

World Journal of *Gastroenterology*

World J Gastroenterol 2015 October 21; 21(39): 10931-11204



TOPIC HIGHLIGHT

- 10931** Anticancer effect of adenosine on gastric cancer *via* diverse signaling pathways
Tsuchiya A, Nishizaki T
- 10936** Evaluation and treatment of malignant ascites secondary to gastric cancer
Maeda H, Kobayashi M, Sakamoto J
- 10948** Companion diagnostics for the targeted therapy of gastric cancer
Yoo C, Park YS
- 10956** Dysregulation of non-coding RNAs in gastric cancer
Yang Q, Zhang RW, Sui PC, He HT, Ding L
- 10982** Sarcopenia and liver transplant: The relevance of too little muscle mass
Kallwitz ER
- 10994** Liver transplantation for alcoholic liver disease: Lessons learned and unresolved issues
Ursic-Bedoya J, Faure S, Donnadieu-Rigole H, Pageaux GP
- 11003** Current status and perspectives in split liver transplantation
Lauterio A, Di Sandro S, Concone G, De Carlis R, Giacomoni A, De Carlis L
- 11016** Clinical significance of donor-specific human leukocyte antigen antibodies in liver transplantation
Cuadrado A, San Segundo D, López-Hoyos M, Crespo J, Fábrega E
- 11027** Candidates for liver transplantation with alcoholic liver disease: Psychosocial aspects
Telles-Correia D, Mega I
- 11034** Posttransplant lymphoproliferative disorders following liver transplantation: Where are we now?
Dierickx D, Cardinaels N
- 11044** Non-invasive assessment of liver fibrosis in patients with alcoholic liver disease
Lombardi R, Buzzetti E, Roccarina D, Tsochatzis EA
- 11053** Endocrine causes of nonalcoholic fatty liver disease
Marino L, Jornayvaz FR
- 11077** Liver fibrosis in non-alcoholic fatty liver disease - diagnostic challenge with prognostic significance
Stål P

REVIEW

- 11088** Genetic background in nonalcoholic fatty liver disease: A comprehensive review
Macaluso FS, Maida M, Petta S

MINIREVIEWS

- 11112** Active vaccination to prevent *de novo* hepatitis B virus infection in liver transplantation
Lin CC, Yong CC, Chen CL

ORIGINAL ARTICLE

Basic Study

- 11118** Hepatic differentiation of rat induced pluripotent stem cells *in vitro*
Sun C, Hu JJ, Pan Q, Cao Y, Fan JG, Li GM
- 11127** Effect of fibulin-5 on adhesion, migration and invasion of hepatocellular carcinoma cells *via* an integrin-dependent mechanism
Tang JC, Liu JH, Liu XL, Liang X, Cai XJ

Case Control Study

- 11141** Low-density lipoprotein receptor genetic polymorphism in chronic hepatitis C virus Egyptian patients affects treatment response
Naga M, Amin M, Algendy D, Elbadry A, Fawzi M, Foda A, Esmat S, Sabry D, Rashed L, Gabal S, Kamal M
- 11152** Serum vitamin D₃ does not correlate with liver fibrosis in chronic hepatitis C
Ren Y, Liu M, Zhao J, Ren F, Chen Y, Li JF, Zhang JY, Qu F, Zhang JL, Duan ZP, Zheng SJ

Retrospective Study

- 11160** Prediction of the indication criteria for endoscopic resection of early gastric cancer
Park JH, Lee SH, Park JM, Park CS, Park KS, Kim ES, Cho KB

Clinical Trials Study

- 11168** Prognostic significance of plasma interleukin-6/-8 in pancreatic cancer patients receiving chemoimmunotherapy
Tsukinaga S, Kajihara M, Takakura K, Ito Z, Kanai T, Saito K, Takami S, Kobayashi H, Matsumoto Y, Odahara S, Uchiyama K, Arakawa H, Okamoto M, Sugiyama H, Sumiyama K, Ohkusa T, Koido S
- 11179** Effects of daily telephone-based re-education before taking medicine on *Helicobacter pylori* eradication: A prospective single-center study from China
Wang CH, Liao ST, Yang J, Li CX, Yang YY, Han R, Chen DF, Lan CH

SYSTEMATIC REVIEWS

- 11185** Managements of recurrent hepatocellular carcinoma after liver transplantation: A systematic review
de'Angelis N, Landi F, Carra MC, Azoulay D

CASE REPORT

- 11199** Case report of primary splenic angiosarcoma with hepatic metastases
Chen F, Jin HF, Fan YH, Cai LJ, Zhang ZY, Lv B

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Nobuhiro Ohkohchi, MD, PhD, Professor, Department of Surgery, Division of Gastroenterology and Hepatobiliary Surgery and Organ Transplantation, University of Tsukuba, Tsukuba, Japan 305-8575, Japan

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1377 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. According to the 2014 Journal Citation Reports® released by Thomson Reuters (ISI), the 2014 impact factor for *WJG* is 2.369, ranking 41 among 76 journals in gastroenterology and hepatology, quartile in category Q2.

FLYLEAF

I-IX Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Xiao-Mei Liu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Yuan Qi*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

Stephen C Strom, PhD, Professor, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA

Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLICATION DATE
October 21, 2015

COPYRIGHT
© 2015 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
<http://www.wjgnet.com/esps/>

Case Control Study

Low-density lipoprotein receptor genetic polymorphism in chronic hepatitis C virus Egyptian patients affects treatment response

Mazen Naga, Mona Amin, Dina Algendy, Ahmed Elbadry, May Fawzi, Ayman Foda, Serag Esmat, Dina Sabry, Laila Rashed, Samia Gabal, Manal Kamal

Mazen Naga, Mona Amin, Dina Algendy, Ahmed Elbadry, May Fawzi, Ayman Foda, Serag Esmat, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Dina Sabry, Laila Rashed, Department of Biochemistry, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Samia Gabal, Department of Pathology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Manal Kamal, Department of Clinical and Chemical pathology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

Author contributions: Naga M designed the study; Amin M, Algendy D, Elbadry A, Fawzi M, Foda A, Esmat S, Sabry D, Rashed L, Gabal S, Kamal M contributed to the drafting of the manuscript; Naga M, Amin M, Algendy D, Elbadry A, Fawzi M, Foda A, Esmat S revised the manuscript and contributed to patient selection; Amin M and Esmat S collected the data; Amin M, Algendy D, Elbadry A, Fawzi M, Foda A, Esmat S, Sabry D, Rashed L, Gabal S and Kamal M contributed to data analyses and interpretation under the supervision of Naga M; Amin M, Fawzi M and Algendy D performed the abdominal ultrasounds and liver biopsies; Esmat S, Elbadry A and Foda A performed the clinical examinations; Gabal S performed the histopathological analyses; Sabry D and Rashed L performed laboratory analyses; Kamal M performed statistical analyses; all authors approved the present version for publication.

Supported by the Science and Technology Development Fund, Ministry of Scientific Research Egypt, Project No. 1587; and Cairo University.

Institutional review board statement: The review board of the Department of Internal Medicine, Faculty of Medicine, Cairo University approved the study protocol, which was performed according to the Declaration of Helsinki.

Informed consent statement: All study participants provided informed written consent prior to study enrolment.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at monasleman@kasralainy.edu.eg. No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Mona Amin, Professor, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo 11562, Egypt. monasleman@hotmail.com
Telephone: +20-23-646394
Fax: +20-23-657104

Received: March 8, 2015
Peer-review started: March 11, 2015
First decision: April 24, 2015
Revised: May 25, 2015
Accepted: August 29, 2015
Article in press: August 29, 2015
Published online: October 21, 2015

Abstract

AIM: To correlate a genetic polymorphism of the low-density lipoprotein (LDL) receptor with antiviral responses in Egyptian chronic hepatitis C virus (HCV) patients.

