Evaluation of the role of different Apolipoprotein-E genotypes in the outcome of liver disease caused by Hepatitis C virus

Thesis

Submitted for Partial Fulfillment of Master Degree in Internal Medicine

By

Ahmed Magdy Fath-Allah Mansour

MB.B.Ch. Ain Shams University, 2008

Under Supervision of

Prof. Dr. Mohammed A. M. Makhlouf

Professor of Internal Medicine and Gastroenterology

Faculty of Medicine, Ain Shams University

Prof. Dr. Ehab Hasan Nashaat

Professor of Internal Medicine and Gastroenterology

Faculty of Medicine, Ain Shams University

Assistant Prof. Dr. Moataz Mohammed Sayyed

Assistant Professor of Internal Medicine and Gastroenterology

Faculty of Medicine, Ain Shams University

Faculty of Medicine

Ain Shams University

2013

Acknowledgement

First of all, all thanks is to ALLAH for granting me the power and ability to finish this work, and for blessing me with his help all through my life.

I can hardly find the words to express my deep and sincere gratitude to Prof. Dr. Mohammed Aly Mar'y Makhlouf, Professor of Internal Medicine and Gastroenterology at Faculty of Medicine, Ain Shams University, for his kind supervision, continuous support, and great effort throughout this work.

Also, I'd like to express my great appreciation for Prof. Dr. Ihab Hasan Nashaat, Professor of Internal Medicine and Gastroenterology at Faculty of Medicine, Ain Shams University, for his guidance, sincere support and insightful comments during the production of this work.

I'm also indebted to Assistant Prof. Dr. Moataz Mohammed Sayyed, Assistant Professor of Internal Medicine and Gastroenterology at Faculty of Medicine, Ain Shams University for his enormous day-to-day effort, continuous support, sincere supervision and persistent encouragement throughout this study.

I wish also to thank Assistant Prof. Dr. Sarah Abu-Agwa, together with Dr. Marwa and Dr. Aya, and the rest of the team at Ain Shams University Medical Research Center, for their unbelievable effort, continuous support, and help in resolving any problems that faced this study.

I'd like to thank all my professors, colleagues, friends and anyone who helped by encouragement, support or even criticism, and to all our mentors who gave us their knowledge, skills and experience, and whom with their efforts we reached this moment.

Finally, I dedicate this work to all of my family, my father, my mother, my wonderful wife, and my cute little angel, Salma.

LIST OF CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AFP	Alpha-Fetoprotein
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Transaminase
ANOVA	Analysis Of Variance
ASO	Allele-Specific Oligonucleotides
AST	Aspartate Transaminase
ApoE	Apolipoprotein-E
Bp	Base Pairs
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CM	Chylomicrons
CNS	Central Nervous System
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic acid
EDTA	Ethylene-Diamine-Tetraacetic Acid
ELISA	Enzyme-Linked Immunosorbant Assay
ER	Endoplasmic Reticulum
ESLD	End-Stage Liver Disease

Hb	Hemoglobin
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HDL	High Density Lipoproteins
HFL	Hepatic Focal Lesion
HIV-1	Human Immunodeficiency Virus 1
HLA	Human Leukocyte Antigen
HSPG	Heparin Sulfate Proteoglycans
HSV	Herpes Simplex Virus
HVAP-A	Human VAMP-Associated Protein A
HVR1	Hypervariable Region 1
IDL	Intermediate Density Lipoprotein
IL-2	Interleukin 2
iNOS	Inducible Nitric Oxide Synthase
INR	International Normalization Ratio
IRES	Internal Ribosomal Entry Site
LDL	Low Density Lipoproteins
LDLRs	Low Density Lipoprotein Receptors
LRP	LDL Receptor-Related Protein
LVP	Lipo-Viro Particle

