

Apolipoprotein E Genotype and Expression Correlated with Hepatitis C Virus Genotype and Infection

Gyu-Nam Park¹, Hyun-Jung Jo¹, Hye-Ran Kim¹, Min-Ju Kim¹, Kyung-A Shin²,
Seung-Bok Hong³ and Kyung- Soo Chang^{1*}

¹Department of Clinical Laboratory Science, Catholic University of Pusan, Busan, Republic of Korea

²Department of Clinical Laboratory Science, Shinsung University, Dangjin, Republic of Korea

³Department of Clinical Laboratory Science, Chungbuk Health & Science University, Cheongwon, Republic of Korea

Corresponding

Kyung Soo Chang

Department of Clinical Laboratory
Science, College of Health Sciences,
Catholic University of Pusan, Bugok
3-dong, Geumjeong-gu, Busan 609-757,
Korea.

Phone : +82-51-510-0565

Fax : +82-51-510-0565

E-mail : kschang@cup.ac.kr

Received : February 15, 2019

Revised : March 19, 2019

Accepted : March 20, 2019

The hepatitis C virus (HCV) is a globally prevalent human pathogen that causes persistent liver infections in most infected individuals. Several studies reported that HCV particles are enriched in apolipoprotein E (apoE) and that apoE is required for HCV infectivity and production. However, the relationship between apoE gene polymorphisms and HCV genotypes in patients with HCV is less well understood. The aim of this study was to investigate the association between apoE gene polymorphism and HCV genotypes in patients. The HCV genotypes were identified among the 124 patients infected with HCV, and the genetic characteristics of the HCV genotype were analyzed. In addition, the results of the clinical laboratory test were comparatively analyzed according to the classified genotypes. Both HCV 1b (n=80) and 2a (n=42) patients had higher AFP, AST, ALT, ALP, γ -GTP, apoB, and apoE values compared with the normal control group. In particular, apoB and apoE levels were statistically significantly higher in the HCV 2a patients ($P<0.05$) and apoE levels were significantly higher in the HCV 1b patients ($P<0.000$). According to the results the patients with HCV genotype 1b showed higher values of liver damage related indicators and apoB expression than the patients with HCV genotype 2a. The fat related indicators and apoE expression were not different between the two major HCV genotypes (2a and 1b). We anticipate that the apoE ϵ 3 allele is the most common type in HCV genotype 1b (89.2%) and 2a (91.7%). As a result of apoE genotyping, we confirmed an association with HCV infection and the apoE ϵ 3 allele. However, the ratios of the apoE ϵ 3 allele among the patients with genotype 1b and 2a were similar to each other.

Key Words: Apolipoprotein E, Hepatitis C virus, Genotype

No potential conflict of interest relevant to this article was reported.

Copyright © 2018 Journal of Bacteriology and Virology

©This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>).

INTRODUCTION

The hepatitis C virus (HCV) belongs to the family *Flaviviridae* and is a single strand RNA virus. Its total gene length is 9,600 bp, and it is characterized by the high probability of being mutated. The currently known HCV genotypes include 1 – 11, with the subtypes a, b, and c. Currently, genotypes up to HCV 6 can be clinically diagnosed (1, 2).

Globally, the number of hepatitis C patients has reached approximately 180 million, and the prevalence ratio is 2.8 %. Among the genotypes of HCV,

genotype 1 is the most prevalent, followed by genotype 2. These genotypes show differences by region; for example, genotype 1a is the most prevalent in Northern Europe and North America, and genotype 1b is highly prevalent in East Asia and Europe. Genotype 2 also appears frequently in Northern Europe, North America, East Asia, and Europe, but in lower ratios than genotype 1. In South Korea, hepatitis C is an infectious disease under sentinel surveillance, in which approximately 4,000 cases are reported every year, and its prevalence ratio in Korea is approximately 0.78 %. Of the diagnosed cases, genotype 1b accounts for the highest ratio, followed by genotype 2a (1~4).

HCV genotypes have important meanings for diagnoses, because their sensitivity levels to antiviral agents are different from each other, leading to different treatment decisions. The antiviral agent's treatment performances against genotypes 1 and 4 have been reported to be lower, when compared to genotypes 2, 3, and 6 (5).

The correlations between HCV and the cholesterol level have been previously proven in many studies. Low cholesterol values are shown in HCV patients, and it has been reported that cholesterol plays an important role when HCVs infiltrate liver cells. More detailed studies have reported that low density lipoproteins (LDLs) and apolipoproteins from cholesterol contribute to an HCV infection and proliferation. Apolipoprotein E (apoE) and B are both components of very low density lipoprotein (VLDL) and are thought to play important roles in the HCV life cycle. In particular, ApoE plays an important role in moving the HCV viral particles to the liver cells (6~9). Based on these reports, studies to develop HCV treatment agents through anti-cholesterol functions are being actively conducted (10).

