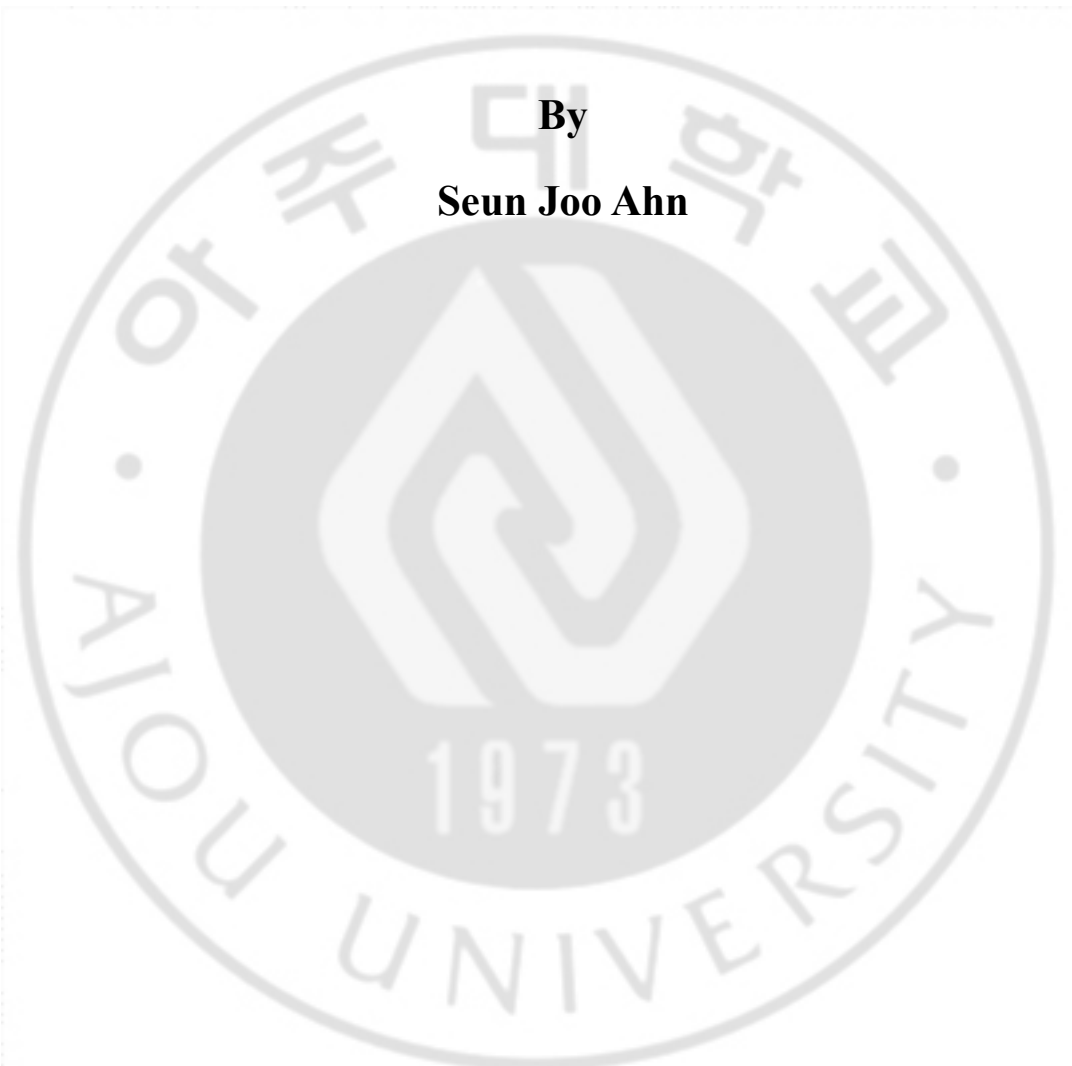
Association of apolipoprotein E genotypes with disease progression in hepatitis B virus infected patients

By

Seun Joo Ahn



Major in Medicine

Department of Medical Sciences

The Graduate School, Ajou University

Association of apolipoprotein E genotypes with disease progression in hepatitis B virus infected patients

by

Seun Joo Ahn

A Dissertation Submitted to The Graduate School of Ajou University in Partial Fulfillment of the Requirements for the Degree of Master of Medicine

Supervised by

Jae Youn Cheong, M.D.

