Genetic Polymorphism of Low-Density Lipoprotein Receptor Did Not Affect Treatment Outcome of Chronic Hepatitis C Genotype 3

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Background: *The low-density lipoprotein receptor (LDL-R) has been proposed to function as a receptor for the hepatitis C virus (HCV) entry. Polymorphism of LDL-R gene may influence the clearance of virus and response to treatment. This study was conducted to evaluate the association of LDL-R gene polymorphism and the response to antiviral treatment in patients with chronic HCV infection.*

Material and Method: *A total of 112 naïve patients with HCV genotype 3 were enrolled in the study. All patients were treated with a combination of pegylated interferon and ribavirin for 24 weeks. Polymerase chain reaction combined with restriction fragment length polymorphism was used to detect the polymorphism at the LDL-R gene intron 11 loci, including intron1, intron 3.1, intron 3.2, intron 4, intron 6, exon 8, intron 11, intron 13, intron 14 and 3'UTR-2 SNPs in intron 16 region. Comparisons of genotype and allele frequency between responders and nonresponders were analyzed.*

Results: *Patients had a mean age of 54 years and 43% were male. Mean HCV RNA viral load and alanine aminotransferase level were 6.3 $\log_{10}$ IU/mL and 100 IU/L, respectively. Sustained virological response, relapse and no response were documented in 68.7%, 17.9% and 13.4%, respectively. Baseline characteristics including age, sex, body weight, aminotransferase levels and HCV RNA viral load were similar between responders and nonresponders. No statistical difference was found for either genotype distribution or allele frequency among responders and nonresponders.*

Conclusion: *This study did not provide the evidence for a role of LDL-R polymorphism the response to antiviral treatment in patients with HCV genotype 3. This indicates that a genetic component via the LDL-R may not control HCV treatment outcome in HCV genotype 3*

Keywords*: Hepatitis C virus, Low-density lipoprotein receptor, Low-density lipoprotein receptor polymorphism, Hepatitis C virus treatment response*

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Hepatitis C virus (HCV) is a major global health problem with up to 3 % of the world population or approximately 170 million people infected. Only approximately 20-30% of infected individuals will spontaneously clear the virus (self-limiting infection). Persistent HCV infection may progress to cirrhosis and hepatocellular carcinoma. The major goal in the treatment of hepatitis C virus infection is to arrest disease progression, prevent the development of decompensated liver disease and death. At present, the standard treatment of chronic hepatitis C consists of the combination of interferon or pegylated interferon and ribavirin[1,2].

The clinical outcome of hepatitis C infection

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and response to current treatment is variable and remains poorly understood. Recent study has shown that sustained virological response was seen in 84% of patients with genotype 2 or 3 virus who were treated with 24 weeks combination of pegylated interferon α-2a and ribavirin and in 52% of patients with genotype 1 virus who were treated with 48 weeks of combination therapy[2]. Clearance of the virus depends on both viral factors and host factors. For the HCV genotype 3a, which is considered an easy-to-treat genotype, that means more favorable viral factors for the treatment. In those who did not response to the treatment, host factors may have a predominant role in preventing clearance of the virus.

The mechanism of viral entry into host hepatocytes is not well understood. Several studies have shown that HCV presence in infected human sera was associated with the low-density lipoprotein receptor (LDL-R), suggesting that the virus might utilize LDL-R as a receptor[3-5]. The role of LDL-R in immune responses and LDL-R polymorphisms provides an important candidate for the study of genetic susceptibility to HCV infection[6-11]. Since LDL-R is proposed as receptor for HCV, LDL-R may play some roles in immune responses and viral clearance by affecting viral entry. The presence of known polymorphisms in LDL-R provides an important candidate for the study of genetic susceptibility to hepatitis C and disease outcome. Most studies have demonstrated that LDL-R is a crucial receptor or co-receptor for cell entry and replication of HCV. There are studies which show that single nucleotide polymorphisms (SNPs) in LDL-R gene exon 8, exon 10, exon 13 and 3'UTR loci are associated with viral clearance, degree of inflammation[12,13]. These studies show that 3'UTR (G→A) may be associated with better treatment response in HCV genotype 1. However, it is not known whether LDL-R polymorphism will affect response to HCV genotype 3. This study was conducted to determine whether LDL-R polymorphisms may affect the treatment response in chronic hepatitis C, genotype 3.

