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A Polymorphism in the Microsomal Triglyceride Transfer Protein Can Predict the Response to Antiviral Therapy in Egyptian Patients with Chronic Hepatitis C Virus Genotype 4 Infection

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Background/Aims: A polymorphism in the microsomal triglyceride transfer protein (MTP) is associated with hepatic fibrosis, and carriers showed higher levels of steatosis, higher levels of hepatitis C virus (HCV) RNA and advanced fibrosis. The aim of this study was to study MTP expression pattern in HCV patients and impact of the MTP polymorphism on the response to antiviral therapy. **Methods:** One hundred consecutive naive HCV genotype 4 patients were recruited to receive antiviral therapy, and 40 control subjects were also recruited. Demographic, laboratory, and histopathology data were collected. DNA was isolated, and the samples were subjected to polymerase chain reaction analysis and genotyping for MTP by restriction fragment length polymorphism analysis. **Results:** Patients and controls were age- and sex-matched (male/female, 56/44, age, 39.2±7.8 years for patients with HCV; male/female, 18/22, age, 38.1±8.1 years for controls). MTP single nucleotide polymorphisms (SNPs) (GG, GT, TT) and alleles (G, T) in the patients versus the controls were 70%, 21%, 9% & 80.5%, 19.5% versus 10%, 87.5%, 2.5% & 53.8%, 46.3%, respectively (p=0.0001). The sustained viral response (SVR) of the patients was 60%. SNPs in MTP genotypes (GG, GT, and TT) and alleles (G and T) in the responders and nonresponders were 71.7%, 25%, 3.3% & 84.2%, 15.8% versus 67.5%, 15%, 17.5% & 75%, 25% (p=0.038 and p=0.109, respectively). A multivariate analysis showed that the GT genotype was an independent predictor of SVR (area under the curve 90% and p=0.0001). **Conclusions:** MTP could be a new predictor for SVR to antiviral therapy in patients with HCV genotype 4 infection. **(Gut Liver 2014;8:655-661)**

Key Words: Microsomal triglyceride transfer protein polymorphism; Hepatitis C virus; Predictor; Response

INTRODUCTION

Current treatment of chronic hepatitis C virus (HCV) is a combination of pegylated interferon-α-2a or pegylated interferon-α-2b and ribavirin. Several factors have been shown to influence response: these include viral factors (particularly genotype) and host factors: HLA type, cytokine polymorphism, sex, age, presence of cirrhosis and race.[1] Hepatic steatosis is a high risk factor for reduced response to antiviral treatment and for evolution towards fibrosis.[2]

The microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein that consists of a large unique 97 kDa subunit and protein disulfide isomerase.[3] MTP is present in high concentration on the luminal side of the endoplasmatic reticulum in the liver, intestine, and heart.[4] The function of MTP is to lipidate the growing apolipoprotein B (apoB) polypeptide chain during translation, allowing apoB to fold correctly and assemble a lipoprotein with a neutral lipid core before secretion.[5-7] The MTP -493G/T polymorphism has been implicated in the susceptibility to develop steatohepatitis in patients with type 2 diabetes.[8] The G allele was more frequently found in patients with nonalcoholic steatohepatitis (NASH) compared with healthy controls, and NASH patients with the homozygous genotype GG showed more severe degrees of liver steatosis.[9] More recently, the role of MTP polymorphism has been investigated in patients chronically infected with HCV.

Many studies suggest that HCV-related steatosis might be the result of a direct interaction between the virus and MTP. It has been demonstrated that MTP is critical for the secretion of HCV particles and that inhibition of its lipid transfer activity reduces HCV production. Higher degrees of hepatic steatosis were found in chronic hepatitis C patients carrying the T allele of MTP -493G/T polymorphism that seems to be associated with

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increased MTP transcription. Liver steatosis in hepatitis C could be a storage disease induced by the effects of the virus and of its proteins on the intracellular lipid machinery and on MTP. Available data support the hypothesis that HCV may modulate MTP expression and activity through a number of mechanisms such as inhibition of its activity and transcriptional control.[10]