ApoE is one of the plasma protein, and it functions in the transport of cholesterol and lipids. apoE is classified as having $\epsilon 2$, $\epsilon 3$, or $\epsilon 4$ alleles, and since these genetic polymorphisms are associated with some diseases, they are used in the diagnoses as well (11~13). However, the relationship between apoE gene polymorphisms and HCV genotypes in patients with HCV is less well understood.

Therefore, the purpose of the present study was to classify the genotypes of the HCVs isolated in Korea. Additionally, the genotypes would be compared with those isolated overseas, a quantitative analysis would be conducted on the blood apoB and apoE according to the HCV genotypes, and genetic polymorphisms of apoE would be identified to study the correlations between them.

MATERIALS AND METHODS

Samples

HCV (n=124) samples (serum and whole blood) were collected at Bundang Jaesang Hospital in 2012, and the HCV samples were defined by anti-HCV tests, looks for antibodies to the HCV. The normal control group (n=100) was selected from clinically healthy people between 45 - 60 years old.

The samples were immediately stored at -70°C, and the patient's medical records were collected for the analysis of the clinical characteristics. This study was approved by the Institutional Review Board of Bundang Jaesang Hospital.

HCV RNA isolation and amplification from serum

The HCV RNA was isolated using Accuzol™ RNA extraction solution (Bioneer, Seoul, Korea), and the isolated RNA was resuspended in 50 µl of 0.1% diethylpyrocarbonate (DEPC). To amplify the 5'UTR region of the HCV RNA for genotyping, a nested PCR was conducted. An initial denaturation was performed at 42°C for 60 min, and at 95°C for 5 min. Thereafter, reverse-transcriptase PCR (RT-PCR) was performed for 35 cycles, with denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min using a RT-PCR premix (Bioneer, Seoul, Korea). The primer set consisted of forward CTG TGA

GGA ACT ACT GTC TT and reverse ACT CGC AAG CAC CCT ATC AG. A second PCR was performed for 25 cycles, with denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec using a PCR premix (Bioneer, Seoul, Korea). The primer set consisted of forward TTC ACG CAG AAA GCG TCT AG and reverse TAT CAG GCA GTA CCA CAA GG.

HCV genotype analysis

The amplicon was purified using a PCRMix purification kit (Cosmogenotech, Seoul, Korea), and the product was transferred for sequencing to Cosmogenotech (Seoul, Korea). The sequence of the HCV isolates and the sequences available in the GenBank database were used for the comparison. The sequences were aligned using MEGA 4.1 software, and their phylogenetic relationship was analyzed. For this, the Kimura-2-parameter distances were calculated using the neighbor-joining (NJ) algorithm. The reliability of the various phylogenetic groupings was evaluated by using the bootstrap test with 1,000 replications.

Quantitative analysis of apolipoprotein B and E

The Apolipoprotein B Human ELISA kit and Apolipoprotein E Human ELISA kit were used for analysis (Abcam, Cambridge, UK). The obtained serum was used for testing, and we followed the protocols thoroughly.

ApoE genotyping

The DNA was isolated using a DNA isolation kit (Cosmogenotech, Seoul, Korea) from whole blood. The isolated DNA was used for apoE genotyping, and an EzWay™ Direct ApoE Genotyping kit (Komabiotech, Seoul, Korea) was used. This kit is based on the multiplex PCR method, and the genotype was determined by electrophoresis in a 2 % agarose gel (Fig. 1.)

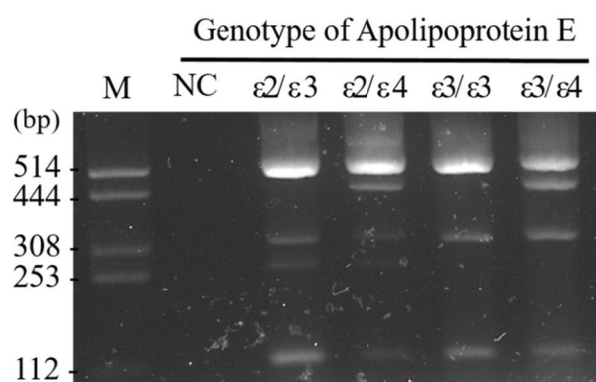


Figure 1. Electrophoresis results of apoE PCR products. lane 3: $\epsilon 2/\epsilon 3$ (253, 308 bp), lane 4: $\epsilon 2/\epsilon 4$ (253, 308, 444 bp), lane 5: $\epsilon 3/\epsilon 3$ (308 bp), lane 6: $\epsilon 3/\epsilon 4$ (308, 444 bp)
M, size marker (112, 253, 308, 444, 514 bp); NC, negative control.