Major in Medicine

Department of Medical Sciences

The Graduate School, Ajou University

August, 2012

This certifies that the dissertation
of Seun Joo Ahn is approved.

SUPERVISORY COMMITTEE

_____
Jae Youn Cheong

_____
Sung Won Cho

_____
Kee Myoung Lee

The Graduate School, Ajou University

June, 22nd, 2012

- ABSTRACT -

Association of Apolipoprotein E Genotypes with Disease Progression in Hepatitis B Virus Infected Patients

Background/aims: Apolipoprotein E (ApoE) plays an important role in regulating lipid and lipoprotein metabolism, and ApoE genotypes are known to affect plasma lipoprotein concentrations. We investigated whether ApoE genotype determine disease outcome in hepatitis B virus (HBV) infected individuals, and to verify the association with the occurrence of hepatocellular carcinoma (HCC) in patients with chronic liver diseases of various etiologies.

Methods: This hospital based case-control study enrolled 183 subjects (47 healthy controls, 50 HBV originated liver cirrhosis, 86 HCC). ApoE genotypes were determined by ApoE genotyping kit, using a PCR method. To verify the biological significances of ApoE genotype, a serum ApoE levels were measured by ELISA kit.

Results: The ε3 allele was the most common allele, with allele frequencies of 5.7% for ε2 allele, 84.7% for the ε3 allele and 9.6 % for ε4 allele in all participants. We identified that ApoE genotype was associated with the progression to liver cirrhosis in chronic HBV carriers. Being an ApoE4 carrier was associated with a lower probability of developing liver cirrhosis. No influence of ApoE genotypes on the susceptibility to the occurrence of HCC was found. The serum ApoE measurements revealed a significantly higher level of ApoE in patients with liver cirrhosis than those in the healthy controls, but we observed no significant difference in the serum ApoE levels, with regard to ApoE genotype.

Conclusion: This study indicates that ApoE genotypes may be a part of genetic variation underlying the susceptibility of individuals to disease progression of chronic HBV infection.

Keywords: apolipoprotein E, hepatitis B virus, genotype, liver cirrhosis

TABLE OF CONTENTS

ABSTRACT i
TABLE OF CONTENTS ii
LIST OF FIGURES iii
LIST OF TABLES iv
I. INTRODUCTION 1
II. MATERIALS AND METHODS 3
A. Study subjects 3
B. ApoE genotyping and serum ApoE measurements 3
C. Statistical analysis 4
III. RESULTS 5
A. Patient characteristics 5
B. ApoE genotype in case-control population 5
C. Serum ApoE levels in study subject 8
IV. DISCUSSION 11
V. CONCLUSION 14
REFERENCES 15
국문요약 18

LIST OF FIGURES

Fig. 1. Apolipoprotein E (ApoE) levels in serum from subjects with different groups…… 9

Fig. 2. Serum ApoE levels in subjects with different ApoE genotype…… 10

LIST OF TABLES

Table 1. Baseline characteristics of the enrolled subjects…….. 5

Table2. Distributions of Apo E genotypes and alleles in enrolled patients…….. 7

I. INTRODUCTION

Hepatitis B virus (HBV) infection is now the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) in the Asian countries (Kwon et al., 2011). Acute self-limiting HBV infection usually has little impact on morbidity, whereas persistent HBV infection is a major public health problem in Korea (Choi et al., 2011). The natural history of HBV infection varies, according to the age at which the infection was contracted. Perinatal transmission of HBV, particularly mother-to-child transmission of the virus during the perinatal period, is a common source of chronic infection (Kwon et al, 2011).

Host genetic factors may significantly influence the ability to clear HBV following infection. In recent years, increasing attention has been drawn to the role of host genetic factors in the natural course of viral hepatitis. Previously, we demonstrated that polymorphisms in TNF-α, IL-10, IFN-γ genes were correlated with the susceptibility to chronic HBV infection (Cheong et al.,2006 ; Cheong et al., 2006). However, the exact role of host genetic factors in the clearance of HBV and the risk of HCC occurrence remains uncertain.

