The Role of IL28B Gene in Treatment Response of HCV Infected Patients

Thesis

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In Internal Medicine

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List of Abbreviations

AA	:	Amino Acid
AASLD	:	American Association For The Study Of Liver Disease
AFP	:	Alpha Fetoprotein
Ag	:	Antigen
ALT	:	Alanine Aminotransferase
AP	:	Alkaline Phosphatatase
AST	:	Aspartate Aminotransferases
AT	:	Antithrombin
BMI	:	Body Mass Index
bp	:	Base pairs
CCP	:	Anti-cyclic citrullinated peptide
CD 81	:	Cluster Of Differentiation 81 (Target Of Anti Proliferation Antibody)
CD4	:	Cluster Of Differentiation 4
CLD	:	Chronic Liver Disease
cDNA	:	Complementary DNA
CTL	:	Cytotoxic T-lymphocytes
D. Bil	:	Direct Bilirubin
DAA	:	Direct-Acting Antiviral Agent
DNA	:	De-Oxy Ribonucleic Acid
DVR	:	Delayed Virological Response
ECM	:	Extra Cellular Matrix
EIA	:	Enzyme Linked Immunosorbent Essay
ER	:	Endoplasmic Reticulum
EVR	:	Early Virologic Response
FDA	:	Food And Drug Administration
GGT	:	Gamma Gluteryl Transferase
HBsAg	:	Hepatitis B Surface Antigen
HBV	:	Hepatitis B Virus
HBeAg	:	Hepatitis B E Antigen
HCC	:	Hepatocellular Carcinoma

List of Abbreviations (Cont.)

HCV	:	Hepatitis C Virus
Hgb	:	Hemoglobin
HIV	:	Human Immune Deficiency Virus
HLA	:	Human Leucocyte Antigen
HRS	:	Hepato-Renal Syndrom
IFN α	:	Interferon alfa
IFN-3	:	Interferon-3
IgM	:	Immunoglobulin M
IL28B	:	Interleukin 28b
INF	:	Interferon
INR	:	International Normalization Ratio
ITP	:	Immune Thrombocytopenic Purpura
IU	:	International Unit
KD	:	Kilo Dalton
LKM	:	liver/kidney microsomes
mRNA	:	Messenger Ribonucleic Acid
Mb	:	Mega base
NI	:	Nucleoside Analogue Inhibitor
NK	:	Natural Killer
NIHC	:	National Institutes of Health Consensus Development Conference
NNI	:	Non Nucleoside Inhibitor
NRTI	:	Neucloside Reverse Transcriptase Inhibitor
NS	:	Non Structural
PCR	:	Polymerase Chain Reaction
PEG-IFN	:	Pegylated Interferon
PHTN	:	Portal Hypertension
PLT	:	Platelets
PT	:	Prothrombin Time
PVT	:	Portal Vein Thrombosis
RBV	:	Ribavirin
RDRP	:	RNA Dependent RNA Polymerase
RNA	:	Riboneucleic Acid Of Hepatitis C Virus

List of Abbreviations (Cont.)

RVR	:	Rapid Virologic Response
SBP	:	Spontaneous Bacterial Peritonitis
SNPs	:	Single nucleotide polymorphisms
STAT-C	:	Specially Targeted Antiviral Therapy For HCV
SVR	:	Sustained Virologic Response
T.bil	:	Total Bilirubin
TE	:	Transient Elastography
Te	:	Transient Elastography
TGF	:	Transforming Growth Factor
TMA	:	Transcription Mediated Amplification
TSH	:	Thyroid Stimulating Hormone
U/S	:	Ultra Sound
USA	:	United States Of America
UTR	:	Untranslated Regions
WBC	:	White Blood Cells
WHO	:	World Health Organization
EDHS	:	Egyptian Demographic Health Survey

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الملخص العربي

لا يزال الفيروس الكبدي سي مشكلة صحية عامة تؤثر على ما يقرب من 3% من سكان العالم أي ما يقرب من 170مليون شخص حامل للمرض حسب إحصائيات منظمة الصحة العالمية 2007م.