METHODS: Our study included 657 HCV-infected patients with genotype 4 who received interferon-based combination therapy. Patients were divided into two groups based on their response to therapy: 356 were responders, and 301 were non-responders. Patients were compared to 160 healthy controls. All patients and controls underwent a thorough physical examination, measurement of body mass index (BMI) and the following laboratory tests: serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total bilirubin, direct bilirubin, prothrombin time, prothrombin concentration, INR, complete blood count, serum creatinine, fasting blood sugar, HCV antibody, and hepatitis B surface antigen. All HCV patients were further subjected to the following laboratory tests: HCV-RNA using quantitative polymerase chain reaction (PCR), antinuclear antibodies, thyroid-stimulating hormone, an LDL receptor (LDLR) genotype study of LDLR exon8c.1171G>A and exon-10c.1413G>A using real-time PCR-based assays, abdominal ultrasonography, ultrasonographic-guided liver biopsy, and histopathological examination of liver biopsies. Correlations of LDL receptor polymorphisms with HAI, METAVIR score, presence of steatosis, and BMI were performed in all cases.

RESULTS: There were no statistically significant differences in response rates between the different types of interferon used or LDLR exon10c.1413G>A. However, there was a significant difference in the frequency of the LDL receptor exon8c.1171G>A genotype between cases (AA: 25.9%, GA: 22.2%, GG: 51.9%) and controls (AA: 3.8%, GA: 53.1% and GG: 43.1%) ($P < 0.001$). There was a statistically significant difference in the frequency of the LDLR exon 8C:1171 G>A polymorphism between responders (AA: 3.6%, GA: 15.2%, GG: 81.2%) and non-responders (AA: 52.2%, GA: 30.6%, GG: 17.2%) ($P < 0.001$). The G allele of LDL receptor exon8c.1171G>A predominated in cases and controls over the A allele, and a statistically significant association with response to interferon was observed. The frequency of the LDLR exon8c.1171G>A allele in non-responders was: A: 67.4% and G: 32.6 *vs* A: 11.2% and G: 88.8% in responders ($P < 0.001$). Therefore, carriers of the A allele exhibited a 16.4 times greater risk for non-response. There was a significant association between LDL receptors exon8 c.1171G>A and HAI ($P < 0.011$). There was a significant association between LDL receptors exon8c.1171G>A and BMI. The mean BMI level was highest in patients carrying the AA genotype ($28.7 \pm 4.7 \text{ kg/m}^2$) followed by the GA genotype ($28.1 \pm 4.8 \text{ kg/m}^2$). The lowest BMI was the GG genotype ($26.6 \pm 4.3 \text{ kg/m}^2$) ($P < 0.001$). The only significant associations were found between LDL receptors exon8 c.1171G>A and METAVIR score or steatosis ($P < 0.001$).

CONCLUSION: LDL receptor gene polymorphisms play a role in the treatment response of HCV and the modulation of disease progression in Egyptians

infected with chronic HCV.

Key words: Hepatitis C virus; Genetic polymorphisms; Low-density lipoprotein receptor; Egypt; Hepatitis C virus response to treatment

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Two molecules may function as hepatitis C virus (HCV) receptors, namely the low density lipoprotein receptor (LDLR) and CD81. This work assessed the role of genetic polymorphisms of the LDLR in Egyptian chronic HCV patients and its correlation with antiviral responses to treatment with pegylated interferon /ribavirin therapy. The study demonstrated that LDLR gene polymorphisms play a role in the response to viral treatment. The G allele of LDLRs exon8c.1171G>A predominated in cases and controls over the A allele. Carriers of the A allele exhibited a 16.4 times greater risk for non-response.

Naga M, Amin M, Algendy D, Elbadry A, Fawzi M, Foda A, Esmat S, Sabry D, Rashed L, Gabal S, Kamal M. Low-density lipoprotein receptor genetic polymorphism in chronic hepatitis C virus Egyptian patients affects treatment response. *World J Gastroenterol* 2015; 21(39): 11141-11151 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i39/11141.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i39.11141>

INTRODUCTION

Hepatitis C exhibits a worldwide prevalence of approximately 3%^[1]. Hepatitis C causes severe morbidity and mortality, and the majority of infected subjects fail to eliminate the virus and develop chronic hepatitis C. Chronic infections are estimated at approximately 80% of infected subjects, and this chronicity will lead to cirrhosis and /or hepatocellular carcinoma^[2-8].

Egypt has the largest epidemic of hepatitis C virus (HCV) worldwide with a percentage of 14.7%, which is ten times greater than any other country^[9-14].

The strong prevalence of HCV subtype (4a) in Egypt suggests that this chronic infection began as an epidemic. A history of injection treatment was implicated as a risk factor for HCV, and the prime culprit is the past practice of parental therapy for schistosomiasis^[15,16].

The rate of disease progression varies widely, and it is not known what factors precisely determine the clinical outcome of the disease in the long term. The mechanism of viral entry into the host hepatocytes is not known.

Much effort was made to identify the receptors involved in viral entry into host cells.

Two molecules were proposed to function as HCV

receptors, namely the low density lipoprotein receptors (LDLRs) and CD81^[17-20].

The LDLR gene family functions as a receptor for the minor group of common cold viruses and other viruses^[21,22].

LDLR plays an important role in cholesterol homeostasis^[23,24], and it is firmly established that mutations and polymorphisms in the LDLR gene are associated with familial hypercholesterolemia, obesity and atherosclerosis^[25-31].

A direct interaction of HCV E2 envelope protein and LDL mediates the binding and internalisation of lipovirions by LDLR in conjunction with CD81^[32]. LDLR, CD81 and HCV E2 are co-localized on cells^[33], and viral binding and endocytosis are inhibited by free LDL, free recombinant LDLR peptides, and antibodies against E2 and LDLRs^[32,34]. Moreover, intracellular HCV RNA levels positively correlate with LDLR expression in cultured primary hepatocytes and *in vivo*^[34,35]. These findings demonstrate that LDLRs are crucial co-receptors for HCV cell entry and replication. Furthermore, the role of LDLRs in immune responses and the incrimination of LDLR polymorphisms in disease processes makes these receptors an important candidate for the study of the genetic susceptibility to hepatitis C.

Several studies investigated nine single nucleotide polymorphisms in the *LDLR* gene and their possible influence on clinical parameters of HCV infection, such as viral clearance, overall inflammation, fibrosis severity and treatment response^[36-38].

Our study assessed genetic polymorphisms of LDLR in chronic HCV genotype 4 Egyptian patients and the effects of polymorphisms on antiviral response.

MATERIALS AND METHODS

The review board of the Department of Internal Medicine, Faculty of Medicine, Cairo University approved the study protocol, which was performed according to the Declaration of Helsinki.

All study participants provided informed written consent prior to study enrolment. The study included 657 chronic HCV patients who were candidates for pegylated-interferon/ribavirin therapy and 160 healthy age- and sex-matched subjects as a control group. Patients were recruited from the outpatient clinics of the Internal Medicine Department, Kasr Al Aini Hospital, Cairo University, Beni-Suef Public Hospital and the National Liver Institute.

All patients and control group underwent thorough physical examinations, measurements of body mass index (BMI) and the following laboratory tests: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total bilirubin, direct bilirubin, prothrombin time (PT), prothrombin concentration (PC), INR, complete blood count (CBC), serum creatinine, fasting

blood sugar (FBS), HCV antibody (anti-HCV), and hepatitis B surface antigen (HBsAg).