Apolipoprotein (ApoE) is mainly found in the lipoprotein bound form, present in chylomicrons, very low density lipoproteins, and high density lipoproteins, as well as a key regulator of lipid and cholesterol metabolism (Minihane et al., 2007 ; Jofre-Monseny et al., 2008). ApoE is a polymorphic protein, which arises from three alleles at a single gene locus. The three major isoforms, apo-ε2, apo-ε3, and apo-ε4, differ from one another by single amino acid substitutions, a change, which has profound functional consequences at both the cellular and molecular levels (Mahley et al., 2000). ApoE has raised particular concern since ApoE polymorphisms are related to several chronic disorders, such as Alzheimer's disease and cardiovascular disease (Corder et al., 1993 ; Bennet et al., 2007).

ApoE genotype could be an important host genetic factor affecting disease progression in chronic liver disease. ApoE isoform appears to have a hepatitis C virus (HCV)-specific protective effect on liver disease, and the outcome of chronic HCV infection is better among the ε4 carriers, due to slow fibrosis progression (Wozniak et al., 2002; Mueller et al., 2003). Since HBV may use apolipoprotein pathways to recycle and infect hepatocytes, it is possible

that apolipoprotein polymorphism may play a role in the natural history of HBV infection. However, evidence regarding the contribution of ApoE genotype to outcome of HBV infection is limited (Toniutto et al., 2010).

The aims of this study were to assess if ApoE functional polymorphisms determine disease outcome in HBV infected individuals, and to verify the association with the occurrence of HCC in patients with chronic liver diseases of various etiologies.

II. MATERIAL AND METHODS

A. Study subjects

This study is a retrospective case-control study, in which ApoE genotyping and measuring of serum ApoE were performed on the enrolled subjects who were randomly selected. A total of 183 subjects were enrolled in this study, which included 136 case subjects who were diagnosed with liver cirrhosis (LC) or HCC at Ajou University Hospital, between September 2007 and May 2009. Diagnosis of HCC was based on imaging findings of nodules larger than 1 cm, which showed an intense arterial uptake, followed by a washout of contrast in the venous-delayed phases in 4-phase multi-detector CT scan or dynamic contrast enhanced MRI and/or biopsy. Cirrhosis of the liver, on the other hand, was diagnosed pathologically or based on the clinical evidence of portal hypertension, such as visible collateral vessels on the abdominal wall, esophageal varices on esophagogastroscopy, palpable splenomegaly, and sonographically definite findings of cirrhotic liver or ascites. Samples from 47 healthy volunteer, without history of liver disease, were collected as a control. A total of 183 participants underwent Apo E genotyping and of those, 162 participants (89.5%) underwent serum ApoE measurements. Informed consent to participate in the study was obtained from all of the participating subjects.

B. ApoE genotyping and serum ApoE measurements

Genotyping for ApoE polymorphisms was carried out by PCR. Amplification reactions were performed in a thermal cycler (Veriti, Life technologies co.,CA,USA), using ApoE genotyping PrimerMix Kit (Genotech co., Daejeon, Korea) as a reaction mix. Each reaction mixture was heated at 94°C for 10 minutes, followed by 30 cycles of amplification (94°C for 45 s, 60°C for 45 s, and 72°C for 45 s). Upon completion of PCR, the products were analyzed by electrophoresis on a 2% ethidium bromide-stained agarose gel.

Serum ApoE levels were measured in study subjects with the use of an ELISA Kit (ApoE4/Pan-ApoE ELISA Kit; minimum detection limit =4 ng/mL; MLB international co., Woburn, MA, USA) on the absorbance reader (Sunrise, Tecan Group Ltd., Männedorf,

Switzerland).

C. Statistical analysis

All categorical variables are reported as counts and percentages, and comparisons were conducted using a chi-square or Fisher's exact test, as appropriate. Continuous variables are reported as the means ± standard deviation with its ranges, and comparisons were conducted using ANOVA with Bonferroni-Dunn test. The homogeneity of variance was tested by Levene's test. Two-sided *P* values <0.05 were considered statistically significant. All statistical analyses were performed with SPSS software version 16.0.