وتعتبر مصر من أعلي دول العالم فى الإصابة حيث أنه يوجد ما يقرب من 11–14% من السكان يحملون الأجسام المضادة للفيروس مما قد يؤدي إلى تليف الكبد ودوالي المرئ ومن ثم إلى النزف والوفاة. ويرجع ذلك إلى تفشى مرض البلهارسيا والاستخدام الغير آمن والمتكرر للحقن.

وإيماناً من العلماء بفداحة هذا المرض وعواقبة الوخيمة فقد كرث الباحثون مجهوداتهم لبحث طرق الكشف المبكر والعقارات الجديدة فى علاج هذا المرض القاتل.

من أمثله الأبحاث أنهم وجدوا أنواع جديدة من العقارات مثل مثبطات الـ Ns3-4Aو Boceprevir , Ceprevir, Telaprevir والتي يجرى عليها تجارب الآن للموافقة على استخدامها والتى من المتوقع أن تثبت نجاح مبهر فى العلاج الجذري للمرض خاصة مرضى النوع الأول من فيروس C.

أما مثبطات الـNs5b و Ns5A اعطت نتائج مبشرة فى علاج الأنواع الأخرى من فيروس C ولكن هذه العقارات مازالت فى طور التجربة وتحتاج إلى معرفة أعراضها الجانبية الأخرى وتكلفتها قبل طرحها فى الأسواق.

ويجرى الباحثون الآن على الجيني IL28b تجاربهم والتي اثبت بما لا يدع مجالاً للشك أن هناك علاقة وطيدة بينهم ومدى استجابة المرضى للعلاج حيث أنه يزيد من معدل الاستجابة 2 إلى 3 أضعاف وقد أصبح الكشف عن هذا الجين المتواجد فى الجينوم البشري ويحتوى على نوعين من (rs12979860, rs809917) واللذان يعطيان الترميز لثلاث جينات خلوية هما IL28b, IL28a,

IL29 وينتمون لعائلة IFN-K حيث يعمل IFN-K على منع الفيروس سي من الانقسام وبالتالي الانتشار.

وقد هدفت هذه الدراسه إلى كشف ما إذا كان هناك علاقة بين الجين IL28b ومدى استجابة مرضى فيروس من النوع الرابع حيث يتراوح معدل الأعمار من 20–60سنة قد تلقى ثلاثون مريضاً شملتهم الدراسة علاج الانترفيرون إلى جانب الريبافيرون بالجرعات والمقاييس العالمية فى عيادات الكبد بمركز الأستاذ الدكتور/يس عبدالغفار وتم عمل الآتي لكل المرضى:

1- أخذ التاريخ المرضى بالكامل.
2- تحليل وظائف كبد وكلى وصورة دم كاملة.
3- تحليل فيروس بي وسي.
4- عد كمي لفيروس سي.
5- تحليل جيني لنوع الفيروس سي.
6- فحص للحين IL28b.

وتم تقسيم المرضى إلى ثلاث مجموعات:

CC (1) CT (2) TT (3)

وقد أظهرت الدراسة ما يلي :

أن المرضى الحاملون للنوع CC وعددهم 11 حققوا شفاء بنسبة 90.9% والحاملون للنوع CT وعددهم 6 حققوا شفاء بنسبة 66.7% وبالنسبة للنمط TT وعددهم 10 لم يتم تحقيق شفاء لأي منهم أي أن الاستجابة للعلاج كانت ذات دلالة إحصائية قوية حيث حقق المرضى من النمط الجيني CC أعلى معدلات استجابة عن أمثالهم من النوع TT بقيمة P> 0.001.

وبناء على ذلك فإنه من المهم أن يتم الكشف عن نوع النمط الجيني IL28b لكل مرضى الالتهاب الكبدي الوبائي C لبيان مدى استجابتهم للعلاج قبل أخذهم إياه.