All HCV patients were further subjected to the following tests: HCV-RNA using quantitative polymerase chain reaction (PCR), antinuclear antibodies (ANA), thyroid-stimulating hormone (TSH), LDLR genotype study, abdominal ultrasonography and ultrasonographic-guided liver biopsy.

Patients received a course of antiviral therapy that consisted of pegylated interferon subcutaneously once weekly and oral ribavirin (10.6 mg/kg) daily. Patients with a reduction of more than 2 logs in PCR results after 12 wk of treatment were deemed responders, and treatment was continued for a total of 48 wk. Patients with PCR scores that were not at least 2 logs lower than baseline after 12 wk of therapy were deemed non-responders. Responders were re-tested 6 mo after the end of therapy using PCR to ensure a sustained virological response (SVR). We divided the patients into two groups based on treatment response: non-responders ($n = 301$) and responders ($n = 356$).

Types of interferon used

The following types of pegylated interferon were used based on availability: Peginterferon alfa-2a (PEGASYS® 180 mcg) was used in 287 cases, Peginterferon alfa-2a (Reiferon Retard® 160 mcg) was used in 59 cases, and Peginterferon alfa-2b (PEGINTRON® 1.5 mcg/kg per week) was used in 311 cases.

Blood sampling

Venous blood (10 mL) was collected from each patient in EDTA vacuum whole blood sample tubes. Samples were divided into 2 parts: 5 mL for HCV and biochemical parameters assessments and the other 5 mL for molecular LDLR gene polymorphism assessments.

Biochemical markers assessment

AST, ALT, T. bilirubin, D. bilirubin, albumin, ALP, FBS and creatinine were assessed using commercially available kits (Randox Laboratories Limited, Country Antrim, United Kingdom). AFP was evaluated using an ELISA kit (DRG International Inc., Springfield, New Jersey, United States). HbA 1c, PC, PT and INR were detected using kits (Stanbio Laboratory, Boerne, TX, United States). HB, TLC, ANC and platelets were detected using a cell counter (Sysmex XT-4000i Automated Hematology Analyzer Lincolnshire, IL, United States).

HCV RNA extraction and quantitative PCR detection

HCV RNA was extracted from 140 µL serum using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Absolute quantitation of the concentration of HCV RNA was based on an external standard curve (HCV Standards IU/mL) in the presence of an internal positive control (IPC). IPC was added to a mixture of

Table 1 The primers and probes used for the low density lipoprotein receptor genes

LDLR primers	exon 8 (c.1171G > A)	F5'-CTACAAGTGCCAGTGTGAGGAA-3' R5'-CCCACCACTCTGCTGTGAAGGCGTGAGGCCGCC-3' Allele 1 specific probe VIC-ACACGAAGGCCTGC-NFQ Allele 2 specific probe 6-FAM-ACACGAAGACCT GC-NFQ
	exon 10 (c.1413G > A)	F5'-CGGCGTCTCTTCTATGACA-3' R5'-GTCCAGTAGATGTTGCTGTGGAT-3' Allele 1 specific probe VIC-ATCAGCAGGGAC ATC-NFQ Allele 2 specific probe 6-FAM-TCAGCAGAGACA TC-NFQ

lysis buffer and sample material during RNA extraction of clinical blood samples. TaqMan assay was used in the AgPath-ID™ One-Step RT-PCR kit (Applied Biosystems, Foster City, CA, United States). The One-Step RT-PCR kit included an enzyme mixture, buffer and detection enhancer for one-step quantitative reverse transcription PCR (qRT-PCR). Amplification was performed in 25 µL of reaction mixture containing 2 × TaqMan Universal RT-PCR Master Mix, 20 µmol/L of each primer and probes for the sample and ICP and 8.5 µL of extracted RNA. All samples were performed in duplicate. Amplification began with an incubation at 50 °C for two min with uracil N'-glycosylase to inactivate possible contaminating amplicons, followed by 45 °C for 10 min for cDNA synthesis using reverse transcriptase, and 10 min at 95 °C to activate the AmpliTaq Gold polymerase and inactivate uracil N'-glycosylase. The PCR cycling program consisted of 45 cycles of 15 s at 95 °C and 45 s at 60 °C (universal conditions).

DNA extraction

Total DNA was isolated from whole blood mononuclear cells (MNCs) using an extraction kit (Qiagen, United States) according to manufacturer's instructions. DNA purity (A260/A280 ratio) and concentration were calculated using spectrophotometry (dual wavelength Beckman, Spectrophotometer, United States). The extracted and purified DNA samples were stored at -80 °C for further use.

LDLR genotyping

LDLR allelic discrimination variants were genotyped using real-time PCR and the QuantiTect® Probe PCR Kit (Qiagen, Hilden, Germany) and a Step-One instrument (Applied Biosystems, Foster City, United States). PCR products were generated under standard conditions in a 15 µL reaction containing 1 × reaction buffer, 1-2 mmol/L MgCl₂, 0.32 mmol/L dNTPs, 0.12 µmol/L of forward, reverse primer and probes, 1 unit Taq polymerase, 50 ng DNA and dH₂O. The PCR thermal cycling profile included an initial incubation at 50 °C for 2 min, 15 min at 95 °C, and 40 amplification cycles (15 s at 95 °C, 1 min at 60-64 °C). Table 1 lists primers and probes. Finally, 1 µL of each LDR amplification product was run on an ABI prism 310 sequencer (Applied Biosystems, Warrington, United Kingdom), and sample genotypes were analysed using Genotyper software

programmes (Applied Biosystems).

DNA Sequencing: PCR product samples were sequenced with a forward primer using a Big Dye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, United States) according to the manufacturer's instructions. Briefly, PCR sequencing cycling reactions were performed in a final total volume of 20 µL, which included 8 µL Big Dye terminator, 3.2 µL of 1 pmol diluted forward primer, 1 µL PCR product and 7.8 µL nuclease free water. The thermal profile conditions were 94 °C for 4 min, 95 °C for 15 s, 55 °C for 30 s and 60 °C for 4 min for 25 cycles. The sequencing cycling PCR products were purified using centri-sep nucleic acid gel-purified columns (Life Technology, Invitrogen). Ten microlitre of Hi Di formamide were added to 10 µL of purified PCR products. The mix was denatured in a PCR thermal cycler (Biometra, Germany) at 95 °C for 5 min. The reactions were performed in an automatic Sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif.). The sequences obtained were analysed using the GenBank BLAST tool. The sequences were edited and aligned using the BioEdit Sequence Alignment. Table 1 shows the primers and probes for the *LDLR* genes.

Liver biopsy

Liver biopsies were performed using 18-gauge Tru-Cut Biopsy Needles under ultrasonographic guidance with a 3.5-MHz convex probe of a GE LOGIQ® P5 ultrasound machine after 6 h of fasting under complete aseptic conditions and the use of a local anaesthesia (5 mL of 2% lidocaine). Patients' vital signs of all patients were controlled for 2-4 h after the procedure to control probable complications. Biopsy specimens were placed in 10% formaldehyde and transferred to our pathology laboratory on the same day.

Histopathological examination of liver biopsies

All liver core biopsies were fixed for 24 h in a 10% neutral-buffered formalin solution, processed in ascending grades of ethyl alcohol (70%, 90%, and 100%) and xylene, and placed in paraffin blocks. Three paraffin wax sections were cut at 5 microns and stained using the following reagents: (1) hematoxylin and eosin for routine histopathological examination, diagnosis and necro-inflammatory scoring; (2)

Masson Trichrome to detect the extent of fibrosis. This stain imparts a blue colour to collagen against a red background of hepatocytes and other structures. It highlights the presence and distribution of reactive fibrosis as a result of liver injury. It is used for the staging of chronic liver diseases; and (3) prussian blue is a common and reliable stain for the detection of iron, which appears as blue granules in the cytoplasm.

