs1012068 G allele variant was associated with liver fibrosis stage in Asian subjects.[14] Also, it was found that the prevalence of DEPDC5 rs1012068 T/G gene polymorphism was higher in subjects with moderate/severe fibrosis than in those with no/mild fibrosis.[10] Therefore, the authors suggested that DEPDC5 rs1012068 G allele variant was an independent risk factor for moderate/severe fibrosis susceptibility in European subjects with chronic HCV infection predicting faster fibrosis progression. This was ascertained by another study which reported 40% increased risk of severe fibrosis with DEPDC5 rs1012068 T/G gene polymorphism.[15]

Hepatic fibrogenesis is a pathophysiological outcome of chronic liver injury characterized by excessive accumulation of extracellular matrix proteins.[16] An in vitro study on immortalized hepatic stellate cells (HSCs) with high DEPDC5

expression was performed.[10] The authors reported that down-regulation of DEPDC5 resulted in increased expression of b-catenin through the Wnt/b-catenin pathway and production of its target profibrotic molecule matrix metallopeptidase 2 (MMP2); a secreted enzyme inducing remodeling of extracellular matrix in favor of type 1 collagen and involved in fibrosis progression. Accordingly, our findings can find its explanation based on researchers who reported that DEPDC5 gene pathogenic G variant is an inactivating variant leading to down-regulation of the encoded DEPDC5 protein.[15] Furthermore, down-regulation of the DEPDC5 protein caused by DEPDC5 gene polymorphism leads to decreasing its blocking effect on mTOR signaling pathway.[8, 15] The consequent increased activity of the mTOR pathway promotes fibrosis through transforming growth factor- β (TGF- β) independent mechanism,[17]which is considered as another explanation of the molecular basis of genetic association of DEPDC5 variants with fibrosis progression. Fibrosis and distortion of hepatic architecture following endoplasmic reticulum stress, liver injury and inflammation is caused by HCV proteins particularly core and NS5A, [18, 12] which was described to activate the mTOR signaling pathway induced by HCV-infected hepatocytes.[19]

Liver fibrosis is a considerable complication of HCV infection and can progress to cirrhosis, liver failure, and HCC.[20] The presence of fibrosis affects the development of hepatocellular carcinoma being a dynamic process that involves cross-talk between hepatocytes, HSCs, sinusoidal endothelial cells and both resident and infiltrating immune cells; through the influence of cytokines, and chemokines; and mitochondria and metabolic changes in hepatic stellate cells modulating this process.[16] Finally, DEPDC5 (rs1012068) could be a valuable indicator in diagnosing the progression of liver disease to HCC risk related HCV patients.[6]

In conclusion, this study suggested an association between DEPDC5 rs1012068 T/G gene polymorphism and liver fibrosis in chronic HCV Egyptian patients. G allele may be a risk factor for liver fibrosis among Egyptian patients.

Author Contributions

HMF and HFG contributed to the study conception and design. KA, HMF and HFG contributed to material preparation, data collection and analysis. DA provided clinical support. HMF wrote the manuscript draft. All authors read and approved the final 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 study protocol was reviewed and approved by the Research Ethical Committee, Faculty of Medicine, Beni-Suef University (January 2019).

Informed consent

An informed consent was obtained from each participant.

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