III. RESULTS

A. Patient characteristics

The comparisons of the baseline characteristics, between the three groups who had assessed their ApoE genotypes, are summarized in Table 1. A total of 183 subjects were included (138 men and 45 women), and their ages ranged from 9 to 82 years. The mean age of patients with LC and HCC was higher than that of the healthy controls. The cause of LC in the enrolled subjects was exclusively HBV infection. The most common cause of HCC in the study subjects was HBV infection (68.6 %), followed by alcohol (14.0 %) and HCV infection (15.1 %) and, much less, idiopathic (2.3 %).

Table 1. Baseline characteristics of the enrolled subjects.*

	Group (n)			
	Control (n=47)	**LC (n=50)**	**HCC(n=86)**	**P value**
Age(yr)				<0.0001
Mean	45.3±16.2	48.1±9.4	57.7±11.5	
Range	9-82	31-81	29-79	
Male/Female,n(%)	31/16(66/34)	40:10(80/20)	67:19(78/22)	0.210
Etiology,n(%)		HBV 50(100)	HBV 59(68.6) Alcohol 12(14.0) HCV 13(15.1) Cryptogenic 2(2.3)	

*Values are expressed as the means± SD for continuous variables and n(%) for categorical variables
HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus

B. ApoE genotype in case-control population

The ApoE genotype distribution and allele frequencies of the subjects are shown in Table 2. Among the 183 subjects, the most common genotype was ε3/3, accounting for 71%, followed by ε3/4 and ε2/3, accounting for 16.9% and 10.4%, respectively. The genotypes of ε2/4 and ε4/4 were minor genotypes, which only accounted for 1.6% together. The ε3 allele was the most common allele, with allele frequencies of 5.7% for ε2 allele, 84.7% for the ε3

allele and 9.6 % for ε4 allele in all participants.

To examine the effect of ApoE genotype on the progression to LC or occurrence of HCC, we analyzed the genotype frequencies among the control, the LC and the HCC subjects. The ApoE genotype was associated with the progression to liver cirrhosis. The odds of developing LC associated, with the carrying of the ε3/3 genotype, were significantly increased (OR 2.71, CI 1.10-6.21)(Table2). Compared with the subjects, who were ε4 carrier, ε4 non-carrier had an increased susceptibility to LC development (OR .26, CI 0.09-0.80). There was no significant difference in the allele frequencies or genotype distribution of ApoE, between liver cirrhosis and HCC patients (Table 2).

Table2. Distributions of Apo E genotypes and alleles in enrolled patients.

	Group						Group			
	Control (n=47)	LC (n=50)	HCC (n=86)	LC+HCC (n=136)	All (n=183)		Control vs LC		LC vs HCC	
Genotype						**Genotype**	P value	OR(95% CI)	P value	OR(95% CI)
ε2/2	0	0	0	0	0		-		-	-
ε2/3	5(10.6)	5(10)	9(10.5)	14(10.3)	19(10.4)	ε2/3, no ε2/3	1.00	0.93 (0.25-3.46)	0.93	1.05 (0.33-3.33)
ε3/3	28(59.6)	40(80)	62(72.1)	102(75)	130(71)	ε3/3, no ε3/3	0.03	2.71 (1.10-6.21)	0.31	0.65 (0.18-1.49)
ε3/4	12(25.5)	5(10)	14(16.3)	19(14)	31(16.9)	ε3/4, no ε3/4	0.04	0.32 (0.10-1.01)	0.31	1.75 (0.59-5.19)
ε2/4	2(4.3)	0	0	0	2(1.1)	ε2/4, no ε2/4	0.23	-	-	-
ε4/4	0	0	1(1.2)	1(0.7)	1(0.5)	ε4/4, no ε4/4	-	-	1.00	-
Allele						**Allele**				
ε2	7(7.4%)	5(5%)	9(5.2%)	14(5.1%)	21(5.7%)	ε2, no ε2	0.46	0.64 (0.19-2.16)	0.91	1.05 (0.33-3.33)
ε3	73(77.7%)	90(90%)	147(85.5%)	237(87.1%)	310(84.7%)	ε3, no ε3	0.14	-	1.00	-
ε4	14(14.9%)	5(5%)	16(9.3%)	21(7.7%)	35(9.6%)	ε4, no ε4	0.01	0.26 (0.09-0.80)	0.24	1.90 (0.65-5.59)
All	94	100	172	272	366					