Statistical analyses

SPSS 18.0 statistical software was used for the data analysis, and continuous variables, like the clinical characteristics, were compared with the Student's t-test. Categorical variables, like apoE genotype, were compared with a cross tabulation test. P-values of <0.05 were considered statistically significant.

RESULTS

HCV genotype distribution and gene characteristics

The HCV genotypes in the 124 HCV patient' serum samples were analyzed, and as a result, the HCV genotypes could be identified in a total of 124 samples. The most frequently identified genotype was genotype 1b, which was identified in a total of 80 samples, accounting for 64.5% of all samples. Genotype 2a was identified in 42 samples, accounting for 33.9%, and genotype 1a was identified in 2 samples, accounting for 1.6% (Table 1).

Table 1. Distribution of HCV genotypes in HCV samples

HCV genotype	n	%
1a	2	1.6
1b	80	64.5
2a	42	33.9
Total	124	100

The isolated HCV genotypes were classified as shown in Table 2. Genes that were similar to each other based on the results of the gene analysis were grouped for analyses. Genotype 1b sequences formed five major separated groups (namely KOR-A to E). Group KOR-A (n=33) and KOR-B (n=21) contained the majority of 1b isolates. Five isolates identified genotype 1b, was not contained any other groups. Genotype 2a sequences formed four major separated groups (namely KOR-A to D). Group KOR-A (n=16) and KOR-B (n=19) contained the majority of 2a isolates. Three isolates identified genotype 2a, was not contained any other groups.

The isolated genes were compared with the isolates by the country identified in the NCBI (Table 3). The phylogenetic tree according to HCV genotypes is shown in Fig. 2.

Table 2. Sample number and group of HCV isolates by HCV genotype

HCV genotype (n)	Group (n)	Sample No.
1a (2)	-	4, 110
1b (80)	KOR-A (33)	1, 33, 35, 36, 39, 40, 42, 43, 44, 45, 47, 49, 50, 51, 53, 55, 56, 57, 60, 61, 62, 64, 67, 68, 69, 70, 71, 73, 78, 85, 87, 88, 96
	KOR-B (21)	5, 6, 8, 9, 11, 19, 38, 48, 66, 72, 81, 83, 86, 91, 94, 97, 98, 101, 106, 116, 125
	KOR-C (3)	15, 113, 122
	KOR-D (6)	37, 84, 95, 118, 119, 124
	KOR-E (12)	41, 104, 107, 108, 109, 111, 112, 114, 115, 117, 121, 126
	34, 93, 103, 120, 127 (5)	-
2a (42)	KOR-A (16)	2, 10, 12, 13, 14, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27
	KOR-B (19)	28, 29, 30, 31, 32, 46, 52, 58, 59, 63, 75, 76, 77, 79, 80, 82, 89, 99, 100
	KOR-C (2)	54, 123
	KOR-D (2)	90, 92
	3, 65, 74 (3)	-

Table 3. NCBI number and origin of HCV gene by HCV genotype

HCV genotype	Abbreviation	Origin	NCBI No.
1a	CZE	Czech Republic	AY725958
	IRN	Iran	HM852067
	ARG	Argentina	JQ065780
	CHN	China	DQ087244
	GBR	UK	AY766661
	USA	USA	EU862840
1b	IRN	Iran	HM852066
	CHN	China	AY702953
	ARG	Argentina	JQ273005
	ZAF	South Africa	HQ396517
	CAN	Canada	EF115582
	BRA	Brazil	AY306266
	RUS	Russia	AY764169
	DEU	Germany	EF175786
	USA-1	USA	FJ390398
	USA-2	USA	GU133617
	JPN	Japan	M58335
2a	CAN-1	Canada	EF115605
	CAN-2	Canada	JQ318145
	MCO	Morocco	HQ833239
	JPN	Japan	AB047639
	GBR	UK	AY766689
	DNK	Denmark	KC967476
	CHN	China	HQ639945

