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Association of LDL-R Polymorphism and Treatment Outcome of Chronic Hepatitis C Genotype 3: a Pilot Study

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ABSTRACT

Host genetic factor has been shown to be influential at least partially in determining treatment responsiveness. For HCV infection, LDL-R has been proposed to promote hepatitis C virus endocytosis. Polymorphisms within the LDL-R gene are associated with familial hypercholesterolemia, atherosclerosis and obesity. Previous studies have shown a correlation between single nucleotide polymorphism (SNP) in LDL-R gene and treatment response as well as degree of inflammation and fibrosis. We conducted a study to determine whether in Thai patients, LDL-R polymorphisms may also exert such effect. A total of 111 patients with chronic hepatitis C genotype 3 were included. Demographic data, HCV genotype, and treatment results at 11 separated LDL-R SNPs were collected. Our results showed SNP in intron3.2 to be borderline associated with response to treatment. Further study is needed, however, owing to the small sample size in this study. More data on severity of inflammation and fibrosis should also be added to identify other possible correlations.

Key words : chronic hepatitis C, low-density lipoprotein receptor, LDL-R polymorphism, single nucleotide polymorphisms

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INTRODUCTION

Hepatitis C virus (HCV) is a major global health problem, with 3% of the world's population, or approximately 170 million persons, chronically infected.(1) The prevalence of HCV infection in Thailand is 1-2%, of which HCV genotype 3a is most common (40-50%). Persistent HCV infection has variable outcome, with 5-20% of infected persons developing liver cirrhosis within 20-25 years. Only approximately 20-30% of infected individuals will spontaneously clear the virus (self-limiting infection), whereas the remaining 70-80% proceed to chronic HCV infection.(12) Approximately 1-2% of cirrhotic patients per year will develop hepatocellular carcinoma. The major goal in the treatment of HCV infection is to prevent the development of decompensate liver disease and death. This strategy

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includes eradication or prolonged suppression of viral replication, reduction of hepatic inflammation, and slowing of the rate of progressive liver injury. At present, the standard treatment of chronic hepatitis C is a combination of interferon or pegylated interferon and ribavirin.[3]

The mechanism of viral entry into the host hepatocytes is not fully understood, as there have been very few attempts to study HCV replication in vitro. The lack of a perfect animal model and a reliable cell culture system for HCV have hindered analysis of the mechanism by which HCV infects liver cells and assembles into virions. In recent years, many studies have been made to identify the receptors involved in viral entry into host cells. Several studies showed that the presence of HCV in infected human sera was associated with low-density lipoprotein receptor (LDL-R), suggesting that the virus might utilize LDL-R as a receptor.[12,14,16,17] Because of its proposed function as a receptor for HCV, its role in immune responses and its presence of known polymorphisms, the LDL-R is an important candidate for the study of genetic susceptibility to HCV infection.[13,15,18-20]

The outcome of a large number of infectious diseases is affected by a multitude of factors, including environmental factors, interaction with pathogens and host factors, particularly genetic variability. Disease association studies based on polymorphisms are widely used in the elucidation of host genetic factors influencing multifactorial diseases, and may help in identifying individuals at risk of developing a disease or a specific disease outcome.[21] The clinical outcome of hepatitis C infection and the response to current treatment are variable and remain poorly understood. A recent study has shown that the sustained virological response was seen in 84% of patients with viral genotype 2 or 3, who were treated for 24 weeks with a combination of pegylated interferon α-2a and ribavirin, and in 52% of genotype 1 patients who were treated for 48 weeks with the combination therapy.[3] Viral factors probably play a role also in the outcome of infection,

Chronic hepatitis C is characterized by varying degrees of hepatic inflammation and fibrosis. The degree of inflammation, the severity of fibrosis and the response to therapy are likely to be influenced by host factors. The latter which include genetic susceptibility may contribute to the variable manifestations of the disease as well as treatment response. There is increasing research activity focusing on the elucidation of host genetic factors that may affect disease progression in hepatitis C.[22,23]