Initially, the MTP -493G/T polymorphism was examined among a set of eight genes that have been reported to have an association with hepatic fibrosis in patients with chronic hepatitis C (CHC).[11] Homozygosity of either the G or the T allele revealed an adjusted odds ratio of 4.1 associated with a more rapid progression to liver fibrosis. More recently[12] patients infected with HCV-3 and carriers of the MTP T allele showed higher degrees of steatosis, higher serum levels of HCV RNA and more advanced fibrosis. In HCV genotype non-3 patients, the MTP T allele was the strongest predictor for severe steatosis, in contrast with what is seen in patients with metabolic syndrome or type 2 diabetes[8] or NASH,[9] in whom genotype MTP -493 GG and the G allele were associated with more severe steatosis. Till now no sufficient data about correlation of MTP variants with response to therapy in HCV, so to justify this issue, the current study aimed to determine the pattern of *MTP* gene polymorphisms in naive HCV genotype 4 patients then to identify the impact of MTP polymorphism on the response to combined pegylated interferon–ribavirin therapy in chronic HCV genotype 4.

MATERIALS AND METHODS

1. Population samples

This study was conducted on 100 consecutive naive patients with chronic HCV genotype 4 infection (in Egypt 92% of our infected population had HCV genotype 4)[13,14] who were candidates for treatment with pegylated interferon-α and ribavirin in addition to 40 healthy subjects served as control (normal transaminases, negative viral hepatitis markers and normal hepatic sonography). Inclusion criteria were naive patients 18 to 60 years, body mass index ≤30, HCV-RNA-positive with abnormal alanine aminotransferase (ALT), liver biopsy performed within 6 months prior to enrolment. Exclusion criteria were those who are not fit for interferon therapy (coinfection with hepatitis B virus, alcohol intake, clinically evident liver cirrhosis, any end organ failure, hematological diseases, major psychiatric disorder, pregnant, and breast feeding women). Informed consent was obtained from all participants before enrolment in the study. The study was performed in accordance with the principles of the Declaration of Helsinki, and its appendices, and with local and national laws. All the patients were subjected to clinical assessment, laboratory investigations, abdominal ultrasound examination, liver biopsy and molecular tests then all the patients were treated with pegylated interferon α-2a, 180 μg/wk subcutaneously, plus ribavirin (1,000-1,200 mg orally/day based on body weight). Response to antiviral therapy was defined as negative HCV viremia by polymerase chain reaction (PCR) 24 weeks after the end of 48 weeks of therapy (sustained viral response [SVR]).

2. Blood sample collection and storage

A 10-mL peripheral blood sample was withdrawn by venipuncture in a dry sterile vacotainer tube. Serum was separated and used for biochemical characterization of HCV specific antibody titers by enzyme-linked immunosorbent assay and enzymatic evaluation. Viral RNA was extracted using viral RNA extraction kit (Qiagen, Valencia, CA, USA) and stored at -80°C, DNA was extracted from EDTA blood for genotyping of MTP.

3. Laboratory tests

Before starting therapy, laboratory investigations included complete liver profile, kidney function, international normalized ratio, and complete blood count. HCV PCR was quantitated in all patients' sera using real-time PCR (Stratagene, Foster City, CA, USA). α-Fetoprotein (AFP) was done by using Axyam-Abbot (Irving, TX, USA). Antinuclear antibody and anti-DNA was done by using immunoflurescence kits.

4. Abdominal ultrasound

It was done for all the enrolled patients after at least 8 hours fasting.

5. Percutaneous liver biopsy

It was performed under ultrasound guidance using 16-gauge needles. Specimens of at least 2.5 cm in length, including a minimum of 12 portal tracts were considered reliable for adequate grading and staging using modified Knodell's score. Reading of liver biopsies was done by a single pathologist who was blind to the clinical data, Metavir classification was used for assesment of necroinflammation and stage of fibrosis.