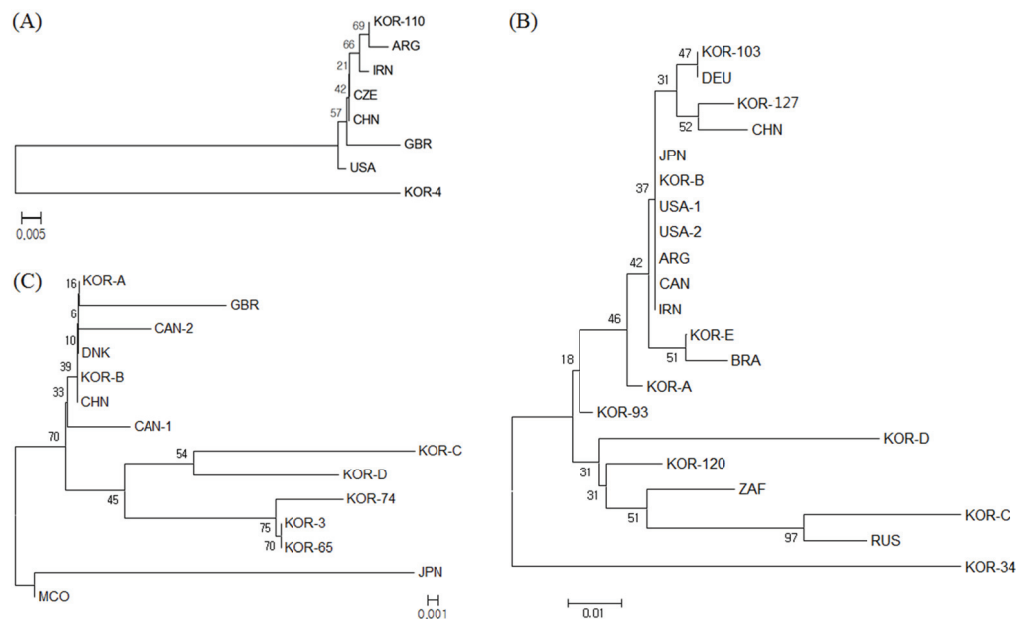


Figure 2. Phylogenetic trees based on HCV isolates and HCV reference strains from other countries
 (A) HCV genotype 1a isolates and references from NCBI; (B) HCV genotype 1b isolates and references from NCBI;
 (C) HCV genotype 2a isolates and references from NCBI.

Analysis of age and gender according to HCV genotype

The ages and genders were analyzed according to the HCV genotypes, and the results showed that the mean age of the patients with HCV genotype 1a was 51.5 years, which was younger by approximately 10 years when compared to the patients with genotypes 1b or 2a. However, the mean ages of the patients with genotype 1b or 2a were both 60.8 years. The gender distributions according to the genotypes were analyzed and according to the results, all of the patients with HCV genotype 1a were males. The ratio of males was higher by at least 10% than the ratio of females among the patients with HCV genotype 1b, and the ratios of males and females were similar to each other among the patients with HCV genotype 1a, although the ratio of females was slightly higher (Table 4).

Table 4. Comparison of age and gender by HCV genotype

Characteristics	1a (n=2)	1b (n=80)	2a (n=42)	Total (n=128)
Age (mean±SD)	51.5±12.0	60.8±14.4	60.8±12.9	60.3±14.1
Gender (n, %)				
Male	2 (100.0)	44 (55.0)	20 (47.6)	69 (53.9)
Female	0 (0.0)	36 (45.0)	22 (52.4)	59 (46.1)

Comparison of the clinical laboratory test results of HCV genotypes 1b and 2a

The clinical properties and states of the patient groups with HCV genotypes 1b or 2a were compared with each other. In addition, each group was compared with the normal control group. The patients with HCV genotype 1a were excluded from this analysis, since the number of isolated patients was too small, and was judged as not being statistically significant (Table 5).

Both HCV 1b and 2a patients had higher AFP, AST, ALT, ALP, γ -GTP, apoB, and apoE values compared with the normal control group. In particular, apoB and apoE levels were statistically significantly higher in the HCV 2a patients ($p < 0.05$) and apoE levels were significantly higher in the HCV 1b patients ($p < 0.000$). Total cholesterol, HDL, and LDL levels were lower in the both HCV 1b and 2a patients ($p < 0.05$) and TG levels were higher in the HCV 2a patients. However, there were no significant differences in the all clinical biomarkers between the HCV 1b and 2a patients ($p > 0.05$) (Table 5).