*Values are expressed as n(%) for categorical variables
HCC, hepatocellular carcinoma;LC, liver cirrhosis
OR: odd ratio(95% CI)

C. Serum ApoE levels in study subjects

To investigate the functional significance at the protein level, we had performed serum ApoE measurements among the162 individuals in the same set of subjects. The level of serum ApoE was significantly higher in the LC group ($P<0.001$) and the HCC group ($P=0.001$) compared with that of the control group (Figure 1). The mean ApoE level of the LC group tended to be higher than that of the HCC group, but significant differences between the two groups were not observed (Figure 1).

We analyzed whether the serum levels of ApoE correlate with that of ApoE genotypes. Figure 2 demonstrates the serum ApoE levels with regard to ApoE genotype. There was no significant difference in the serum ApoE levels among the different ApoE genotypes

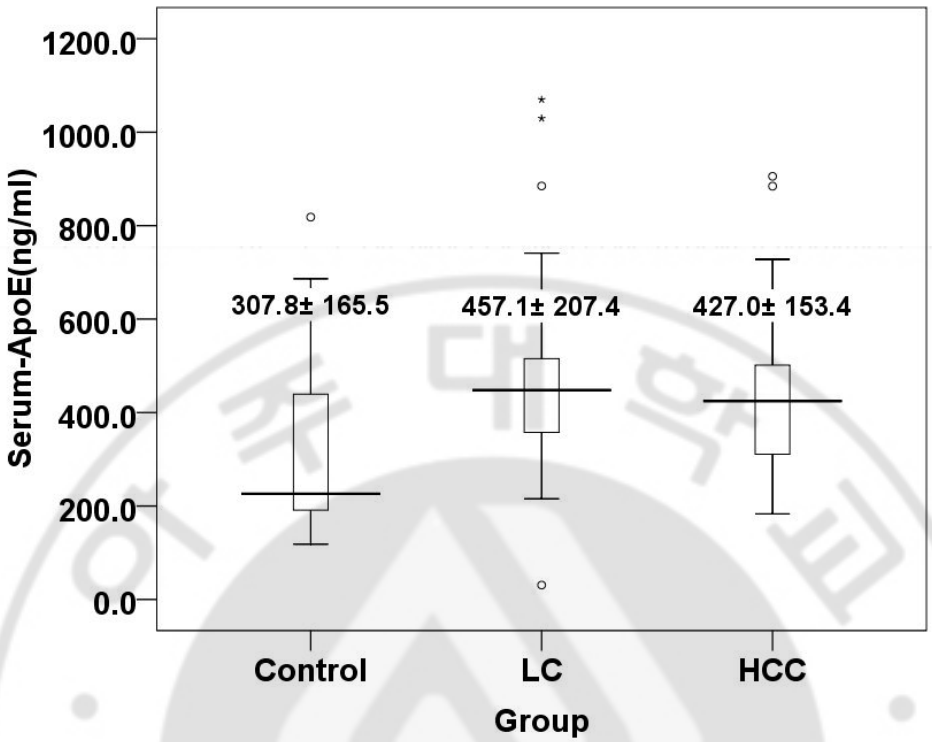


Fig. 1. Apolipoprotein E (ApoE) levels in serum from subjects with different groups. The level of serum ApoE was significantly higher in liver cirrhosis group (P<0.001) and hepatocellular carcinoma group (P=0.001) compared with the control group.