Necro-inflammatory scoring and staging of the studied cases was performed using a modified HAI score and Metavir systems^[39].

Statistical analysis

Data obtained from the study were coded and entered using SPSS (Statistical package for social science) software version 21. Parametric data are summarized using means and standard deviation, and non-parametric data are summarized as medians and percentiles for quantitative variables. Frequencies and percentages were used for qualitative variables. Comparisons between groups were performed using the χ^2 and Fischer's exact tests for qualitative variables, and Student's *t* test and the non-parametric Mann-Whitney test were used to compare two groups. ANOVA and a non-parametric test (Kruskal Wallis test) were used to compare multiple groups. The odds ratio (OR) and 95%CI were calculated to estimate the strength of associations between each genotype and alleles and patients and controls. *P* values were considered significant when *P* < 0.05.

RESULTS

Patient characteristics

A total of 657 chronic active hepatitis C patients were used in this study. Mean patient age was 42.5 ± 10.2 years. There were 399 males and 258 females. The control population included 160 volunteers. Patients were divided into 301 non-responders and 356 responders based on their responses to therapy. Median levels of BMI, ALT, and AST were significantly higher in the non-responders vs responders. There were no statistically significant differences in levels of albumin, viral load, platelet count, or ANA, and the presence of co-morbidities or the type of interferon used between groups. Liver biopsies from patients prior to treatment revealed a significant difference between responders and non-responders. Responders exhibited fewer disturbances in liver architecture as evidenced by HAI stage and Metavir A and F. There was also a significantly greater percentage of non-responders who exhibited liver steatosis than responders (Figures 1 and 2).

Frequency of LDLR genotype polymorphisms between responders, non-responders and controls

The frequency of the LDLR exon 8 C: 1171 G>A in the patient group was distributed as follows: AA: 170

(25.9%), GA: 146 (22.2%), and GG: 341 (51.9%). This result was significantly different from the control group: AA: 6 (3.8%), GA: 85 (53.1%) and GG: 69 (43.1%). Responders exhibited the following frequency of LDLR exon 8C:1171 G>A polymorphism: AA: 13 (3.6%), GA: 54 (15.2%), and GG: 289 (81.2%). Non-responders exhibited the following distribution: AA: 157 (52.2%), GA: 92 (30.6%), and GG: 52 (17.2%). These differences were statistically significant (*P* < 0.001).

LDLR exon 10 C1413 G>A revealed no significant differences between cases and controls, and there was no difference between responders and non-responders (*P* = 0.354) (Table 2).

Allele frequency and risk ratio in non-responders vs responders

There was a statistically significant association between the A allele of LDLR exon 8 C1171 G>A gene polymorphism and the response to treatment (standard of care treatment). Carriers of the A allele exhibited a 16.4 times greater risk for non-response to treatment than carriers of the G allele (*P* < 0.001). There was no significant association between response to therapy and LDLR exon 10 C:1413 G>A (Table 3).

Comparisons of HAI stage, BMI, steatosis and Metavir score to LDLR gene polymorphisms

The LDLR exon 8 C1171 G>A polymorphism was significantly associated with the HAI stage of liver fibrosis. There was a statistically significant association between the AA variant of the LDLR exon 8 genotype and increased BMI. There was no significant association between the different genotypes of LDLR exon 10 polymorphisms and liver fibrosis as evidenced by HAI and Metavir scores. There was a statistically significant association between LDLR exon 8 and Metavir A and F scores and the pathological evidence of steatosis. Carriers of the AA genotype exhibited a significantly higher incidence of fibrosis and steatosis. There was no association between the various genotypes of LDLR exon 10 gene polymorphisms and Metavir A or F scores or the presence of steatosis (Figure 3 and Table 4). There was no association between LDL receptor genotypes and the type of interferon in responders and non-responders.

DISCUSSION

Many studies suggested that HCV likely utilizes cell receptors to facilitate entry into cells^[40]. The discrepancy in the rate of progression and clinical outcome of this disease was extensively investigated, and this step of viral entry is most incriminated in the development of chronicity^[17]. The LDL receptor is a transmembrane glycoprotein that acts as a receptor for the uptake of cholesterol containing serum lipoproteins^[41].

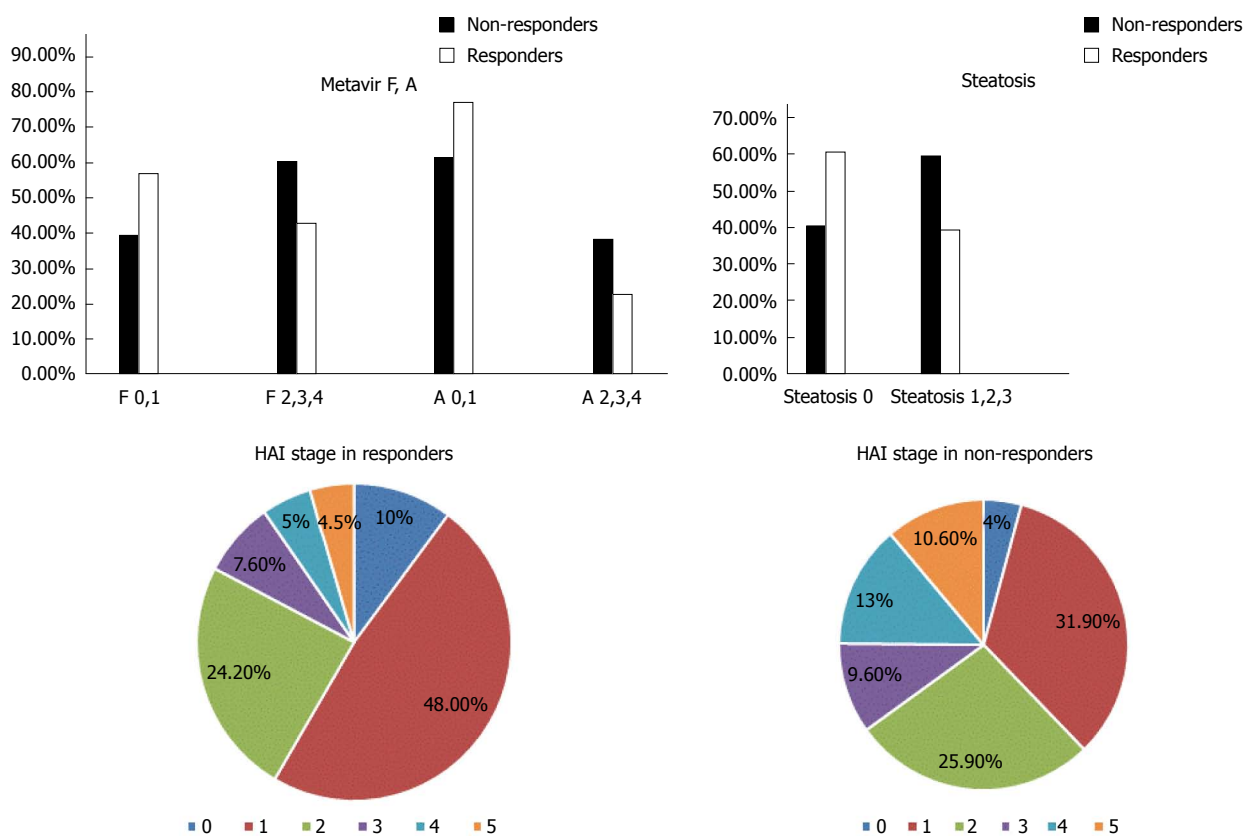


Figure 1 The HAI stage, Metavir score, steatosis grade in the responders and non-responders.