Material and Method

One hundred and twelve chronic hepatitis C genotype 3 patients who had been treated with peginterferon/ribavirin for 24 weeks with at least 24 weeks of follow-up were identified from October 2005 to December 2006. Treatment response was assessed at the end of treatment and 24 weeks posttreatment. The response was classified into responder when HCV RNA was negative at the end of treatment and at 24 weeks follow-up and non-responder, if HCV RNA was positive at the end of treatment or at 24 weeks of follow-up (relapser). All patients gave consent and the study was conducted with approval of Siriraj's Ethic Committee.

DNA extraction

Five milliliters of fasting venous blood from each subject was drawn in EDTA tube. According to the instruction provided by the manufacturer, genomic DNA was extracted by Phenol Chloroform method and the DNAs were stored at -20°C until tested.

Target sequences and primers

Eleven polymorphisms of current interest were chosen: SNP in intron1, intron 3.1, intron 3.2, intron 4, intron 6, exon 8, intron 11, intron 13, intron 14, 3'UTR, and 2 SNPs in intron 16 region. Designed primers by Oligo 6 program for the amplification of 11 fragments were grouped into 3 sets and as described in Table 1. The primers for extension reaction (3 sets) were shown 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. When the PCR was completely done, the PCR products were run and analyzed in the electrophoresis gel, as showed in Fig. 1. Then, the PCR products were analyzed in DHPLC machine to identify the LDL-R genotype as shown in Fig. 2.

Statistical Analysis

Patient characteristics were analyzed by descriptive statistics and reported as mean ± SD, range, and percent. For the results, we compared sustained virological responders and non-responders with Chi-square test, Fisher's exact test, student t-test and Mann-Whitney tests as appropriate. To determine cutoff levels of the quantitative factors with significant differences between the response groups, we also analyzed 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 SNPs were assessed by using Hardy-Weinberg equilibrium (HWE) and haplotypes analysis was assessed by Hapstat to defined haplotype-disease association.

Results

There were 112 patients, 48 male and 64 female

Table 1. The 3 primer sets for PCR for LDL-R polymorphisms

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

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

with mean age of 54 years old. Seventy seven patients (68.7%) were sustained responders and 35 patients (31.2%) were either relapsers (17.9%) or non-responders (13.4%) as shown in Table 3. Baseline characteristics of all patients were summarized in table 3. Most of patients (66.9%) had no risk factors. There was no statistically significant of demographic characteristic in both responders and those who did not respond.

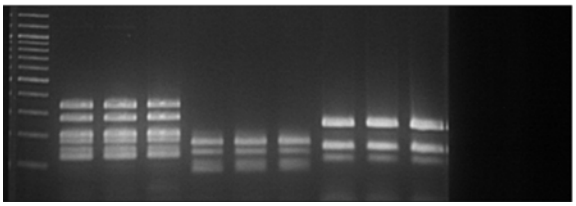

Fig. 1 PCR product Set I, II and III when analyzed by electrophoresis

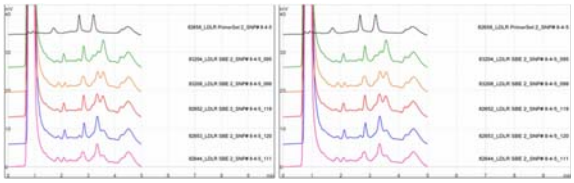

Fig. 2 Sample of SBE Set II, III when analyze in DHPLC machine to identify LDL-R genotype

Table 3. Baseline characteristics of the patients

Characteristic	Number of Patients (n = 112)
Age, years	54 ± 10
Gender (Male)	48 (42.9)
Body weight (kg)	64.3 ± 12.1
Risk factors identified	
Blood transfusion	30 (26.8)
IVDU	5 (4.5)
Tattooing	2 (1.8)
Unknown	75 (66.9)
Mean AST (U/L)	91 ± 74
Mean ALT (U/L)	101 ± 78
Mean HCV RNA (log_{10} IU/mL)	6.3 ± 6.6
Response to treatment	
Sustained virological response	77 (68.7)
Relapse	20 (17.9)
No response	15 (13.4)