MELD	Model of End-Stage Liver Disease
MPGN	Membranoproliferative Glomerulonephritis
mRNA	Messenger Ribonucleic Acid
NASH	Non-Alcoholic Steatohepatitis
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health in the United States
NS	Non-Structural
OR	Odds Ratio
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PLT	Platelets
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RDRP	RNA-Dependent RNA Polymerase
RNA	Ribonucleic Acid
SD	Standard Deviation
SPSS	Statistical Package for Special Science
SR-BI	Scavenger Receptor Class B Type I
SVR	Sustained Viral Response
Tat	Transactivator Protein

TGFβ1	Transforming Growth Factor β1
TLC	Total Leukocytic Count
TNF-A	Tumour Necrosis Factor A
UTR	Untranslated Region
UV	Ultraviolet
VAMP	Vesicle-Associated Membrane Protein
VLDL	Very Low Density Lipoproteins

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health problem. More than 170 million people worldwide are infected with HCV ***(Poynard et al., 2003, Shepard et al., 2005, Kamal and Nasser, 2008)***. HCV is a causative agent of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) ***(Hishiki et al., 2010)***. In Egypt, HCV prevalence is ~15%, which is considered the highest prevalence worldwide, with genotype 4 being the most common, and responsible for 90% of HCV infections in Egypt ***(Egyptian Ministry of Health, 2007, Kamal and Nasser, 2008, El-Zanaty and Ann Way., 2009)***.

There are several possible outcomes after infection with HCV ***(Alter et al., 1992)***. Approximately 15% to 20% of infected individuals clear the virus, as evidenced by the presence of anti-HCV antibodies, but absence of viral RNA in serum. The remaining individuals (~85%) become chronically infected, with HCV RNA readily detectable in serum ***(Seeff and Hoofnagle, 2002)***.

The consequences of chronic infection also vary: some individuals develop minor or no liver damage, whereas others suffer from progressive chronic hepatitis, leading to liver cirrhosis, and even HCC. The reasons for the diversity of outcomes of HCV infection are still unclear.

At least 6 major HCV genotypes are identified ***(Robertson et al., 1998, NIH Consens State Sci Statements, 2002)***. However, infection with a particular genotype is not thought to influence disease outcome ***(Alric et al., 2000)***. Alternatively, host factors may be important. Some factors include male sex, an older age at

infection, increased alcohol intake, coinfection with Human Immunodeficiency Virus 1 (HIV-1) or Hepatitis B virus (HBV), and insulin resistance ***(Sanchez-Quijano et al., 1995, Poynard et al., 1997, Wiley et al., 1998, Zarski et al., 1998, Mohsen and the Trent HCV Study Group, 2001, Sud et al., 2004, Price et al., 2006)***.

HCV particles circulating in the blood of HCV carriers associate with lipoproteins, such as low-density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons (CM); thus, it is termed a lipo-viro particle (LVP) ***(Andre et al., 2002, Nielsen et al., 2006, Hishiki et al., 2010)***.

It has been suggested that the virus might gain entry into cells via a hitchhiker method with the lipoproteins ***(Agnello V et al., 1999)***. Specifically, entry might involve LDL receptors (LDLRs) ***(Price et al., 2006)***. One of the trials that supported this theory was done using COS-7 cells, which are fibroblast-like cells derived from monkeys kidney tissue and used in production of different recombinant proteins. These cells were transfected with the human gene for LDLR, and were found to bind more HCV particles than untransfected cells. Another trial used antibodies that block binding of ligands to LDLRs, and it was found that they also block binding of HCV to cells ***(Monazahian M et al., 1999)***. The virus also has been shown to bind directly to certain apolipoproteins, which might facilitate entry ***(Depla E et al., 1998, Sabile A et al., 1999)***.

Several lipoproteins groups contain Apolipoprotein-E (ApoE), a polymorphic and multifunctional protein with numerous roles in lipoproteins metabolism. The three common isoforms: ApoE2, ApoE3 and ApoE4 show isoform-specific functional properties