Table 5. Comparison of clinical characteristics in HCV genotype 1b and 2a

Characteristics	1b	2a	Control	p^a	p^b	p^c
AFP (ng/ml)	109.2±14.4	32.0±107.2	2±1.1	0.721	0.636	0.412
Total protein (g/dl)	7.4±0.8	7.5±0.6	6.8±1.6	0.517	0.476	0.663
Albumin (g/dl)	3.9±0.6	4.1±0.5	4.4±0.1	0.346	0.362	0.120
AST (IU/L)	59.5±57.7	56.7±80.6	21.4±4.6	0.000	0.176	0.826
ALT (IU/L)	48.7±45.6	47.7±62.8	16.5±3.6	0.000	0.003	0.919
Total bilirubin (mg/dl)	1.3±2.0	0.8±0.3	0.8±0.3	0.709	0.531	0.044
ALP (IU/L)	316.7±2.0	264.0±70.6	242.5 ±46.3	0.584	0.557	0.114
γ -GTP (IU/L)	73.5±91.1	59.46±94.8	19.2±6.4	0.000	0.190	0.469
BUN (mg/dl)	19.5±14.7	19.9±16.2	16.2 ±3.6	0.663	0.661	0.903
Creatinine (mg/dl)	1.3±0.9	1.5±1.7	0.954±0.1	0.293	0.297	0.362
TG (mg/dl)	127.2±106.9	115.1±48.0	112 ±66.9	0.668	0.872	0.562
Total cholesterol (mg/dl)	164.7±49.8	166.2±30.9	224.3 ±32.0	0.000	0.000	0.871
HDL (mg/dl)	47.1±13.2	49.8±15.0	74.8 ±27.2	0.012	0.002	0.445
LDL (mg/dl)	104.1±33.5	102.7±22.8	135.2 ±43.8	0.021	0.048	0.684
ApoB (μ g/ μ l)	9.2±16.0	5.4±5.6	1.93 ±1.3	0.155	0.001	0.056
ApoE (μ g/ μ l)	0.220±0.173	0.198±0.167	0.058 ±0.016	0.000	0.000	0.504

AFP, α -fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ -GTP, γ -glutamyltransferase; BUN, TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoB, apolipoprotein B; ApoE, apolipoprotein E.

^acompared with patients of HCV genotype 1b and Normal control

^bcompared with patients of HCV genotype 2a and Normal control

^ccompared with patients of HCV genotype 1b and 2a

The apoE genetic polymorphisms were determined according to the classified HCV genotypes. According to the results of all of the patients with HCV genotype 1a, they were apoE type $\epsilon 3/\epsilon 3$. Among the patients with HCV genotype 1b, type $\epsilon 3/\epsilon 3$ was the most prevalent at 78.5%, followed by $\epsilon 2/\epsilon 3$ and $\epsilon 3/\epsilon 4$ in order of precedence. Among the patients with HCV

genotype 2a, type $\epsilon 3/\epsilon 3$ was the most prevalent at 85.7%, followed by $\epsilon 3/\epsilon 4$, $\epsilon 2/\epsilon 3$, and $\epsilon 2/\epsilon 4$ in order of precedence. Among the patients with HCV genotype 2a, type $\epsilon 2/\epsilon 4$ was specifically identified, and the isolation ratio of $\epsilon 3/\epsilon 3$ was shown to be higher by at least 7.0% than in genotype 1b. The isolation ratios of the other subtypes were lower. The apoE genotypes according to the HCV genotypes were cross-analyzed, and the correlations were found to be statistically significant ($p < 0.05$) (Table 6).

Table 6. Comparison of apolipoprotein E genotype by HCV genotype

HCV genotype	Apolipoprotein E genotype (n, %)						Total	χ^2 (p)
	$\epsilon 2/\epsilon 2$	$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	$\epsilon 4/\epsilon 4$		
1a	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	19.918 (0.018)
1b	0 (0.0)	10 (12.5)	0 (0.0)	63 (78.7)	7 (8.8)	0 (0.0)	80 (100.0)	
2a	0 (0.0)	2 (4.8)	1 (2.4)	36 (85.7)	3 (7.1)	0 (0.0)	42 (100.0)	

The apoE genotypes were identified using the apoE alleles according to the classified HCV genotypes, and the results indicated that the ratios of the apoE $\epsilon 3$ and $\epsilon 4$ alleles among the patients with genotype 1b and 2a were similar to each other. However, the ratio of the $\epsilon 2$ allele among the patients with genotype 1b was higher by approximately 2 times that among the patients with genotype 2a (Table 7).

Table 7. Comparison of apolipoprotein E gene allele by HCV genotype

HCV genotype	ApoE allele (%)			Total
	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	
1a	0.0	100.0	0.0	100.0
1b	6.3	89.2	4.4	100.0
2a	3.6	91.7	4.8	100.0

In the HCV 1b patients, the $\epsilon 3/\epsilon 3$ group had higher AFP and ALP levels than the $\epsilon 2/\epsilon 3$ apoE genotype group and the $\epsilon 2/\epsilon 3$ group had higher AST and ALT levels than the $\epsilon 3/\epsilon 3$ apoE genotype group. In the HCV 2a patients, the $\epsilon 2/\epsilon 3$ group had higher AFP and ALP levels than the $\epsilon 3/\epsilon 3$ apoE genotype group. And the $\epsilon 2/\epsilon 3$ group and $\epsilon 3/\epsilon 3$ apoE genotype groups had the same levels of AST, and ALT.