6. Molecular biology tests

1) DNA extraction

DNA was extracted from whole blood using DNA extraction kit and stored at -80°C in aliquots until required. This was done using Qia-amplification extraction kit (Qiagen).

2) Amplification of MTP gene using the PCR

The presence of the MTP -493G/T mutation was determined by analyzing the restriction fragment length polymorphism utilizing the HphI restriction enzyme, after PCR amplification of the genomic DNA fragment of interest. The sequence of primers used for amplification of MTP was: forward, 5'-AGTTTCACA-CATAAGGACAATCATCTA-3'; reverse, 5'-GGATTTAAATTTA-AACTGTTAATTCATATCAC-3'. PCR was performed using 100 ng genomic DNA templates in a total PCR reaction volume of 50 μL. The cycling condition was denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30

seconds, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes. Final extension cycle of 72°C for 7 minutes was also done.

3) Restriction enzyme cleavage for PCR amplified product of MTP gene

It was done by digestion of 25 μL of PCR product with 5 μL of enzyme HphI, The mixture was incubated at 37°C for 24 hours. HphI digestion of the 109 base pair (bp) PCR product produces major 89 bp and a minor 20 bp fragments. Bands were resolved on 2% agarose gel electrophoresis (Fig. 1). The restriction digestion gives rise to one full-length fragment of 109 bp for homozygotes for the T variant, two fragments of 89 and 20 bp for homozygotes for the G variant, and three fragments of 109, 89, and 20 bp for G/T heterozygotes. The size of 20 bp was not appearing in the gel.

7. Statistical methods

Analysis of data was performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA); description of quantitative variables was in the form of mean and standard deviation, description of qualitative variables was in the form of numbers and percents. Comparison between parametric quantitative variables was carried out by Student t-test of two independent samples. Repeated measures analysis of variance test was used instead of t-test when comparing more than two groups of independent variables. Comparison between qualitative variables was carried out by chi-square test. Fisher exact test was used instead of chi-square test when one expected cell or more were ≤5. The significance of the results was assessed in the form of p-value that was differentiated into nonsignificant when p≥0.05, significant when p<0.05.

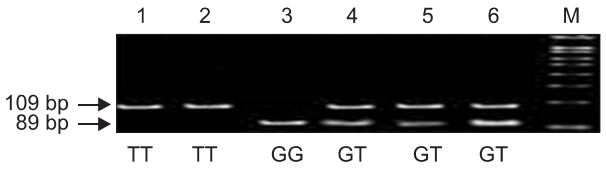

Fig. 1. Agarose gel electrophoresis (2%) stained with ethidium bromide shows the genotype of the microsomal triglyceride transfer protein gene after digestion by restriction endonucleases. Lanes 1,2: TT homozygous genotype; Lane 3: GG homozygous genotype; Lanes 4-6: GT heterozygous genotype; M: molecular DNA marker.

Table 1. Demographic and Laboratory Parameters of the Studied Groups

Variable	HCV patients (n=100)	Control (n=40)	p-value
Age	39.18±7.80	38.10±8.06	0.300
Sex, female/male	44/56	22/18	0.239
ALT, U/L	90.55±48.33	29.50±5.46	<0.001*
AST, U/L	112.64±75.66	31.30±7.71	<0.001*
T bil, mg/dL	1.26±0.65	0.75±0.20	<0.001*
ALP, U/L	113.93±25.05	43.05±7.00	<0.001*
Albumin, g/dL	3.52±0.39	3.85±0.21	<0.001*
AFP, ng/mL	16.11±10.83	5.86±2.00	<0.001*

Data are presented as mean±SD.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; T bil, total bilirubin; ALP, alkaline phosphatise; AFP, α-fetoprotein.
*Indicates a statistically significant difference.