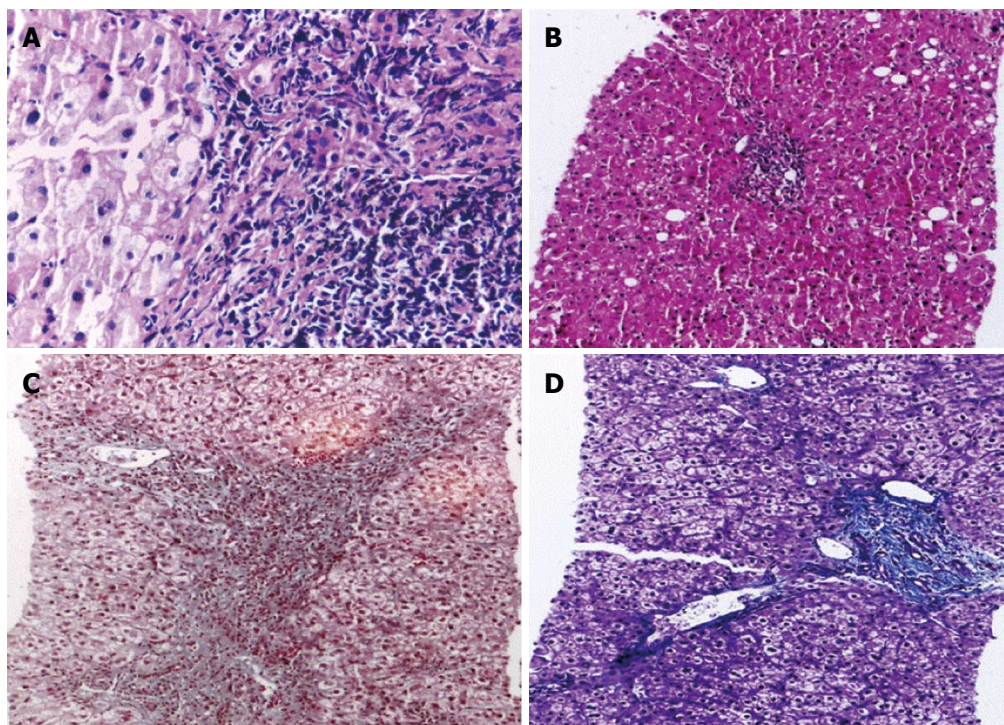


Figure 2 Histopathology of the liver biopsy tissues of patient group. A: Hematoxylin-eosin (HE) stain showing Portal tract s with piecemeal necrosis; B: HE stain showing moderate portal inflammation, the hepatocytes shows mild steatosis; C: MassomTrichrom stain showing Portal tract with bridging fibrosis; D: MassomTrichrom stain showing fibrous expansion with septation.

Table 2 The frequency of the genotypes of gene polymorphisms in the studied groups *n* (%)

	Non responders (<i>n</i> = 301)	Responders (<i>n</i> = 356)	Control (<i>n</i> = 160)	<i>P</i> value
LDR exon8 c.1171G>A				< 0.001
AA	157 (52.2)	13 (3.6)	6 (3.8)	
GA	92 (30.6)	54 (15.2)	85 (53.1)	
GG	52 (17.2)	289 (81.2)	69 (43.1)	
LDR exon10 c.1413G>A				0.204
AA	48 (15.9)	51 (14.3)	15 (9.4)	
GA	191 (63.5)	215 (60.4)	109 (68.1)	
GG	62 (20.6)	90 (25.3)	36 (22.5)	

Table 3 Allele frequency and risk ratio in non-responders vs responders

	Non responders <i>n</i> (%)	Responders <i>n</i> (%)	<i>P</i> value	OR (95%CI)
LDR exon 8 c.1171G>Aallele				
A	406 (67.4)	80 (11.2)	< 0.001	16.4 (12.3-21.8)
G	196 (32.6)	632 (88.8)		1
exon 10 c.1413 G>Aallele				
A	287 (47.7)	317 (44.5)	0.253	1.13 (0.9-1.4)
G	315 (52.3)	395 (55.5)		1

The LDL receptor is a proposed receptor for HCV entry into hepatocytes. However the exact mechanism of HCV entry is not clear. The association of the virus with a host's lipoprotein component may facilitate this interaction^[42]. The endoplasmic reticulum is the likely site of production of lipid droplets that associate with HCV after its replication and assembly^[43]. Mature viruses are released from infected cells, and they are found in the low-density fraction of the serum of infected persons^[44]. Hepatocytes endocytosed HCV-RNA in the LDL fraction of HCV-infected patients. Anti-LDL and anti-ApoE antibodies block viral entry into cells^[17]. Several *in vitro* studies confirmed this hypothesis and concluded that the binding and entry of HCV particles into cultured hepatocytes was strongly associated with the level of LDLR expression^[45]. HCV infection propagates LDLR expression, which further promotes lipid uptake from the blood and enriches the hepatocyte content of lipids to facilitate HCV replication^[44,46].

Some studies indicate that the LDLR functions as a receptor of HCV binding and entry through interactions between the HCV/lipoprotein virion complex^[47]. Other studies suggest that the LDLR is essential only for post-entry events, such as viral replication^[48]. These studies note the possible role of other host receptors in HCV binding, attachment, endophagocytosis and viral replication, whether sequentially or individually. However, the viral envelope glycoproteins E1 and E2 are the main binding sites for the host receptors^[49].

Direct interaction of HCV_{E2} envelope proteins and LDL mediates the binding and internalisation of lipovirions by LDLR^[32]. Atherosclerosis, obesity and familial hypercholesterolaemia are definitely related to mutations and polymorphisms in the gene encoding for LDLR^[25]. This fact led investigators to probe the significance of gene polymorphisms of LDLR and the development of fibrosis, and the response to the standard of care treatment.

This study assessed gene polymorphisms at exon 8 G>A 1171 and exon 10 G>A 1413. The study was conducted on 657 patients with HCV infection and 160 healthy controls. The genotype distribution and allele frequency of exons 8 and 10 of the LDLR was studied and related to liver inflammation, the presence of steatosis, and BMI.

Our results demonstrated that the AA gene polymorphism of the 3 types of gene polymorphisms found in exon 8 of the LDLR (AA, GA, GG) was significantly different ($P < 0.001$) between HCV patients and the healthy control group, but the GA and GG gene polymorphisms were not significantly different. LDLR exon 10 also revealed no significance in gene polymorphisms (either AA, GA or GG), which indicates that the presence of the AA gene polymorphism of LDLR exon 8 was clearly associated with a higher susceptibility to HCV infection. Furthermore, the LDLR exon 8 AA gene polymorphism occurred more frequently in non-responders of interferon-based treatment than responders, and this difference was highly statistically significant ($P < 0.001$). A total of 157 patients (52.2%) of the non-responders had AA genes, but only 13 (3.6%) of the responders had AA genes.

However, no such discrepancy was detected in patients with GA or GG genes. exon 10 also exhibited no differences between gene polymorphisms between responders and non-responders.

One of the most important outcomes of the present study was the statistically significant association between the A allele of LDLR exon 8 gene polymorphism and the response to interferon, with carriers of the A allele exhibiting a 16.4 times greater risk for non-response to interferon-based treatment than patients who did not carry the A allele ($P < 0.001$), regardless of the type of interferon used.

The study identified a significant association between LDLR exon 8 and HAI staging and Metavir A and Metavir F, but this statistically significant difference was not present in LDLR exon 10.

Steatosis and BMI levels were significantly associated with LDLR exon 8, and both factors were higher in patients carrying the AA genotype. However, no such association was found in LDLR exon 10.