The $\epsilon 2/\epsilon 3$ group in the HCV 1b, and 2a patients had higher levels of γ -GTP. The $\epsilon 3/\epsilon 3$ group in the HCV 1b, and 2a patients had higher levels of TG. The $\epsilon 2/\epsilon 3$ group in the HCV 1b, and 2a patients had the lowest LDL levels. HDL levels were generally low when not accounting for apoE genotype or hepatitis type. In the HCV 1b patients, apoB and apoE levels were higher in the $\epsilon 3/\epsilon 3$ group. In the HCV 2a patients, apoB and apoE levels were higher in the $\epsilon 3/\epsilon 3$ and $\epsilon 3/\epsilon 3$ groups, respectively (Table 8).

Table 8. Comparison of clinical characteristics in HCV genotype by apoE polymorphism

Characteristics	HCV genotype	$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$
n	1b	10	0	63	7
	2a	2	1	36	3
AFP (ng/ml)	1b	3.3 \pm 1.4	-	111.1 \pm 131.2	188.6 \pm 82.3
	2a	62.8 \pm 71.3	3.8	14.6 \pm 89.2	3.0 \pm 4.1

Total protein (g/dl)	1b	7.8±1.0	-	7.4±0.5	7.1±0.9
	2a	7.1±0.9	6.7	7.5±1.0	7.4±0.7
Albumin (g/dl)	1b	4.1±0.9	-	3.9±0.2	3.8±0.8
	2a	3.7±0.2	3.8	4.2±0.9	3.8±0.6
AST (IU/L)	1b	59.6 ±67.1	-	62.4 ±43.3	33.7±48.7
	2a	56.2 ±58.3	19.0	60.2 ±66.8	34.3±46.1
ALT (IU/L)	1b	51.5 ±49.3	-	49.9 ±40.8	34.4±28.9
	2a	47.9 ±62.2	33.0	51.6 ±50.1	19.3±21.7
Total bilirubin (mg/dl)	1b	0.9±0.1	-	1.3±2.1	0.9±0.4
	2a	0.9±0.3	1.0	0.8±1.1	0.8±0.1
ALP (IU/L)	1b	291.1±132.2	-	327.3 ±189.1	267.3±112.1
	2a	271.7±122.2	188.0	266.9±105.2	277.0 ±141.2
γ-GTP (IU/L)	1b	93.4 ±81.1	-	72.1±66.1	57.7±38.2
	2a	89.8 ±71.3	23.0	61.4±81.1	38.0±31.4
BUN (mg/dl)	1b	26.4±11.2	-	18.7±10.1	16.1±10.8
	2a	25.3±13.1	17.6	19.1±9.7	33.0 ±21.5
Creatinine (mg/dl)	1b	1.2±0.8	-	1.3±1.5	1.1±0.9
	2a	1.2±0.6	0.9	1.4±1.1	3.7±2.1
Total cholesterol (mg/dl)	1b	154.4±41.7	-	167.8 ±41.6	152.0±32.8
	2a	148.9±26.6	214.0	162.8±44.5	170.3 ±31.3
TG (mg/dl)	1b	107.0±88.0	-	134.0 ±82.5	106.3±41.6
	2a	112.3±68.2	92.0	114.6±94.0	99.5±48.8
HDL (mg/dl)	1b	46.2±12.6	-	46.0 ±13.0	54.4±17.5
	2a	42.5 ±10.6	-	50.0±12.8	54.0±8.5
LDL (mg/dl)	1b	81.6 ±38.2	-	108.3±30.8	106.8±11.1
	2a	86.1 ±32.4	-	99.3±27.7	114.5±14.5
ApoB (μg/μl)	1b	5.1±6.6	-	10.1 ±11.4	6.9±11.0
	2a	5.6±6.7	1.6	5.5±7.9	2.9±3.9
ApoE (μg/μl)	1b	0.180±0.092	-	0.236 ±0.150	0.133±0.111
	2a	0.199 ±0.117	0.126	0.198 ±0.177	0.109±0.096

AFP, α-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase; BUN, TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoB, apolipoprotein B; ApoE, apolipoprotein E.