RESULTS

Baseline features for studied individuals were shown in Table 1, both patients and control subjects were age and gender matched. Single nucleotide polymorphisms (SNPs) pattern in MTP genotypes (GG, GT, TT) and Alleles (G and T) showed significant predominance of G alleles in HCV patients compared to control (70%, 21%, 9% & 80.5%, 19.5% versus 10%, 87.5%, 2.5% & 53.8%, 46.3%) (p=0.0001) (Table 2). Out of 100 patients 60 patients showed SVR for combined antiviral therapy while 40 showed no response or relapse, no patient withdrew from therapy because of side effects and no patients received <80% of the therapeutic schedule.

Responders and nonresponders' demographics, laboratory and histological parameters were shown in Table 3. Both groups were age- and sex-matched, with no significant difference as regard ALT and HCV viral load in responders versus nonresponders while nonresponders had significant higher fibrosis score and more elevated billirubin, aspartate aminotransferase (AST), and AFP compared with responders. SNPs pattern in MTP genotypes (GG, GT, and TT) and alleles (G and T) in responders versus nonresponders were 71.7%, 25%, 3.3% & 84.2%, 15.8% versus 67.5%, 15%, 17.5% & 75%, 25% (p=0.038 and p=0.109, respectively) (Table 4). Classifying the studied patients according to MTP gene polymorphism was shown in Table 5, mild and moderate fibrosis score (F1-2) were associated with GG

Table 2. Single Nucleotide Polymorphism Patterns in the MTP Gene among Hepatitis C Virus Patients and Normal Individuals

Variable	HCV patients	Control	p-value
Genotypes			
GG	70 (70)	4 (10)	<0.001*
GT	21 (21)	35 (87.5)	
TT	9 (9)	1 (2.5)	
Alleles			
G alleles	161 (80.5)	43 (53.8)	<0.001*
T alleles	39 (19.5)	37 (46.3)	

Data are presented as number (%).
MTP, microsomal triglyceride transfer protein; HCV, hepatitis C virus.
*Indicates a statistically significant difference.

pattern of MTP polymorphism compared to GT and TT pattern which was associated with severe fibrosis and cirrhosis (F3-4) (p=0.0001).

On multivariate analysis for predictors of response to combined antiviral therapy for HCV genotype 4 in our patients; AST, fibrosis score and GT were the only predictors of response with (p=0.022, p=0.029, and p=0.016, respectively) and odds ratio (p=1.029, p=0.215, and p=54.086, respectively), as shown in Table 6. Predicted probability score of MTP polymorphism as predictor of response to antiviral therapy showed area under the curve 90% with cutoff=0.546; p-value=0.0001 with 90% sensitivity and 82.5% specificity (Table 7, Fig. 2).

Table 3. Demographic, Laboratory, and Histopathological Features in Responders and Nonresponders to Antiviral Therapy

Variable	Responders (n=60)	Nonresponders (n=40)	p-value
Age	39.53±8.39	38.65±6.90	1.000
Sex			
Female	26 (43.3)	18 (45)	0.493
Male	34 (56.7)	22 (55)	
ALT, U/L	92.40±55.76	87.78±34.81	0.645
AST, U/L	107.55±87.57	120.28±53.22	0.001*
T bil, mg/dL	1.02±0.55	1.62±0.63	0.0001*
AFP, ng/mL	14.34±11.93	18.75±8.38	0.0001*
HCV RNA, IU/mL	$3.34\times10^{7}\pm1.03\times10^{8}$	$7.37\times10^{7}\pm2.16\times10^{8}$	0.155
Fibrosis			
F1	8 (13.3)	0	0.0001*
F2	31 (51.7)	6 (15)	
F3-4	21 (35)	34 (85)	

Data are presented as mean±SD or number (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; T bil, total bilirubin; AFP, α-fetoprotein; HCV, hepatitis C virus.
*Indicates a statistically significant difference.