Due to its proposed function as receptor for HCV, its role in immune responses, and the presence of known polymorphisms, LDL-R is an important candidate for the study of genetic susceptibility to hepatitis C. We reported here the investigation of 13 single nucleotide polymorphisms (SNPs) in the LDL-R gene, and their possible influence on the clinical parameters of HCV infection. Previous studies[5,10] showed that SNP in LDL-R gene exon8, exon10, exon13 and 3' UTR loci are associated with viral clearance, degree of inflammation, and response to treatment. Specifically, we classified genotyped SNPs in the promoter region 3'UTR, intron1, intron3.1, intron3.2, intron4, intron6, intron11, intron14, intron16, exon8, exon10 and exon13. All selected SNPs are shown to be in the same haplotype block from an Asian population analysis. An assessment was made on possible associations between genetic variation and viral clearance, overall inflammation, and response to treatment.

Materials and Methods

Study subjects

One-hundred-and-twelve patients with chronic hepatitis C (HCV genotype 3) were enrolled. The diagnosis of chronic hepatitis C was based on elevated serum aminotransferase levels at least 2 times of upper limit of normal (40 U/L) for at least 6 months, histological examination, anti-HCV positivity as assessed by third-generation enzyme-linked immunosorbent assay version 3.0 (Abbott Axym), and consistently detectable serum HCV RNA by quantitative reverse-transcriptase polymerase chain reaction with the lowest detection sensitivity of 600 IU/ml (Cobas Amplicor Monitor HCV version 2.0, Roche diagnostics). HCV genotypes were determined using reverse hybridization assay (INNO LiPA HCVC-II, Innogenetics, Zwijnaarde, Belgium). Patients were excluded for any of the following reasons: other liver diseases (such as chronic hepatitis B, autoimmune hepatitis, alcohol-induced liver disease, drug-induced hepatitis, decompensated liver disease), co-infection with human immunodeficiency virus, current pregnancy or breast feeding, or previous antiviral therapy. Data were also collected on patient's age, sex, body weight, route of HCV infection, CBC, blood chemistry, liver function tests, HCV viral load and liver histology (if available) at

before, during and after complete treatment and follow up period. Informed written consent was obtained from every patient. The study was approved by the Ethics Committees of Siriraj Hospital, Mahidol University, Thailand.

Antiviral Therapy

All patients received their first course of treatment with standard antiviral therapy at the Hepatology Outpatient Clinic, Siriraj Hospital. One of three different recommended standard regimens were used: 1) a combination of interferon α-2b 3 mU by subcutaneous injection thrice a week plus daily oral ribavirin (in divided doses administered twice daily; 1000-1200 mg, depending on weight <75 kg or ≥75 kg) for 48 weeks 2) a combination of pegylated interferon α-2a 180 μg by subcutaneous injection once a week plus 800 mg daily of oral ribavirin (in divided doses administered twice daily) for 24 weeks 3) a combination of pegylated interferon α-2b 1.5 μg/kg by subcutaneous injection once a week plus at least 10.6 mg/kg daily of oral ribavirin (in divided doses administered twice daily) for 24 weeks. Routine laboratory tests such as complete blood count and liver function tests were performed at baseline, 24 or 48 weeks (the end of treatment), and 48 or 72 weeks (24 weeks after the end of treatment), depending on the treatment regimens. The virological response to therapy was assessed by a qualitative HCV RNA, and the patients were defined as (1) sustained virological responders (no detectable HCV RNA at the end of treatment and 24 weeks afterwards), (2) virological non-responders (detectable HCV RNA at the end of treatment or detectable HCV RNA within 24 weeks after the end of treatment response), (3) relapsers (no detactable HCV RNA at the end of treatment but detectable HCV RNA within 24 wk post treatment follow up.

DNA extraction

Five milliliters of fasting venous blood from each subject was drawn in vacutainer tube containing EDTA, and was frozen at -20˚C. In accordance with to the instruction provided by the manufacturer, genomic DNA was extracted by Phenol Chloroform method and stored at -20˚C.