DISCUSSION

Many previous studies have investigated the distribution of HCV genotypes, in Korea. Whereas genotypes 1b and 2a have been isolated at similar frequencies in Korea, in the present study, genotype 1b was more frequently isolated by approximately two times than genotype 2a, as the ratio was 64.5% (80/124). This bias is considered attributable to the fact that the clinical samples were obtained from only one medical institution located in the Gyeonggi-do Province. In previous study, Shon et al. reported that the prevalence of HCV infection differs by regions as well as towns in the Korea, and is highest in Busan, Jeonnam, and Gyeongnam. Therefore, the genotypes of the isolated HCVs may be different by region (14~17).

We obtained 124 HCV positive samples that were successfully sequenced. To analyze the distribution of HCV genotypes, three phylogenetic trees were generated, each representing one of the three genotypes identified in this study, 1a, 1b, and 2a (Fig.2.). The phylogenetic tree for genotype 1a was showed two clear separate groups. In the genotype 1b and 2a trees, major groups distributed throughout the trees. These showed that the distribution of HCV genotypes of isolates from this study is complex. Our phylogeny analysis need to be verified in new further studies with large random sampled sequences.

HCV infection is associated with a decrease in serum levels of total cholesterol, LDL cholesterol (18). In previous study, we already confirmed that HCV infection are strongly associated with low levels of main cholesterol biomarker (HDL, LDL, and total cholesterol) (12). We reconfirmed it through this study. In addition, apoB and apoE levels were higher in both HCV 1b and 2a patients than in healthy controls. ApoE and B are both components of VLDL and are thought to play important roles in the HCV life cycles. Some studies have reported that both apolipoproteins are required for HCV assembly and secretion (19~21). HCV 1b has more of an effect than HCV 2a due to high levels of TG, apoB, and apoE. However, there were no statistically significant differences between HCV genotype 1b and 2a.

Price *et al.* reported that apolipoprotein $\epsilon 3$ allele is associated with persistent HCV infection. Also, Gomma et al. suggest that apo $\epsilon 3$ allele is considered as a particular risk factor in HCV patients (22, 23). In the present study, the correlations between the HCV genotypes and apoE genotypes were analyzed. The HCV 1b and 2a both had a high distribution of haplotype $\epsilon 3/\epsilon 3$ 78.7% (63/80), 85.7% (36/42), respectively. The distribution of $\epsilon 3/\epsilon 3$ type is about 75% in normal people (11, 24). Therefore, $\epsilon 3/\epsilon 3$ is more common type in HCV 1b and HCV 2a. Especially, genotype 2a was shown to be higher than the ratio of type $\epsilon 3/\epsilon 3$ generally identified in Korea, indicating that genotype 2a is correlated with type $\epsilon 3/\epsilon 3$. We anticipate that the apoE $\epsilon 3$ allele is the most common type in HCV genotype 1b (89.2%) and 2a (91.7%). As a result of apoE genotyping, we confirmed an association with HCV infection and the apoE $\epsilon 3$ allele. However, the ratios of the apoE $\epsilon 3$ allele among the patients with genotype 1b and 2a were similar to each other (were not statistically significant). The present study has some limitations; The problem of clinical samples (relatively small sample size and limitation of collection region), and exclusion of the patient's underlying medical conditions. Therefore, we need further studies to verify our findings.

In conclusion, the HCV genotypes were identified among the 124 patients infected with HCV, and the genetic characteristics of the HCV genotype were analyzed. In addition, the results of clinical laboratory test were comparatively analyzed according to the classified genotypes. According to the results the patients with HCV genotype 1b showed higher values of liver damage related indicators and apoB expression than the patients with HCV genotype 2a. The fat related indicators and apoE expression were not different between the genotypes. The apoE $\epsilon 3$ and $\epsilon 2$ allele were more frequently identified in the patients with genotype 2a and 1b, respectively. Analyses with large scale samples collected from diversity of region are needed in order to verify the relationship between HCV genotype and apolipoprotein.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012007586) and by Brain Busan 21 (BB21).