DISCUSSION

The role of MTP polymorphism has been reported to have an association with hepatic fibrosis in patients with CHC.[11] Homozygosity of either the G or the T allele revealed an adjusted odds ratio of 4.1 associated with a more rapid progression to liver fibrosis. Till now no sufficient data about correlation of MTP variants with response to therapy in HCV, so to clarify this issue, the current study aimed to determine the pattern of MTP gene polymorphisms in naive HCV genotype 4 patients then

Table 4. Single Nucleotide Polymorphism Patterns in the MTP Gene in the Responders and Nonresponders

Variable	Responders (n=60)	Nonresponders (n=40)	p-value
Genotypes			
GG	43 (71.7)	27 (67.5)	0.038*
GT	15 (25)	6 (15)	
TT	2 (3.3)	7 (17.5)	
Alleles			
G alleles	101 (84.2)	60 (75)	0.109
T alleles	19 (15.8)	20 (25)	

Data are presented as number (%).
MTP, microsomal triglyceride transfer protein.
*Indicates a statistically significant difference.

Table 5. Demographic, Laboratory, and Histological Features of the Studied Patients according to the Genetic Polymorphisms of MTP (N=100)

Variable	GG (n=70)	GT (n=21)	TT (n=9)	p-value
Age	38.79±7.43	40.19±9.15	39.89±7.94	0.743
Sex				0.136
Female	32 (45.7)	6 (28.6)	6 (66.7)	
Male	38 (54.3)	15 (71.4)	3 (33.3)	
ALT, U/L	89.81±49.15	87.62±41.67	103.11±59.31	0.898
AST, U/L	100.5±65.73	123.19±79.29	182.44±103.91	0.002*
T bil, mg/dL	1.28±0.69	1.02±0.47	1.26±0.55	0.046*
AFP, ng/mL	15.18±9.78	15.86±11.57	23.89±14.66	0.071
HCV RNA (IU/mL)	6.19E7±1.79E8	2.59E7±1.02E8	7.99E6±2.14E7	0.693
Fibrosis				0.0001*
F1-2	45 (64.3)	0	0	
F3-4	25 (35.7)	21 (100.0)	9 (100.0)	

Data are presented as mean±SD or number (%).
MTP, microsomal triglyceride transfer protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; T bil, total bilirubin; AFP, α-fetoprotein; HCV, hepatitis C virus.
*Indicates a statistically significant difference.

Table 6. Multivariate Analysis for the Predictors of Response to Interferon Therapy

Variable	p-value	OR	95% CI for OR	
			Lower	Upper
Age	0.360	0.958	0.875	1.050
ALT	0.458	1.008	0.987	1.030
AST	0.022	1.029	1.004	1.054
AFP	0.769	0.987	0.903	1.078
HCV RNA	0.355	1.000	1.000	1.000
Fibrosis	0.029	0.215	0.054	0.854
GG	0.200	6.820	0.363	128.135
GT	0.016	54.086	2.123	1,377.844

OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, α-fetoprotein; HCV, hepatitis C virus.

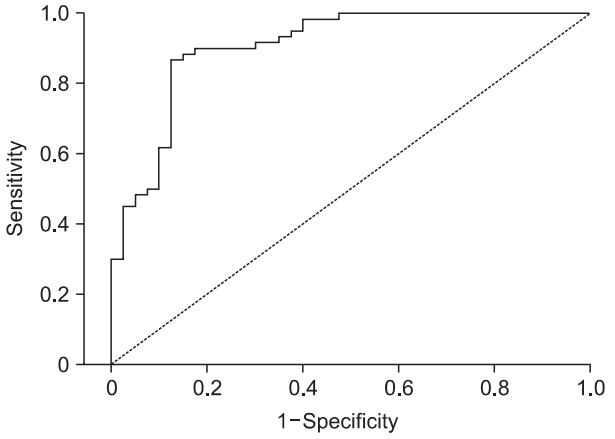

Fig. 2. Receiver operating characteristic curve for the predicted probability score of microsomal triglyceride transfer protein polymorphism.

Table 7. Predicted Probability Score of an MTP Polymorphism as a Predictor of Response to Antiviral Therapy

Area	p-value	Cutoff	Sensitivity	Specificity	PPV	NPV
0.907	0.0001	0.546	0.900	0.825	88.5%	84.6%

MTP, microsomal triglyceride transfer protein; PPV, positive predictive value; NPV, negative predictive value.

to identify the impact of MTP polymorphism on the response to combined pegylated interferon–ribavirin therapy in chronic HCV genotype 4.