Target sequences and primers

Thirteen polymorphisms of current interest to our group were chosen to illustrate the methodology, namely SNP in intron1, intron3.1, intron3.2, intron4, intron6, exon8, intron11, intron13, intron16, 3'UTR - 2 SNPs in intron16 region. Primers for the amplification of 12 fragments were grouped into 3 sets, and were designed by Oligo 6 programmed as described in Table 1.

Table 1. Primer for PCR 3 sets.

Oligo Name	Sequence 5' to 3'
Set I	
LDLR int1F	CTC AAA ACA CCC TCT AGG AAG
LDLR int1R	GGT TAG ATA GAC AAT CCT GG
LDLR int3.1F	GAG AGG GCA GTG GTT CAG AG
LDLR int3.1R	GCA CTT CCC ATC GTG GCA GC
LDLR int3.2F	TCC TGG GGA GTG GTC TGA CT
LDLR int3.2R	CCT TCA TGT TAC GTG GGT CA
3UTRF	AGA CAG ATG GTC AGT CTG GAG
3UTRR	GGC AAT GCT TTG GTC TTC TC
Set II	
LDLR int 6F	ACA TGA ATT CTT TTC CTT AGA TG
LDLR int 6R	CGA CAG AGC AAG ACT CTG TT
LDLR int 4F	GAG ATG GAG TCT CAC TCT GTG
LDLR int 4R	ACA ACC AGC ACC TTC CAA
LDLR int 11F	GCC TTC CAA ACT GCT GGG
LDLR int 11R	GGA CCT AGC AGA AAA GCA CCT
Set III	
LDLR exon 8F	TAC AAG TGC CAG TGT GAG GAA G
LDLR exon 8R	GTG CAA AGT TCA GAG GAT GAA ACT
LDLR int 16F	GGC AGA GGA AAT GAG AAG AAG C
LDLR int 16R	CCC TTA GCT GTC TGA TCT TGT CAC

Set 1 (6 SNP) = 3'UTR (129bp), int 1 (150bp), exon 13 (185bp), int 3.2 (207bp), exon 10 (284bp), int 3.1 (350bp)
Set 2 (3 SNP) = int 6(80bp), int 4 (133bp), int 11 (168bp)
Set 3 (3 SNP) = int14 (108bp), exon 8 (150 bp), int 16 (246bp)

The experimental conditions for genotyping described in this paper have been successfully applied to unpublished polymorphisms.

Polymerase chain reaction

Set I: PCR for the fragment was performed in a 50 μl volume containing 175 ng of genomic DNA, 0.5 μM of each primer, 1000 μM of each dNTP, 3 unit of Taq DNA polymerase (Immolase), 1× PCR buffer in a total volume of 50 μl (The 10× PCR buffer contains: 670 mM Tris-HCl (pH 8.3); 67 mM $MgCl_2$; 166 mM $(NH_4)2SO_4$; 85 mM 2 mercaptoethanol; 1.7 mg/ml BSA). Amplification was performed with an initial denaturation step of 10 min at 95˚C followed by 35 cycles of 95˚C for 30 s, 58˚C for 30 s and 65˚C for 3 min, and then an extension step of 65˚C for 10 min.

Set II: PCR for the fragment was performed in a 25 µl volume containing 175 ng of genomic DNA, 1 mM $MgCl_2$, 1.2 µM of int6 primer, 1 µM of int4 primer, 0.32 µM of int11 primer, 200 µM of each dNTP, 1.5 unit of Taq DNA polymerase (Qiagen), 1× PCR buffer (contains 1.5 mM $MgCl_2$). Amplification was performed with an initial denaturation step of 10 min at 95˚C followed by 37 cycles of 95˚C for 30 s, 54˚C for 30 s and 72˚C for 1 min, and then an extension step of 72˚C for 10 min.