REFERENCES

- 1) Lanini S, Easterbrook PJ, Zumla A, Ippolito G. Hepatitis C: global epidemiology and strategies for control. *Clin Microbiol Infect* 2016;22:833-38.
- 2) Umar S, Yasir W, Muhammad A. Hepatitis B and hepatitis C viruses: a review of viral genomes, viral induced host immune responses, genotypic distributions and worldwide epidemiology. *Asian Pac J Trop Dis* 2014;4:88-96.
- 3) Shin HR, Kim JY, Kim JI, Lee DH, Yoo KY, Lee DS, *et al*. Hepatitis B and C virus prevalence in a rural area of South Korea: the role of acupuncture. *Br J Cancer* 2002;87:314-8.
- 4) Cho EJ, Jeong SH, Han BH, Lee SU, Yun BC, Park ET. Hepatitis C virus (HCV) genotypes and the influence of HCV subtype 1b on the progression of chronic hepatitis C in Korea: a single center experience. *Clin Mol Hepatol* 2012;18:219-24.
- 5) Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. *Antivir Ther* 2005;10:1-11.
- 6) Chang KS, Jiang J, Cai Z, Luo G. Human apolipoprotein e is required for infectivity and production of hepatitis C virus in cell culture. *J Virol* 2007;81:13783-93.
- 7) Fierro NA, Gonzalez-Aldaco K, Torres-Valadez R, Martinez-Lopez E, Roman S, Panduro A. Immunologic, metabolic and genetic factors in hepatitis C virus infection. *World J Gastroenterol* 2014;20:3443-56.
- 8) Felmlee DJ, Hafirassou ML, Lefevre M, Baumert TF, Schuster C. Hepatitis C virus, cholesterol and lipoproteins--impact for the viral life cycle and pathogenesis of liver disease. *Viruses* 2013;5:1292-324.
- 9) Jiang J, Luo G. Apolipoprotein E but not B is required for the formation of infectious hepatitis C virus particles. *J Virol* 2009;83:12680-91.
- 10) Jang ES, Won JE, Jung JI, Lee SH, Kim JW, Jeong SH. The effect of antiviral therapy on serum cholesterol levels in chronic hepatitis C. *Gut Liver* 2011;5:356-62.
- 11) Kim YS, Paeng JR, Woo JT, Kim SW, Yang IM, Kim JW, *et al*. Apolipoprotein E genotypes of normal and hyperlipidemic subjects. *J Korean Med Sci* 1993;8:262-6.
- 12) Jo HJ, Park GN, Kim HR, Kim MJ, Shin KA, *et al*. Differences in Hematological Characteristics, Including Cholesterol and Apolipoprotein B and E, between Hepatitis B Virus and Hepatitis C Virus Patients in Korea. *J Bacteriol Virol* 2016;46:152-8.
- 13) Gause JW, Day RJ, Caraway CA, Poon WW, Rohn TT. Evaluation of Apolipoprotein E fragmentation as a biomarker for Alzheimer's disease. *J Neurol Neurol Disord* 2017;3:204.
- 14) Shon HS, Choi HY, Kim JR, Ryu SY, Lee YJ, Lee MJ, *et al*. Comparison and analysis of the prevalence of hepatitis C virus infection by region in the Republic of Korea during 2005-2012. *Clin Mol Hepatol* 2015;21:249-56.
- 15) Jeong SH, Jang ES, Choi HY, Kim KA, Chung W, *et al*. Current status of hepatitis C virus infection and countermeasures in South Korea. *Epidemiol Health* 2017;13:39:e2017017.

- 16) Seong MH, Kil H, Kim YS, Bae SH, Lee YJ, Lee HC, *et al.* Clinical and epidemiological features of hepatitis C virus infection in South Korea: a prospective, multicenter cohort study. *J Med Virol* 2013;85:1724-33.
- 17) Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, *et al.* Global distribution and prevalence of hepatitis C virus genotypes. *Hepathology* 2015;61:77-87.
- 18) Bassendine MF, Sheridan DA, Bridge SH, Felmlee DJ, Neely RD. Lipids and HCV. *Semin Immunopathol* 2013;35: 87-100.
- 19) 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;82:2120-9.
- 20) Huang H, Sun F, Owen DM, Li W, Chen Y, Gale M Jr, *et al.* Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low density lipoproteins. *Proc Natl Acad Sci U S A* 2007;104:5848-53.
- 21) Icard V, Diaz O, Scholtes C, Perrin-Cocon L, Ramière C, Bartenschlager R, *et al.* Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. *PloS One* 2009;4:e4223.
- 22) Price DA, Bassendine MF, Norris SM, Golding C, Toms GL, Schmid ML, *et al.* Apolipoprotein epsilon3 allele is associated with persistent hepatitis C virus infection. *Gut* 2006;55:715-8.
- 23) Gomaa HE, Mahmoud M, Saad NE, Saad-Hussein A, Ismail S, Thabet EH, *et al.* Impact of ApoE gene polymorphism on HCV therapy related outcome in a cohort of HCV Egyptian patients. *J Genet Eng Biotechnol* 2018;16:47-51.
- 24) Shin MH, Kim HN, Cui LH, Kweon SS, Park KS, Heo H, *et al.* The effect of apolipoprotein E polymorphism on lipid levels in Korean adults. *J Korean Med Sci* 2005;20:361-6.