Results expressed as number (%) or mean □ SD unless specified otherwise. IVDU, intravenous drug users; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus

Genotyping analysis in LDL-R

All available genotype data was analyzed by the comparison between sustained virological response group versus relapser and non-responder group. Table 4 showed summary of overall P-values for all SNPs tested. However, p-value of exon 8 was not able to be calculated due to the presence of only one allele in this locus. The intron 3.2 polymorphism was found to have borderline association with poorer treatment responses from Armitage's trend test. However, the SNPs in 3'UTR (G→A) that previously was associated with better response to treatment were found to be insignificant in this study.

LDL-R haplotype analysis

A total 30 haplotypes were observed but only one haplotype is relatively common, with the frequency of 30% (Table 5). Log-likelihood estimation was done using different genetic models. The difference of haplotype frequencies between responders and non-response groups did not reach a statistically significant level in any model assumption.

Discussion

LDL-R gene polymorphisms have been studied extensively during past several years in many diseases. Recent studies have shown that LDL-R may play an important role in pathogenesis and disease progression of chronic hepatitis C. The virus uptake can be mediated directly by the LDL-R and lipoprotein bound to HCV may function as a ligand. It seems that the LDL-R or its homologues are involved in endocytosis of HCV. Therefore, polymorphisms in the LDL-R could influence the activity of the receptor and thus may affect the level of cellular entry for HCV. This, in turn, may influence the ability of the virus to establish persistent infection, to induce fibrosis and influence treatment response to HCV treatment. Previous studies have shown that some SNP in LDL-R gene, such as exon 8, exon 10, exon 13 and 3'UTR loci, were associated with more viral clearance, degree of inflammation and response to treatment[12,13]. Within the LDL-R gene, there are abundant of polymorphisms, which have been well characterized and studied extensively (details found at http://www.ucl.ac.uk/fh). For the present study, eleven SNPs, as mentioned before, have been selected, base on data from previous studies and their haplotype block from Asian population analysis.

The result of this study showed that there was no correlation between all selected SNPs and treatment response in chronic hepatitis C genotype 3. Only a SNP in intron 3.2 has shown a borderline significant association with poor response to treatment, but the result could not reach a statistically significant level. Moreover, no SNPs in this study led to an amino acid change. The negative correlation between LDL-R polymorphisms and treatment response in this study may be explained by several mechanisms. LDL-R polymorphisms do not play an important role in

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

Genotype		Fisher's exact test for HWE		
		Sustained virological response	Relapse and non-responder	p-value
Intron1:	AA	29	14	0.81
(A→T)	TA	38	10	
	TT	10	8	
Intron3.1:	GG	66	25	> 0.99
(G→A)	GA	12	7	
	AA	0	1	
Intron3.2:	GG	68	29	> 0.99
(G→A)	GA	10	2	
	AA	0	2	
Intron4:	TT	50	20	0.72
(T→G)	GT	26	13	
	GG	2	0	
Intron6:	GG	46	20	0.75
(G→A)	GA	29	10	
	AA	3	3	
Intron11:	GG	52	20	0.45
(G→C)	CG	25	13	
	CC	1	0	
Intron13:	GG	68	27	>0.99
(G→C)	GA	10	6	
Intron16:	CC	34	12	0.33
(C→T)	CT	32	16	
	TT	12	5	
Intron16:	GG	38	18	0.58
(G→A)	GA	35	11	
	AA	5	4	
3'UTR:	CC	35	12	0.32
(C→T)	CT	31	16	
	TT	12	5	

Table 5. Distribution of LDL-R haplotype (showed only haplotype that provided frequency more than 0.05)

LDL-R haplotype	Estimate (f)	Z-stat	p-value
AGGTGGGCGC	0.1562	5.5606	< 0.001
AGGTGGGTGT	0.3115	7.429	< 0.001
AGGTAGGCAC	0.018	1.7453	0.0809
AGGGGCGCGC	0.1498	5.4161	< 0.001
TGGTGGGCAC	0.0984	4.2982	< 0.001

interferon treated viral clearance, or this role is minor and is overshadowed by other factors in this easy-to-treat HCV genotype, including dosage of both peginterferon and ribavirin, severity of liver disease, and other host genetic factors and most importantly, the very high response rate of HCV genotype 3 may mask the effect of LDL-R polymorphisms. However, that some SNP such as intron 3.2 had a trend towards statistical significance may due to small in sample size.