Set III: PCR for the fragment was performed in a 25 µl volume containing 162.5 ng of genomic DNA, 1 mM $MgCl_2$, 1.2 µM of int14 primer, 1 µM of exon8 primer, 1 µM of int16 primer, 200 µM of each dNTP, 1.5 unit of Taq DNA polymerase (Qiagen), 1× PCR buffer (contains 1.5 mM $MgCl_2$). Amplification was performed with an initial denaturation step of 10 min at 95˚C followed by 37 cycles of 95˚C for 30 s, 54˚C for 30 s and 72˚C for 1 min, and then an extension step of 72˚C for 10 min.

The PCR amplification of all 3 sets were then carried out and purified by the Exosap purification kit, preparing for the single base extension.

Multiplex PCR and primer extension reaction

The primers for extension reaction (3 sets) were again designed by Oligo6 programmed as describe in Table 2. The multiplexed primer extension reaction was performed in a single reaction tube, and was essentially the same as the single primer extension reaction described above.

Set I: The final volume was 20 µl, containing reaction buffer, 1mM ddNTP, 2 unit/µl thermosequenase, 10 pmol/µl primer (SNP2), 10 pmol/µl primer (SNP1), 10 pmol/µl primer (SNP13), 10 pmol/µl primer (SNP3), 10 pmol/µl primer (SNP9), 10 pmol/µl primer (SNP7), PCR-product (Exo-Sap treated), and DI-H_2O. The reaction was carried out in a thermal cycling with an initial denaturation step of 1 min at 96˚C followed by 60 cycles of 96˚C for 15 min, 50˚C for 15 min and 60˚C for 1 min, and then an extension step of 96˚C for 1 min.

Set II: The final volume was 20 µl, containing reaction buffer, 1 mM ddNTP, 2 unit/µl thermosequenase, 10 pmol/µl, 10 pmol/µl primer (SNP8), 10 pmol/µl primer (SNP4), 10 pmol/µl primer (SNP5), and DI-H_2O. The reaction was carried out in a thermal cycling with an initial denaturation step of 1 min at 96˚C followed by 60 cycles of 96˚C for 15 min, 50˚C for 15 min and 60˚C for 1 min, and then an extension step of 96˚C for 1 min.

Set III: The final volume was 20 µl, containing Reaction buffer, 1mM ddNTP, 2 unit/µl Thermosequenase, 10 pmol/µl, 10 pmol/µl primer (SNP8), 10 pmol/µl primer (SNP12), 10 pmol/µl primer (SNP11), 10 pmol/µl primer (SNP6), 10 pmol/µl primer (SNP10), and DI-H_2O. The reaction was carried out in a thermal cycling with an initial denaturation step of 1 min at 96˚C followed by 60 cycles of 96˚C for 15 min, 50˚C for 15 min and 60˚C for 1 min, and then an extension step of 96˚C for 1 min.

DHPLC analysis

When the PCR were completely done, the PCR products were run and analyzed in the electrophoresis, as showed in Figure 1.

Table 2. Primers for extension.

Oligo Name	Sequence 5' to 3'
LDLR -int3.1-ext	GTC ACG CAC A GT CAG
LDLR int1-ext	TAA CAA CTT GGG TAA CTG ACT
LDLR 3'UTR-ext	TTT TTT TGT CTT TGA ATA AAA CAA GGC
LDLR int3.2-ext	TTT TTT TTT TTT TTT TTA CCG TGT GAA GTC TCC CA
LDLR int11-ext	GGG GAG TTG CAG GTC A
LDLR int4-ext	TTT TTA ACC AGC ACC TTC CAA ACA G
LDLR int6-ext	TTT TTT TTT TTT TTC CTT AGA TGC CTG CTT CT
LDLR int16-ext	AGG GAA CAG CCC CAC T
LDLR int16.1-ext	TTT TTT TCC CAG GTC ACA GCC TCC
LDLR exon8-ext	TTT TTT TTT TTT TCT GGA CCC CCA CAC GAA G
LDLR int14-ext	TTT TTT TTT TTT TTT TCC AAG GTC ATT TGA GAC TTT C