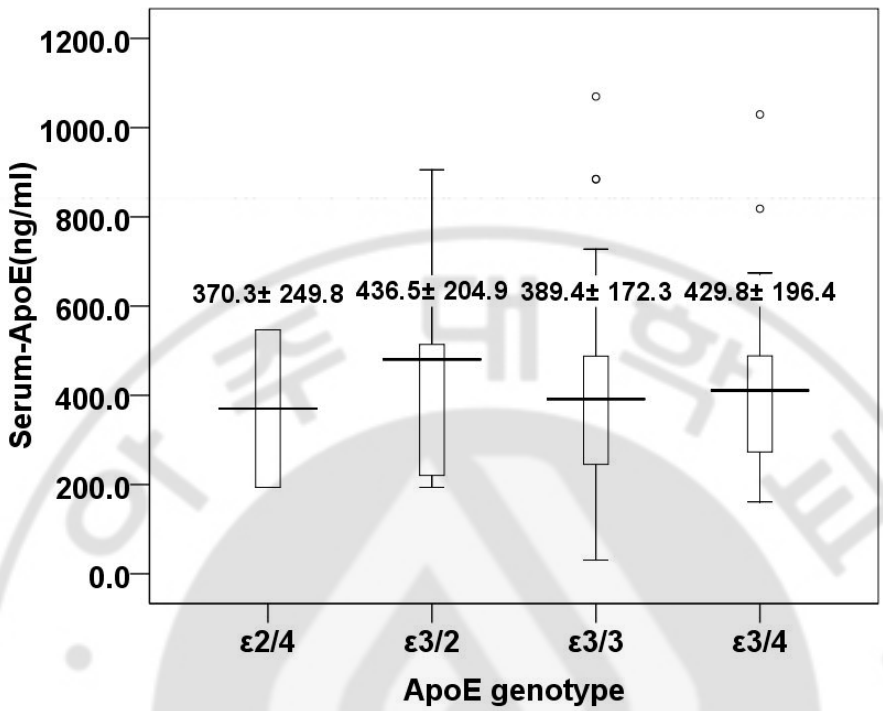


Fig.2. Serum ApoE levels in subjects with different ApoE genotype. There was no significant difference in mean ApoE levels among different ApoE genotypes.

IV. DISCUSSION

Increasing evidence indicates that genetic factors influence the natural history of chronic liver disease. In this study, we have determined whether the ApoE genotypes can predict the outcome of HBV infection and the occurrence of HCC originated from various etiologies. The carriers of apoE4 allelic variant had lower probability of disease progression to liver cirrhosis, but the occurrence of HCC was not affected by ApoE genotypes. Although there is insufficient data to show a direct functional effect of ApoE genotype on the progression to liver cirrhosis, our results suggest a possible genetic association between ApoE genotype and viral clearance in HBV infected patients.

In the current study, ApoE3 allele was associated with a higher probability of progression to HBV-related liver cirrhosis, and ApoE4 allele was a protective factor for developing liver cirrhosis. This result is an agreement with previous report, that a strong association was observed between the apoE3/E3 genotype and the occurrence of more severe liver disease (Toniutto et al., 2010). Another recent investigation of the influence of ApoE polymorphism and outcome of HCV infection also suggested that the apoE4 allele protected against severe liver diseases (Wozniak et al.,2002).

The role of ApoE in the pathogenesis and eradication of chronic HCV is becoming clearer. It is well known that HCV is linked to lipoproteins and mainly uses the LDL receptor pathway to colonize liver cells (Agnello et al., 1999; Pumeechockchai et al., 2002). A strong interaction has been postulated to exist between ApoE and hepatitis C virus infection (Mazumdar et al., 2011). Taken together, ApoE4 seems to be protective against CHC infection and retards the progression of fibrosis. In contrast, HBV interacts with ApoH and possibly interacts with other lipoproteins (Stefas et al., 2001; Mehdi et al., 1994). By modulating the transport and the entry of HBV into hepatocytes, apolipoproteins may influence the course of infection, although the virological effects of HBV and HCV are different.

Polymorphisms should have a functional significance. It is essential to show that the change in the gene causes a relevant alteration in the function or level of gene product to establish

medically useful links between polymorphism and disease (Rosenthal et al., 1998). In this study, we assessed both the ApoE genotype and the serum ApoE levels in the same set of patients and sought to confirm the relationship between the serum ApoE levels and liver cirrhosis progression in the enrolled subjects. Higher ApoE levels are observed in patients with liver cirrhosis than in the healthy controls. These results suggest that genetic factor and its gene product may affect the natural course of viral infection. It is not clear that allelic polymorphisms, in the ApoE, can affect the capacity to product ApoE. We assessed whether ApoE production varies, according to the genetic composition of the ApoE. According to our results, ApoE production was not affected by ApoE genotype.