Introduction

Approximately 3% of the world's population has been infected with the hepatitis C virus (HCV), which represents about 170 million chronic carriers at risk of developing serious complications **(Pradat, 2000).** The presence of HCV in the liver triggers the human immune system, which leads to inflammation. Over time (usually decades), prolonged inflammation may cause scarring. Extensive scarring in the liver is called fibrosis. When the liver becomes fibrotic, the liver fails to perform its normal functions, (liver failure), and this leads to serious complications and even death. Cirrhotic livers also are more prone to become cancerous **(Fung, 2009)**.

It has been estimated that HCV related complications is responsible for approximately 250 000 to 350 000 deaths per year. Following the introduction of screening of blood donors for infection the risk of transmitting HCV by blood products is presently at 1/200 000 units distributed. Intravenous drug users are currently the main risk group with a prevalence rate of about 80% and a yearly incidence varying between 4 and 6%. Vertical and sexual transmissions have also been implicated but data are limited and sometimes controversial. The source of infection for the 30% of cases without an identifiable risk factor remains to be clarified. Prevention of spread includes detailed information of persons at risk of being infected, screening of high-risk populations, and limitting of syringe exchanges among IV drug users and strict application of disinfection procedures for all invasive medical equipment **(Pradat, 2000).**

The combination of a pegylated IFN plus ribavirin is now recognized as the standard of care for chronic HCV infected patients, together they significantly increases sustained virological response rates **(Zeuzem et al .,2009).** It is important in treating the chronic HCV infection to identify

patient and viral characteristics that predict response to current therapies. These factors include; HCV genotype, viral load, body weight, age, liver histology, co-infection with HIV and treatment adherence and patient tolerance **(Nelson et al., 2009).**

In 2009, researchers first reported that specific variations known as single nucleotide polymorphisms (SNPs) in the IL28B gene region-which encodes interleukin 28, also known as interferon lambda -were associated with spontaneous clearance of genotype 1 hepatitis C virus (HCV) and response to interferon-based therapy for chronic infection. Different teams have identified several associated SNPs, but one known as rs12979860 is implicated most often **(Kurbanov et al., 2010).**

Recent studies showed that presence of IL28b affects response of hepatitis C virus to interferon-based therapy as well. Among people with hepatitis C, individuals with the C/C gene pattern, meaning they carry 2 copies of the "C" variant or allele- are more likely to respond well to interferon Alfa plus ribavirin. People with the T/T pattern have the least favorable response, while those carrying 1 "C" and 1 "T" allele fall somewhere in between **(Kurbanov et al ., 2010).**

Aim of the work

The goal of this study is to find out whether there is a relation between the gene IL28b and early virological response (EVR) with interferon based therapy in Egyptian patients infected by HCV genotype 4.

Genetics

Genetics Is the science that studies the patterns of inheritance of specific traits.

Each DNA molecule contains many genes, the basic physical and functional units of heredity. A gene is a specific sequence of nucleotide bases, whose sequences carry the information required for constructing proteins, which provide the structural components of cells and tissues as well as enzymes for essential biochemical reactions. The human genome is estimated to comprise at least 100,000 genes (**Griffiths et al., 2000a)**

Human genes vary widely in length, often extending over thousands of bases, but only about 10% of the genome is known to include the (exons) which are the protein-coding sequences of genes. Interspersed within many genes are intron sequences, which have no coding function. The balance of the genome is thought to consist of other noncoding regions (such as control sequences and intergenic regions), whose functions are obscure (**Hartl and Jones, 2005).**

In humans, as in other higher organisms, a DNA molecule consists of two strands that wrap around each other to resemble a twisted ladder whose sides are made of sugar and phosphate molecules, connected by nitrogen-containing chemicals called bases (Fig.1). Four different bases are present in DNA: adenine (A), thymine (T), cytosine (C), and Guanine (G). The particular order of the bases arranged along the sugar-phosphate backbone is called the DNA sequence, the sequence specifies the exact genetic instructions required to create a particular organism with its own unique traits (Fig.1) **(Lodish et al., 2000)**