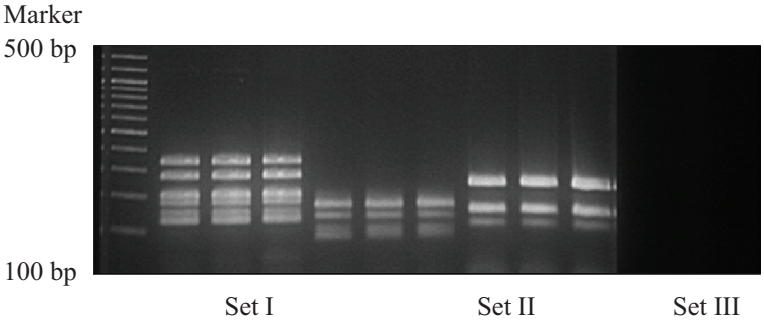

Figure 1. PCR product Set I, II and III when analyzed by electrophoresis.

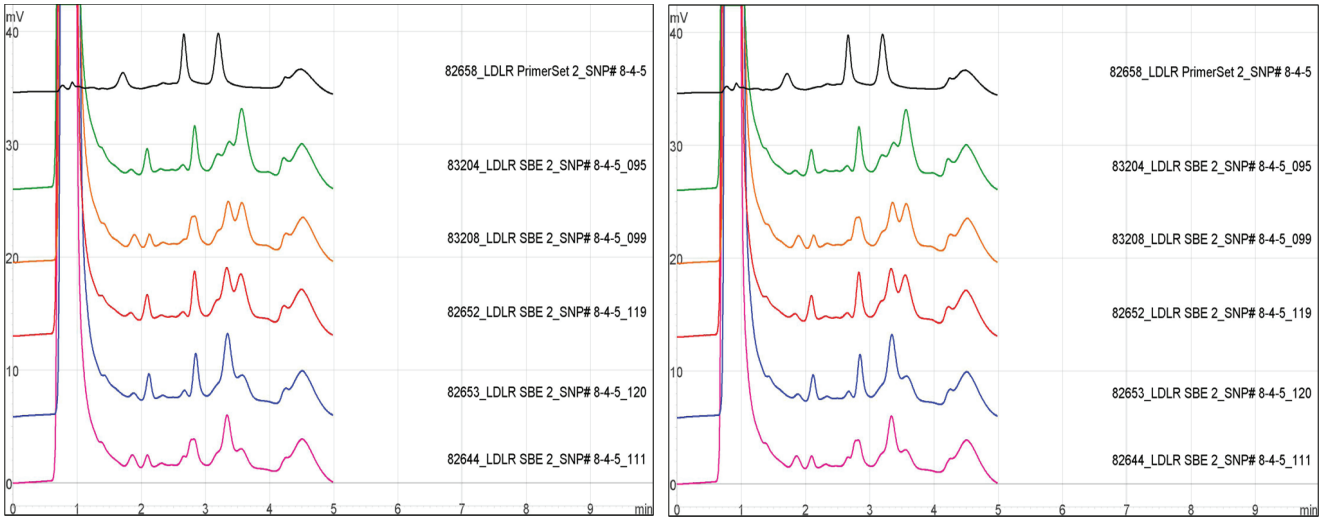

Figure 2. Sample of SBE Set II, III was analyzed in DHPLC machine and then identified for genotype.

Statistical analysis

Patient characteristics were analyzed by descriptive statistics and reported as mean, range, and percent. For the results, sustained virological responders and non-responders were compared, using Chi-Square test, Fisher's exact test, student *t* test, or Mann-Whitney tests as appropriate. To determine the cutoff levels of the quantitative factors with significant differences between the response groups, we also analyzed the odds ratios with 95%-confidence intervals. Two sided *p*-values of less than 0.05 were regarded as significant. All calculations were performed with SPSS software version 13.0 (SPSS Inc.). In addition, the frequency of each SNP was assessed by using Hardy-Weinberg equilibrium (HWE) — http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl. The DeFinetti program and haplotypes analysis were assessed by Hapstat to defined haplotype-disease association.