This difference may be explained by the fact that polymorphisms in the LDLR gene theoretically affect steatosis, which eventually leads to more severe liver disease and the failure of response to treatment in patients with HCV. However, the relationship between

Table 4 Steatosis and body mass index mean level in different *LDLR* genotypes in hepatitis C virus patients *n* (%)

	LDR exon 8 c.1171 G>A			<i>P</i> value	LDR exon 10 c.1413 G>A			<i>P</i> value
	AA	GA	GG		AA	GA	GG	
BMI (kg/m ²)	28.7 ± 4.7	28.1 ± 4.8	26.6 ± 4.3	< 0.001	27.3 ± 4.3	27.5 ± 4.8	27.4 ± 4.4	0.887
Steatosis								
0	64 (37.6)	70 (47.9)	204 (59.8)	< 0.001	50 (50.5)	208 (51.2)	80 (52.6)	0.938
1,2,3	106 (62.4)	76 (52.1)	137 (40.2)		49 (49.5)	198 (48.8)	72 (47.4)	

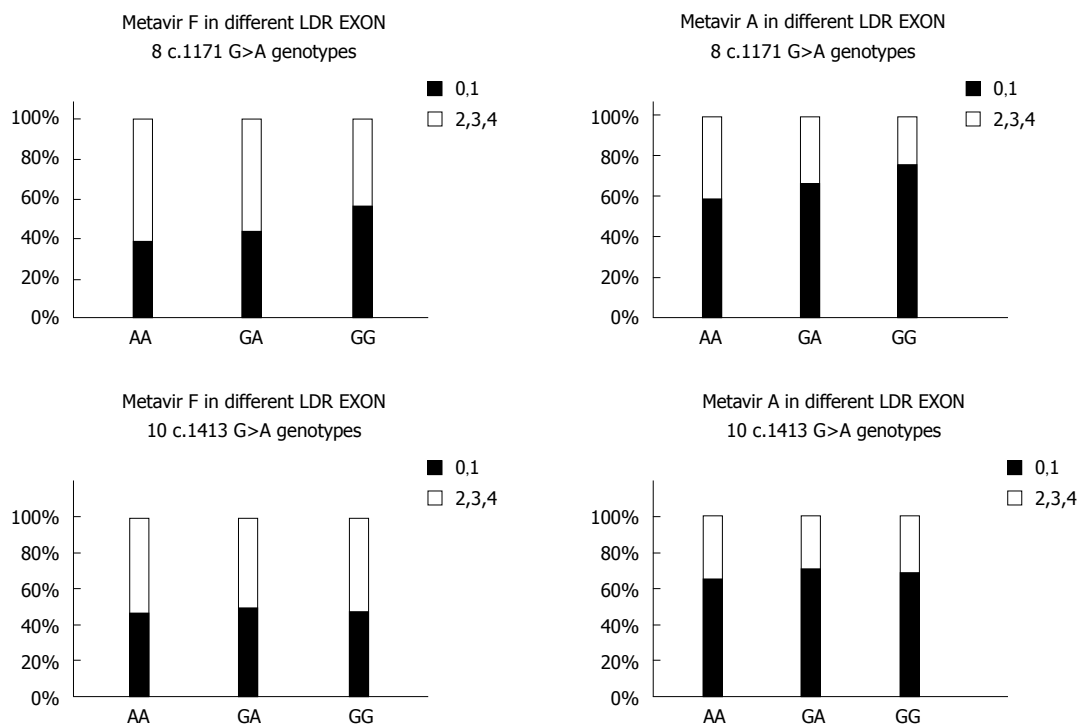


Figure 3 Low density lipoprotein receptor genes studied and Metavir A and F score in patient groups.

exon 8 and steatosis requires further study.

The finding that a single polymorphism in exon 8 was associated with more severe liver disease was also concluded in a study by Li *et al.*^[50] in 2006, who reported a correlation between exon 8 and fibrosis severity. In contrast to our study, Li *et al.*^[50] correlated a polymorphism of exon 10 to viral clearance and overall inflammation, which was not observed in our study. This difference may be explained by the difference in the ethnicity of the group studied and/or the type of viral genome.

The same correlation between exon 10, viral clearance and overall inflammation was also observed in a study by Hennig *et al.*^[36]. This study agreed with our study that the polymorphism in exon 8 exhibited the strongest association with disease severity. However the carriage of the G allele on exon 8 was associated with more severe fibrosis in this study, and heterozygosity at this site appeared protective against inflammation. This result directly contradicts our study in which the presence of the A allele was associated with more severe disease, and the presence of the G allele was somewhat protective.

A more recent study by Mas Marques *et al.*^[51] in

2009 demonstrated that the prevalence of an LDLR exon 8 polymorphism did not alter the grade of inflammation or stage of fibrosis. However, a careful analysis of this study reveals a low prevalence of the A allele of exon 8, which may support our results.

In conclusion, polymorphisms in the LDLR play a key role in the progression of HCV infection. The recent study of different exons identified exons 8 and 10 for further scrutiny. The results that relate polymorphisms of the alleles of these exons to the propagation of HCV infection and disease progression are somewhat contradictory. The need to clarify this dilemma is evident to improve disease outcome and treatment response in patients with HCV. This results of the present and previous studies supports the pivotal role of LDLR exon 8 in the natural course of HCV and its response to treatment. However, it is evident that further studies are needed to clarify whether other exons and alleles play a similar role.

ACKNOWLEDGMENTS

Special thanks to Professor Nouman Elgarim (Professor of internal medicine, Cairo University) and associate

professor Ahmed Abdel Moaaty for their efforts to facilitate the recruitment of patients from Beni-Suef public hospital. We thank Dr. Amr Abdelaziz (Assistant lecturer of internal medicine, Cairo University) for his efforts in sample collection from the Beni-Suef public hospital. We also thank Dr. Ismail Kassem (Assistant Professor of pathology, National Liver Institute, Cairo) for his help in the recruitment of patients from the National Liver Institute.

COMMENTS

Background

Hepatitis C virus (HCV) infection causes severe morbidity and mortality worldwide. Egypt has the largest epidemic of HCV worldwide. It was proposed that low-density lipoprotein receptors (LDLR) function as HCV receptors, and these receptors are implicated in the entry of HCV into hepatocytes.

Research frontiers

Genetic polymorphisms of the LDLR may be involved in treatment response and final disease outcome. Assessment of the frequency of LDLR genotype polymorphisms and the effect of these genetic polymorphisms in Egyptian chronic HCV patients aid predictions of treatment response.

Innovations and breakthroughs

The results demonstrated that the G allele of LDL receptor exon8c.1171G>A predominated in cases and controls over the A allele. Moreover, carriers of the A allele have a 16.4 times greater risk for non-response to anti-HCV therapy. A significant association was also found between LDL receptor exon8 c.1171G>A and the degree of liver fibrosis.

Applications

These findings aid in the selection of patients who will benefit from anti-HCV therapy. The study also confirmed the important role of LDLR exon 8 in the natural course of HCV and patient response to treatment. However, further studies are needed to clarify whether other exons and alleles play a similar role.

Terminology

LDLR is a cell surface receptor that plays an important role in cholesterol homeostasis. Mutations and polymorphisms in the LDLR gene are associated with familial hypercholesterolemia, obesity and atherosclerosis. The LDLR acts as a viral receptor. The LDLR gene family functions as a receptor for the minor group of common cold viruses, and these receptors may function as HCV and other virus receptors.

Peer-review

This report was very interesting study about HCV and LDLR polymorphism. HCV particles are known to be in complex with lipoproteins. Discussion need to be more detailed about LDLR and HCV.