The serum ApoE predominantly originates from the liver and the macrophages (Wu et al.,1979). ApoE has additional roles, as a modulator of the immune function. Proliferation of both CD4 and CD8 lymphocytes is suppressed by ApoE, which reduces the production of biologically active IL-2 (Kelly et al., 1994). In addition, ApoE can have a direct effect on tissue macrophage recruitment, independently, of the lipoprotein metabolism (Shiri-Sverdlov et al., 2006). Several data on the role of ApoE in the regulation of inflammation were reported. ApoE has been observed to increase in the serum of patients with sepsis (Li et al., 2008). Recently, serum proteomic profiling in patients with drug-induced liver injury demonstrated that elevation of the serum ApoE had the diagnostic power for differentiating patients with drug-induced liver injury from that of the controls (Bell et al., 2012). In our study, patients with liver cirrhosis showed higher ApoE levels than that of the controls. This result suggests that the elevated ApoE levels can be caused by repeated hepatic inflammation seen in patients with liver cirrhosis.

ApoE was up-regulated in the tissue of HCC developed in patients with chronic viral hepatitis C and serum of HCC patients (Blanc et al., 2005; Ritorto et al., 2011). The course of HCC development is a multistep process, and cirrhosis has been considered as a precancerous stage. Because only a subset of LC patients develops HCC, it is of great interest to identify factors that affect the transition from LC to cancer. Hence, only patients with cirrhosis were enrolled in our study, however, there were no differences between liver cirrhosis and HCC patients in ApoE genotype and serum ApoE levels.

Our study has some limitations in its study design. First, study subjects did not include the patients with chronic hepatitis or inactive HBV carrier. Thus, this study could not adequately

evaluate the effects of the ApoE genotype on the progression of fibrosis. As a consequence, ApoE genotype could, in fact, be associated with an increased susceptibility to the HBV persistence, not to fibrosis progression. It would be more reasonable to select non-cirrhotic chronic liver disease as a control to find factors of disease progression. Second, sample size was small. Prediction of patients who will develop HCC in patients with chronic liver disease is an important unmet clinical need. We were unable to assess this issue clearly in this current study because of the sample size being small. Further investigations with a larger sample size are needed. Third, the causes of HCC were heterogeneous, even if HBV is the main etiology of HCC. Thus, the differences in etiology of liver disease in cirrhosis and HCC patients may affect the results

V. CONCLUSION

In conclusion, this study suggests that ApoE genotype influence the outcome of HBV infection, with the ApoE4 allele favoring viral clearance. The serum ApoE levels are higher in patients with liver cirrhosis compared with that of the healthy individuals. It appears that the usefulness of ApoE genotype as a predictor of viral clearance after HBV infection and diagnostic value of the serum ApoE levels for the diagnosis of liver cirrhosis need to be defined.

REFERENCE

1. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX : Hepatitis c virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 96:12766-71,1999
2. Bell LN, Vuppalanchi R, Watkins PB, Bonkovsky HL, Serrano J, Fontana RJ : Serum proteomic profiling in patients with drug-induced liver injury. *Aliment Pharmacol Ther* 35:600-12,2012
3. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A:. Association of apolipoprotein e genotypes with lipid levels and coronary risk. *JAMA* 298:1300-11,2007
4. Blanc JF, Lalanne C, Plomion C, Schmitter JM, Bathany K, Gion JM: Proteomic analysis of differentially expressed proteins in hepatocellular carcinoma developed in patients with chronic viral hepatitis c. *Proteomics* 5:3778-89,2005
5. Cheong JY, Cho SW, Chung SG, Lee JA, Yeo M, Wang HJ: Genetic polymorphism of interferon-gamma, interferon-gamma receptor, and interferon regulatory factor-1 genes in patients with hepatitis b virus infection. *Biochem Genet.* 44:246-55,2006
6. Cheong JY, Cho SW, Hwang IL, Yoon SK, Lee JH, Park CS: Association between chronic hepatitis b virus infection and interleukin-10, tumor necrosis factor-alpha gene promoter polymorphisms. *J Gastroenterol Hepatol* 21:1163-9,2006
7. Choi HJ, Ko SY, Choe WH, Seo YS, Kim JH, Byun KS: Clinical features of acute viral hepatitis b in korea: A multi-center study. *Korean J Hepatol* 17:307-12,2011
8. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW : Gene dose of apolipoprotein e type 4 allele and the risk of alzheimer's disease in late onset families. *Science* 261:921-3,1993
9. Jofre-Monseny L, Minihane AM, Rimbach G: Impact of apoe genotype on oxidative stress, inflammation and disease risk. *Mol Nutr Food Res* 52:131-45,2008