RESULTS

Patients characteristics

Of all 112 patients with chronic hepatitis C (genotype 3), 77 patients (68.8%) achieved a sustained virological response (SVR), and 34 patients (20.4%) were non-sustained responders. Of the latter, 19 patients (17%) relapsed after end-of-treatment response while 15 patients (13.4%) never responded during treatment. Baseline characteristics of all patients are summarized in Table 3. Most patients (62.5%) had no risk factors. From the patients characteristic, we found that only previous treatment was associated with a significant chance to be non-responder to treatment or relapser after the end-of-treatment response, odd ratio 7.8 (2.43-26.67) compared to naïve patients. The genotype frequencies for all eleven SNPs investigated did not vary significantly between different age groups.

Table 3. Baseline patient characteristics.

Age (yr)	52
Sex : Male/Female (n, %)	48/64 (42.9/ 57.1)
Body weight (kg) (range)	64 (41-101)
Risk factors :	
- blood transfusion	30 (26.8%)
- IVDU	5 (4.5%)
- tattoo	2 (1.8%)
- combined IVDU and tattoo	4 (3.6%)
AST (U/L)	90 (4-448)
ALT (U/L)	100 (10-390)
HCV RNA (IU/mL)	
mean ± SD	362,500 ± 4,023,834
(range)	(117,750 - 2,212,500)
IFN reduction	26 (23.2%)
Ribavirin reduction	43 (38.4%)
Response to treatment : SVR	77 (68.8%)
Relapse	19 (17%)
No response	15 (13.4%)
EVR	102 (91.1%)
Discontinued treatment	14 (12.5%)

* The data are showed in mean ± SD, HCV RNA are showed in median IQR

Genotyping analysis in LDL-R

All SNPs were in HWE, especially in SVR group, according to Fisher's exact test and De fenetti diagram. All available genotype data was analyzed with the comparison between the sustained virological response (SVR) group, the relapser and non-responder group. Table 4 shows a summary of overall *p*-values for all SNPs tested. Table 5 displays the results for the presence or the absence of specific genotypes and alleles, with the risk associated with carriage of specific alleles.

One SNP in this study was shown to be monomorphic in our population (exon8), which was in contrast to a previous study in which the codon change in exon8 was associated with the severity of fibrosis.[5] There was only few data in our study about the histology and fibrosis score, anyway. The intron3.2 polymorphism was found to be borderline associated with treatment responses from Armitage's trend test (Table 5). This was due to an increased frequency of A alleles, rendering carrier of this alleles more susceptible to resistance to standard treatment. In addition, the SNP in 3'UTR (G→A), which was previously shown to be associated with response to treatment, was found

Table 4. SNP frequencies and Fisher's exact test for deviation from HWE.

Genotype	Fisher's exact test for HWE	
	SVR	relapse and non-responder
Intron1 : (A→T) *		
AA	28	15
TA	38	10
TT	10	8
Intron3.1 : (G→A)		
GG	65	26
GA	12	7
AA	0	1
Intron3.2 : (G→A)		
GG	67	29
GA	10	3
AA	0	2
Intron4 : (T→G)		
TT	47	20
GT	28	13
GG	2	1
Intron6 : (G→A)		
GG	45	22
GA	29	10
AA	3	2
exon8 : (G→A)		
GG	77	33
GA	0	0
AA	0	0
Intron11 : (G→C)		
GG	50	17
CG	26	17
CC	1	0
Intron13 : (G→A)		
GG	67	28
GA	10	6
AA	0	0
Intron16 : (C→T)		
CC	32	12
CT	31	17
TT	14	5
Intron16 : (G→A)		
GG	38	18
GA	34	12
AA	5	4
3'UTR : (C→T)		
CC	35	12
CT	30	17
TT	12	5

* For intron1, 1 patient had technical problems that cannot identify the genotype, so, total number in SVR group in intron1 is 76 patients whereas in other groups are 77

Table 5. Distribution of LDL-R haplotype (only haplotypes with frequencies of more than 0.05 were shown).