REFERENCES

- Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]
- Marcellin P. Hepatitis C: the clinical spectrum of the disease. *J Hepatol* 1999; **31** Suppl 1: 9-16 [PMID: 10622554 DOI: 10.1016/S0168-8278(99)80368-7]
- Lee MH, Yang HI, Yuan Y, L'Italien G, Chen CJ. Epidemiology and natural history of hepatitis C virus infection. *World J Gastroenterol* 2014; **20**: 9270-9280 [PMID: 25071320 DOI: 10.3748/wjg.v20.i28.9270]
- Hajarizadeh B, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]
- Dumortier J, Boillot O, Scoazec JY. Natural history, treatment and prevention of hepatitis C recurrence after liver transplantation: past, present and future. *World J Gastroenterol* 2014; **20**: 11069-11079 [PMID: 25170196 DOI: 10.3748/wjg.v20.i32.11069]
- Lee JJ, Kim PT, Fischer S, Fung S, Gallinger S, McGilvray I, Moulton CA, Wei AC, Greig PD, Cleary SP. Impact of viral hepatitis on outcomes after liver resection for hepatocellular carcinoma: results from a north american center. *Ann Surg Oncol* 2014; **21**: 2708-2716 [PMID: 24806113 DOI: 10.1245/s10434-014-3609-6]
- Lee MH, Yang HI, Lu SN, Jen CL, You SL, Wang LY, L'Italien G, Chen CJ, Yuan Y. Hepatitis C virus genotype 1b increases cumulative lifetime risk of hepatocellular carcinoma. *Int J Cancer* 2014; **135**: 1119-1126 [PMID: 24482200 DOI: 10.1002/ijc.28753]
- Jahan S, Ashfaq UA, Qasim M, Khaliq S, Saleem MJ, Afzal N. Hepatitis C virus to hepatocellular carcinoma. *Infect Agent Cancer* 2012; **7**: 2 [PMID: 22289144 DOI: 10.1186/1750-9378-7-2]
- El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008. Egypt Demographic health survey. Final report, 2009. Egyptian Ministry of Health. El-Zanaty and associates and macro international: Cairo, 2009: 431
- Guerra J, Garenne M, Mohamed MK, Fontanet A. HCV burden of infection in Egypt: results from a nationwide survey. *J Viral Hepat* 2012; **19**: 560-567 [PMID: 22762140 DOI: 10.1111/j.1365-2893.2011.01576.x]
- Cuadros DF, Branscum AJ, Miller FD, Abu-Raddad LJ. Spatial epidemiology of hepatitis C virus infection in Egypt: analyses and implications. *Hepatology* 2014; **60**: 1150-1159 [PMID: 24913187 DOI: 10.1002/hep.27248]
- Reker C, Islam KM. Risk factors associated with high prevalence rates of hepatitis C infection in Egypt. *Int J Infect Dis* 2014; **25**: 104-106 [PMID: 24865321 DOI: 10.1016/j.ijid.2014.02.003]
- Kim DD, Hutton DW, Raouf AA, Salama M, Hablas A, Seifeldin IA, Soliman AS. Cost-effectiveness model for hepatitis C screening and treatment: Implications for Egypt and other countries with high prevalence. *Glob Public Health* 2015; **10**: 296-317 [PMID: 25469976 DOI: 10.1080/17441692.2014.984742]
- Benova L, Awad SF, Miller FD, Abu-Raddad LJ. Estimation of hepatitis C virus infections resulting from vertical transmission in Egypt. *Hepatology* 2015; **61**: 834-842 [PMID: 25366418 DOI: 10.1002/hep.27596]
- Lavanchy D, McMahon B. 10 Worldwide prevalence and prevention of hepatitis C. In: Liang TJ, Hoofnagle JH, eds. Hepatitis C. San Diego: Academic Press, 2000; 2: 185-201. Available from: URL: <http://www.sciencedirect.com/science/article/pii/S1874532600800149>
- Abdel-Rahman M, El-Sayed M, El Raziky M, Elsharkawy A, El-Akel W, Ghoneim H, Khattab H, Esmat G. Coinfection with hepatitis C virus and schistosomiasis: fibrosis and treatment response. *World J Gastroenterol* 2013; **19**: 2691-2696 [PMID: 23674877 DOI: 10.3748/wjg.v19.i17.2691]
- Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci USA* 1999; **96**: 12766-12771 [PMID: 10535997 DOI: 10.1073/pnas.96.22.12766]
- Monazahian M, Böhme I, Bonk S, Koch A, Scholz C, Grethe S, Thomssen R. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. *J Med Virol* 1999; **57**: 223-229 [PMID: 10022791]
- Jahan S, Samreen B, Khaliq S, Ijaz B, Khan M, Siddique MH, Ahmad W, Hassan S. HCV entry receptors as potential targets for siRNA-based inhibition of HCV. *Genet Vaccines Ther* 2011; **9**: 15 [PMID: 21896165 DOI: 10.1186/1479-0556-9-15]
- Rivero-Juarez A, Camacho A, Caruz A, Neukam K, Gonzalez R, Di Lello FA, Perez-Camacho I, Mesa P, Torre-Cisneros J, Peña J, Pineda JA, Rivero A. LDLr genotype modifies the impact of IL28B on HCV viral kinetics after the first weeks of treatment with PEG-IFN/RBV in HIV/HCV patients. *AIDS* 2012; **26**: 1009-1015 [PMID: 22382144 DOI: 10.1097/QAD.0b013e3283528b1c]
- Hofer F, Gruenberger M, Kowalski H, Machat H, Huettinger M, Kuechler E, Blaas D. Members of the low density lipoprotein