Conclusion

There was no association of LDL-R polymorphisms and treatment response in chronic hepatitis C, genotype 3. This result may indicate that the genetic component mediated via the LDL-R may not influence treatment outcome in HCV genotype 3.

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Potential conflicts of interest

None.

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โพลีมอร์ฟีสมพันธุกรรมของ ตัวรับไลโพโปรตีนความหนาแน่นต่ำ (low-density lipoprotein receptor) ไม่มีผลต่อการรักษาในผู้ป่วยไวรัสตับอักเสบ ซี จีโนไทป์ 3

ทวีศักดิ์ แทนวันดี, มานพ พิทักษ์ภากร, นพวรรณ วิภาตกุล, พูลชัย จรัสเจริญวิทยา, ศิวะพร ไชยนุวัติ, สุพจน์ นิ่มอนงค์, วรายุ ปรัชญกุล, สุพจน์ พงศ์ประสบชัย, สถาพร มานัสสถิตย์, สมชาย ลีลากุศลวงศ์, นนทลี เผ่าสวัสดิ์, อุดม คชินทร, ชนินทร์ ลิ่มวงศ์, สุทธิพล อุดมพันธุรัก

ภูมิหลัง: *ตัวรับไลโพโปรตีนความหนาแน่นต่ำ (แอลดีแอล-อาร์) คาดว่าทำหน้าที่เป็นตัวรับการเข้าเซลล์ของไวรัสตับอักเสบ ซี โพลีมอร์ฟีสมของจีน แอลดีแอล-อาร์ อาจมีผลต่อการหายของ ผู้ป่วยไวรัสตับอักเสบ ซี ทั้งที่หายเองหรือจากการรักษา การศึกษานี้เพื่อจะประเมินความสัมพันธ์ของโพลีมอร์ฟีสมของ แอลดีแอล-อาร์จีนกับการหายของผู้ป่วยไวรัสตับอักเสบ ซี เรื้อรัง*

วัสดุและวิธีการ: *ผู้ป่วยไวรัสตับอักเสบ ซี จีโนไทป์ 3 ที่ไม่เคยได้รับการรักษามาก่อนหนึ่งร้อยสิบสองราย ทุกรายได้รับการรักษาด้วยยาเพ็กอินเตอร์เฟอรอนร่วมกับยาไรบาไวรินเป็นเวลา 24 สัปดาห์ การตรวจโพลีมอร์ฟีสม ของจีนแอลดีแอล-อาร์ทำโดยวิธีพีซีอาร์ร่วมกับอาร์เอฟแอลพี 11 ตำแหน่งประกอบด้วย intron 1, intron 3.1, intron 3.2, intron 4, intron 6, exon 8, intron 11, intron 13, intron 14, 3'UTR และ 2 SNP ใน intron 16 ตามลำดับ และเปรียบเทียบระหว่างกลุ่มที่ตอบสนองกับกลุ่มที่ไม่ตอบสนอง*

ผลการศึกษา: *ผู้ป่วยมีอายุเฉลี่ย 54 ปี ร้อยละ 43 เพศชาย ระดับไวรัส ซี และอลานีนอมิโนทรานสเฟอเรสก่อนการรักษาเท่ากับ 2.1 ล้าน ไอยูต่อ มล. และ 100 ไอยูต่อมล. ตามลำดับ อัตราการหายขาดการกลับซ้ำ และไม่ตอบสนองเท่ากับร้อยละ 68.7, 17.9 และ 13.4 ตามลำดับโดยที่ไม่พบความแตกต่างของปัจจัยพื้นฐานก่อนการรักษารวมถึงอายุ เพศ น้ำหนักตัว ระดับเอนไซม์ตับ ปริมาณไวรัส นอกจากนั้นไม่พบความแตกต่างของ จีโนไทป์และอัลลีลของ แอลดีแอล-อาร์ระหว่างผู้ป่วยที่ตอบสนองและไม่ตอบสนอง*