LDL-R haplotype	Estimate (f)		Standard error	Z-stat	*p*-value
	SVR	Non response			
AGGTGGGCGC	0.0909	0.1612	0.0281	5.5606	0
AGGTGGGTGT	**0.3027**	0.2664	0.0419	7.429	0
AGGGGCGCGC	0.1502	0.1226	0.0277	5.4161	0
TGGTGGGCAC	0.0754	0.1101	0.0229	4.2982	0
TGGTAGGCAC	0.0758	0.0766	0.0216	3.9858	0.0001

to be insignificant in our study that the codon changes is C→T.

LDL-R haplotype analysis

A total 30 haplotypes were observed, of which only one is relatively common with a frequency of 30% (Table 6). Log-likelihood estimation was performed using different genetic models. The difference of haplotype frequencies between SVR and non-response group did not reach statistical significant level in any model assumption.

Discussion

It has been proposed that production of soluble variants by host cells may be utilized in order to control virus infections. Overall, it remains uncertain as to whether virus uptake can be mediated directly by the LDL-R, but lipoprotein bound to HCV may function as a ligand. It seems that the LDL-R or its homologues are involved in endocytosis of HCV. However, the exact mechanisms underlying this process are to date not fully understood. Therefore, polymorphisms in the LDL-R could influence the activity of the receptor, and may thus affect the level of cellular entry for HCV. This in turn may influence the ability of the virus to establish persistent infection, to evade interferon therapy and to induce fibrosis.

Genetic variation in the LDL-R gene has been studied intensively over the past two decades or more. The data provided evidence for an involvement of LDL-R polymorphism in the pathogenesis and /or progression of hepatitis C. Within the LDL-R gene there are abundant polymorphisms, which have been well-characterized and studied intensively (details at http://www.ucl.ac.uk/fh). In the present study, we selected 11 SNPs as mentioned before, based on data from a previous study and their haplotype block from an Asian population analysis.

We found a negative correlation between all SNPs and response to treatment, except for the SNP in intron 3.2 that showed borderline significance associated with response to treatment. No SNP in this study led to an amino acid change. Both heterozygous and homozygous changes at these SNPs appeared to have worsened the outcome in response to treatment.

Unfortunately, there were some technical problems in our study evolving the SBE process of the SNPs in exon8, exon10 and exon13 that we were interested in. Therefore, we did not learn about the frequencies and changes in SNPs in those locations, and could not compare the differences or similarities of those SNPs to previous studies.

Our data analysis was limited by the small sample size and the lack of histologic data for correlation with other factors such as severity of fibrosis, inflammation, and steatosis. Further study with a larges sample size is required to increase power of the association study.

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Table 6. Association of single SNP and treatment response.