SNPs are the most common form of genetic variation that has been employed for the prediction of both disease progression and therapeutic response.[15] Polymorphisms in the promoter of MTP lead to decreased MTP transcription, less export of triglyceride from hepatocytes, and greater intracellular triglyceride accumulation.[16]

In the present study, we investigated the polymorphism of MTP; located at -493 of the promoter region. We are trying to find the role of the genetic MTP -493G/T SNP on the efficacy of interferon therapy for HCV genotype 4. To our knowledge this is one of the first studies to detect the MTP polymorphism in responders and nonresponders HCV genotype 4 patients in order to be used as predictor for response to interferon therapy. Our data showed that there was significant difference in the prevalence of SNPs in MTP gene between hepatitis C patients and controls as regards genotypes (GG, GT, and TT) and alleles (G and T alleles).

None of the authors studied the MTP -493G/T SNP and its relation to efficacy of treatment but they investigate the relation of MTP SNP and development of nonalcoholic fatty liver disease (NAFLD) as reported that MTP rs3816873 might be a candidate to determine susceptibility to NAFLD.[17]

However, data regarding the association of MTP -493G/T SNP with histological variables in CHC patients are still conflicting. Initially, Richardson and his colleagues[11] examined this polymorphism among a set of SNPs in eight genes previously associated with hepatic fibrosis in a group of 326 patients with CHC and identified homozygosity for either the G or the T allele of the -493G/T SNP as independent risk factors for more rapid progression of liver fibrosis. This finding is intriguing in view of evidence from functional studies that G and T alleles are associated with different transcriptional activities of the MTP gene. On the other hand, others investigated the influence of a functional polymorphism in the promoter region of the MTP gene (493G/T) on the development of HCV-related steatosis in a small series of 86 HCV-positive patients, they concluded that the functional G/T MTP polymorphism do not seem to play any role in the development of steatosis in chronic hepatitis C.[18]

Meanwhile, analysis of 102 patients infected with HCV genotype 3 and showed higher degrees of steatosis, higher serum levels of HCV RNA and more advanced fibrosis in carriers of the MTP T allele. Chronic hepatitis C patients with the MTP -493T allele reveal higher grades of steatosis, concluding that the presence of T allele of MTP-493G/T gene polymorphism predisposes patients infected with HCV genotype 3 to develop higher degree of fatty liver accumulation.[12,19]

At the same time, Chinese studied the polymorphisms of MTP at the promoter region -493 in populations with hepatitis B virus infection. They concluded that the polymorphism of the MTP gene, T allele at -493, may be involved in determining the hepatitis B virus infection outcomes, of which the mechanism needs to be further investigated.[20]

The present study revealed that there was significant difference in the prevalence of SNPs in MTP gene between responders and nonresponders to interferon therapy of chronic hepatitis C genotype 4 patients as regards genotypes (GG, GT, and TT) (p=0.038). Meanwhile, there is no significant differences were

observed as regards alleles (G and T alleles) (p=0.109).

The polymorphism of MTP-493 G-to-T substitution affects the promoter activity of the MTP gene.[21,22] It was reported that the G allele, which decreases the MTP gene transcription, increases intrahepatic triglyceride content.[23] The T allele is associated with an increased expression of the MTP gene.[21]

In the present study the G allele was more frequently present in HCV genotype 4 patients with lower degrees of fibrosis. Also, genotypes GT and TT were found to be increased in higher degree of fibrosis.

On multivariate logistic regression analysis, it was found that AST (p=0.022), fibrosis (p=0.029), and SNP GT (p=0.031) were found to be significant predictors for antiviral therapy response in HCV genotype 4.

In conclusion, MTP polymorphism can be used as marker for prediction of the response to antiviral therapy in Egyptian patients with HCV genotype 4 patients.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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