- receptor family mediate cell entry of a minor-group common cold virus. *Proc Natl Acad Sci USA* 1994; **91**: 1839-1842 [PMID: 8127891 DOI: 10.1073/pnas.91.5.1839]
- 22 **Bates P**, Young JA, Varmus HE. A receptor for subgroup A Rous sarcoma virus is related to the low density lipoprotein receptor. *Cell* 1993; **74**: 1043-1051 [PMID: 8402880 DOI: 10.1016/0092-8674(93)90726-7]
- 23 **Go GW**, Mani A. Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis. *Yale J Biol Med* 2012; **85**: 19-28 [PMID: 22461740 DOI: 10.1002/9780470015902.a0006138]
- 24 **Morales-Villegas E**. PCSK9 and LDLR The Yin-Yang in the Cellular Uptake of Cholesterol. *Curr Hypertens Rev* 2014; Epub ahead of print [PMID: 24993279 DOI: 10.2174/1573402110666140702092415]
- 25 **Li H**, Zhang Y, Wei X, Peng Y, Yang P, Tan H, Chen C, Pan Q, Liang D, Wu L. Rare intracranial cholesterol deposition and a homozygous mutation of LDLR in a familial hypercholesterolemia patient. *Gene* 2015; **569**: 313-317 [PMID: 25936346 DOI: 10.1016/j.gene.2015.04.071]
- 26 **De Castro-Orós I**, Pérez-López J, Mateo-Gallego R, Rebollar S, Ledesma M, León M, Cofán M, Casanovas JA, Ros E, Rodríguez-Rey JC, Civeira F, Pocoví M. A genetic variant in the LDLR promoter is responsible for part of the LDL-cholesterol variability in primary hypercholesterolemia. *BMC Med Genomics* 2014; **7**: 17 [PMID: 24708769 DOI: 10.1186/1755-8794-7-17]
- 27 **Khan SP**, Ghani R, Ahmed KZ, Yaqoob Z. Two novel mutations in exon 3 and 4 of low density lipoprotein (LDL) receptor gene in patients with heterozygous familial hypercholesterolemia. *J Coll Physicians Surg Pak* 2011; **21**: 403-406 [PMID: 21777527]
- 28 **Zee RY**, Schrader AP, Robinson BG, Griffiths LR, Morris BJ. Association of HincII RFLP of low density lipoprotein receptor gene with obesity in essential hypertensives. *Clin Genet* 1995; **47**: 118-121 [PMID: 7634533 DOI: 10.1111/j.1399-0004.1995.tb03942.x]
- 29 **Sarzani R**, Salvi F, Bordicchia M, Guerra F, Battistoni I, Pagliariccio G, Carbonari L, Dessi-Fulgheri P, Rappelli A. Carotid artery atherosclerosis in hypertensive patients with a functional LDL receptor-related protein 6 gene variant. *Nutr Metab Cardiovasc Dis* 2011; **21**: 150-156 [PMID: 19833493 DOI: 10.1016/j.numecd.2009.08.004]
- 30 **Ding Z**, Liu S, Wang X, Khaidakov M, Fan Y, Deng X, Xiang D, Mehta JL. Lectin-like oxidized low-density lipoprotein receptor-1 regulates autophagy and Toll-like receptor 4 in the brain of hypertensive mice. *J Hypertens* 2015; **33**: 525-533; discussion 533 [PMID: 25380158 DOI: 10.1097/HJH.0000000000000411]
- 31 **Salazar LA**, Hirata MH, Giannini SD, Forti N, Diamant J, Lima TM, Hirata RD. Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. *Clin Chim Acta* 2000; **300**: 139-149 [PMID: 10958870 DOI: 10.1016/S0009-8981(00)00308-9]
- 32 **Wunschmann S**, Muller HM, Stipp CS, Hemler ME, Stapleton JT. In vitro interaction between hepatitis C virus (HCV) envelope glycoprotein E2 and serum lipoproteins (LPs) results in enhanced cellular binding of both HCV E2 and LPs. *J Infect Dis* 2006; **194**: 1058-1067 [PMID: 16991080 DOI: 10.1086/507647]
- 33 **Zhao LJ**, Zhao P, Chen QL, Ren H, Pan W, Qi ZT. Mitogen-activated protein kinase signalling pathways triggered by the hepatitis C virus envelope protein E2: implications for the prevention of infection. *Cell Prolif* 2007; **40**: 508-521 [PMID: 17635518 DOI: 10.1111/j.1365-2184.2007.00453.x]
- 34 **Molina S**, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D, Roitelman J, Barbaras R, Graber P, Ghersa P, Smolarsky M, Funaro A, Malavasi F, Larrey D, Coste J, Fabre JM, Sa-Cunha A, Maurel P. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol* 2007; **46**: 411-419 [PMID: 17156886 DOI: 10.1016/j.jhep.2006.09.024]
- 35 **Carrière M**, Rosenberg AR, Conti F, Chouzenoux S, Terris B, Sogni P, Soubrane O, Calmus Y, Podevin P. Low density lipoprotein receptor transcripts correlates with liver hepatitis C virus RNA in patients with alcohol consumption. *J Viral Hepat* 2006; **13**: 633-642 [PMID: 16907851 DOI: 10.1111/j.1365-2893.2006.00737.x]
- 36 **Hennig BJ**, Hellier S, Frodsham AJ, Zhang L, Klenerman P, Knapp S, Wright M, Thomas HC, Thursz M, Hill AV. Association of low-density lipoprotein receptor polymorphisms and outcome of hepatitis C infection. *Genes Immun* 2002; **3**: 359-367 [PMID: 12209363 DOI: 10.1038/sj.gene.6363883]
- 37 **Matas M**, Picornell A, Cifuentes C, Payeras A, Homar F, González-Candelas F, López-Labrador FX, Moya A, Ramon C, Castro JA. Relating the outcome of HCV infection and different host SNP polymorphisms in a Majorcan population coinfecting with HCV-HIV and treated with pegIFN-RBV. *Int Microbiol* 2014; **17**: 11-20 [PMID: 25296442]
- 38 **Soriano V**, Poveda E, Vispo E, Labarga P, Rallón N, Barreiro P. Pharmacogenetics of hepatitis C. *J Antimicrob Chemother* 2012; **67**: 523-529 [PMID: 22194301 DOI: 10.1093/jac/dkr506]
- 39 **Lefkowitz JH**. Liver biopsy assessment in chronic hepatitis. *Arch Med Res* 2007; **38**: 634-643 [PMID: 17613355 DOI: 10.1016/j.arcmed.2006.08.005]
- 40 **Helle F**, Dubuisson J. Hepatitis C virus entry into host cells. *Cell Mol Life Sci* 2008; **65**: 100-112 [PMID: 17914604 DOI: 10.1007/s00018-007-7291-8]
- 41 **Goldstein JL**, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009; **29**: 431-438 [PMID: 19299327 DOI: 10.1161/ATVBAHA.108.179564]
- 42 **Gastaminza P**, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. *J Virol* 2008; **82**: 2120-2129 [PMID: 18077707 DOI: 10.1128/jvi.02053-07]
- 43 **Miyinari Y**, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007; **9**: 1089-1097 [PMID: 17721513 DOI: 10.1038/ncb1631]
- 44 **Syed GH**, Tang H, Khan M, Hassanein T, Liu J, Siddiqui A. Hepatitis C virus stimulates low-density lipoprotein receptor expression to facilitate viral propagation. *J Virol* 2014; **88**: 2519-2529 [PMID: 24352472 DOI: 10.1128/JVI.02727-13]
- 45 **Martin C**, Nielsen SU, Ibrahim S, Bassendine MF, Toms GL. Binding of liver derived, low density hepatitis C virus to human hepatoma cells. *J Med Virol* 2008; **80**: 816-823 [PMID: 18360895 DOI: 10.1002/jmv.21150]
- 46 **Caruz A**, Neukam K, Rivero-Juárez A, Herrero R, Real LM, Camacho A, Barreiro P, Labarga P, Rivero A, Pineda JA. Association of low-density lipoprotein receptor genotypes with hepatitis C viral load. *Genes Immun* 2014; **15**: 16-24 [PMID: 24173146 DOI: 10.1038/gene.2013.56]
- 47 **Owen DM**, Huang H, Ye J, Gale M. Apolipoprotein E on hepatitis C virion facilitates infection through interaction with low-density lipoprotein receptor. *Virology* 2009; **394**: 99-108 [PMID: 19751943 DOI: 10.1016/j.virol.2009.08.037]
- 48 **Albecka A**, Belouzard S, Op de Beeck A, Descamps V, Goueslain L, Bertrand-Michel J, Tercé F, Duverlie G, Rouillé Y, Dubuisson J. Role of low-density lipoprotein receptor in the hepatitis C virus life cycle. *Hepatology* 2012; **55**: 998-1007 [PMID: 22121002 DOI: 10.1002/hep.25501]
- 49 **Scarselli E**, Ansuini H, Cerino R, Roccasecca RM, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R, Vitelli A. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002; **21**: 5017-5025 [PMID: 12356718 DOI: 10.1093/emboj/cdf529]
- 50 **Li H**, Liu Z, Han Q, Li Y, Chen J. Association of genetic polymorphism of low-density lipoprotein receptor with chronic viral hepatitis C infection in Han Chinese. *J Med Virol* 2006; **78**: 1289-1295 [PMID: 16927291 DOI: 10.1002/jmv.20693]
- 51 **Mas Marques A**, Mueller T, Welke J, Taube S, Sarrazin C, Wiese M, Halangck J, Witt H, Ahlenstiel G, Spengler U, Goebel U, Schott E, Weich V, Schlosser B, Wasmuth HE, Lammert F, Berg T, Schreiber

E. Low-density lipoprotein receptor variants are associated with spontaneous and treatment-induced recovery from hepatitis C virus

infection. *Infect Genet Evol* 2009; **9**: 847-852 [PMID: 19446659
DOI: 10.1016/j.meegid.2009.05.002]

P- Reviewer: Mahmood T, Ogura S **S- Editor:** Yu J
L- Editor: A **E- Editor:** Liu XM





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327