SNP position	Test for deviation HWE		Allele freq. diff.	Heterozygous	Homozygous	Allele positivity	Armitage's trend test
	SVR	Relapse + NR					
Intron1	$p = 0.8089$	$p = 0.0642$	A↔T $p = 0.6823$	A/A↔A/T $p = 0.2051$	Risk allele T A/A↔T/T $p = 0.3779$	A/A↔A/T, T/T $p = 0.5533$	Common OR $p = 0.6924$
			T↔A $p = 0.6823$	T/T↔A/T $p = 0.0551$	Risk allele A T/T↔A/A $p = 0.3779$	T/T↔A/T, A/A $p = 0.1240$	Common OR $p = 0.6924$
Intron3.1	$p = 1$	$p = 0.4665$	G↔A $p = 0.1666$	G/G↔G/A $p = 0.4134$	Risk allele A G/G↔A/A $p = 0.1092$	G/G↔G/A, A/A $p = 0.2671$	Common OR $p = 0.1668$
			A↔G $p = 0.1666$	A/A↔G/A $p = 0.2089$	Risk allele G A/A↔G/G $p = 0.1092$	A/A↔G/A, G/G $p = 0.1225$	Common OR $p = 0.1668$
Intron3.2	$p = 1$	$\mathbf{p = 0.0109}$	G↔A $p = 0.5713$	G/G↔G/A $\mathbf{p = 0.0157}$	Risk allele A G/G↔A/A $\mathbf{p = 0.0343}$	G/G↔G/A, A/A $\mathbf{p = 0.0102}$	Common OR $p = 0.5179$
			A↔G $p = 0.5713$	A/A↔G/A $\mathbf{p = 0.0157}$	Risk allele G A/A↔G/G $\mathbf{p = 0.0343}$	A/A↔G/A, G/G $\mathbf{p = 0.0282}$	Common OR $p = 0.5179$
Intron4	$p = 0.7229$	$p = 0.3071$	T↔G $p = 0.9360$	T/T↔G/T $p = 0.6040$	Risk allele G T/T↔G/G $p = 0.3737$	T/T↔G/T, G/G $p = 0.7272$	Common OR $p = 0.9316$
			G↔T $p = 0.9360$	G/G↔G/T $p = 0.3231$	Risk allele T G/G↔T/T $p = 0.3737$	G/G↔G/T, T/T $p = 0.3533$	Common OR $p = 0.9316$
Intron6	$p = 0.7482$	$p = 0.3452$	G↔A $p = 0.7699$	G/G↔G/A $p = 0.6094$	Risk allele A G/G↔A/A $p = 0.3218$	G/G↔G/A, A/A $p = 0.8729$	Common OR $p = 0.7707$
			A↔G $p = 0.7699$	A/A↔G/A $p = 0.2204$	Risk allele G A/A↔G/G $p = 0.3218$	A/A↔G/A, G/G $p = 0.2640$	Common OR $p = 0.7707$
Intron11	$p = 0.4451$	$p = 0.3071$	G↔C $p = 0.6720$	G/G↔C/G $p = 0.4738$	Risk allele C G/G↔C/C $p = 0.5362$	G/G↔C/G, C/C $p = 0.5410$	Common OR $p = 0.6444$
			C↔G $p = 0.6720$	C/C↔C/G $p = 0.4738$	Risk allele G C/C↔G/G $p = 0.5362$	C/C↔C/G, G/G $p = 0.5135$	Common OR $p = 0.6444$
Intron13	$p = 1$	$p = 1.0$	G↔A $p = 0.5713$	G/G↔G/A $p = 0.4623$	Risk allele A G/G↔A/A $p = 1$	G/G↔G/A, A/A $p = 0.4623$	Common OR $p = 0.4623$
			A↔G $p = 0.5713$	A/A↔G/A $p = 1$	Risk allele G A/A↔G/G $p = 1$	A/A↔G/A, G/G $p = 1$	Common OR $p = 0.4623$
Intron16	$p = 0.3327$	$p = 1.0$	C↔T $p = 0.6218$	C/C↔C/T $p = 0.4425$	Risk allele T C/C↔T/T $p = 0.7919$	C/C↔C/T, T/T $p = 0.4800$	Common OR $p = 0.6337$
			T↔C $p = 0.6218$	T/T↔C/T $p = 0.7664$	Risk allele C T/T↔C/C $p = 0.7919$	T/T↔C/T, C/C $p = 0.9751$	Common OR $p = 0.6337$
Intron16	$p = 0.5817$	$p = 0.3901$	G↔A $p = 0.9930$	G/G↔G/A $p = 0\ 7664$	Risk allele A G/G↔A/A $p = 0.7919$	G/G↔G/A, A/A $p = 0.9751$	Common OR $p = 0.9930$
			A↔G $p = 0.9930$	A/A↔G/A $p = 0.3592$	Risk allele G A/A↔G/G $p = 0.4691$	A/A↔G/A, G/G $p = 0.3137$	Common OR $p = 0.9930$
3'UTR	$p = 0.3191$	p = 1.0	C↔T $p = 0.5583$	C/C↔C/T $p = 0.3700$	Risk allele T C/C↔T/T $p = 0.7563$	C/C↔C/T, T/T $p = 0.4070$	Common OR $p = 0.5744$
			T↔C $p = 0.5583$	T/T↔C/T $p = 0.7275$	Risk allele C T/T↔C/C $p = 0.7563$	T/T↔C/T, C/C $p = 0.9751$	Common OR $p = 0.5744$

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