10. Kelly ME, Clay MA, Mistry MJ, Hsieh-Li HM, Harmony JA : Apolipoprotein e inhibition of proliferation of mitogen-activated t lymphocytes: Production of interleukin 2 with reduced biological activity. *Cell Immunol* 159:124-39,1994
11. Kwon SY, Lee CH: Epidemiology and prevention of hepatitis b virus infection. *Korean J Hepatol* 17:87-95,2011
12. Li L, Thompson PA, Kitchens RL: Infection induces a positive acute phase apolipoprotein e response from a negative acute phase gene: Role of hepatic ldl receptors. *J Lipid Res* 49:1782-93,2008
13. Mahley RW, Rall SC, Jr. Apolipoprotein e: Far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 1:507-37,2000
14. Mazumdar B, Banerjee A, Meyer K, Ray R: Hepatitis c virus e1 envelope glycoprotein interacts with apolipoproteins in facilitating entry into hepatocytes. *Hepatology* 54:1149-56,2001
15. Mehdi H, Kaplan MJ, Anlar FY, Yang X, Bayer R, Sutherland K : Hepatitis b virus surface antigen binds to apolipoprotein h. *J Virol* 68:2415-24,1994
16. Minihane AM, Jofre-Monseny L, Olano-Martin E, Rimbach G: Apoe genotype, cardiovascular risk and responsiveness to dietary fat manipulation. *Proc Nutr Soc* 66:183-97,2007
17. Mueller T, Gessner R, Sarrazin C, Graf C, Halangk J, Witt H: Apolipoprotein e4 allele is associated with poor treatment response in hepatitis c virus (hcv) genotype 1. *Hepatology* 38:1592; author reply -3,2003
18. Pumeechockchai W, Bevitt D, Agarwal K, Petropoulou T, Langer BC, Belohradsky B, Bassendine MF: Hepatitis c virus particles of different density in the blood of chronically infected immunocompetent and immunodeficient patients: Implications for virus clearance by antibody. *J Med Virol* 68:335-42,2002
19. Ritorto MS, Borlak J: Combined serum and tissue proteomic study applied to a c-myc transgenic mouse model of hepatocellular carcinoma identified novel disease regulated proteins suitable for diagnosis and therapeutic intervention strategies. J *Proteome Res* 10:3012-30,2011

20. Rosenthal N, Schwartz RS: In search of perverse polymorphisms. *N Engl J Med* 338:122-4,1998
21. Shiri-Sverdlov R, Wouters K, van Gorp PJ, Gijbels MJ, Noel B, Buffat L: Early diet-induced non-alcoholic steatohepatitis in apoe2 knock-in mice and its prevention by fibrates. *J Hepatol* 44:732-41,2006
22. Stefas I, Rucheton M, D'Angeac AD, Morel-Baccard C, Seigneurin JM, Zarski JP: Hepatitis b virus dane particles bind to human plasma apolipoprotein h. *Hepatology* 33:207-17,2001
23. Toniutto P, Fattovich G, Fabris C, Minisini R, Burlone M, Pravadelli C: Genetic polymorphism at the apolipoprotein e locus affects the outcome of chronic hepatitis b. *J Med Virol* 82:224-331,2010
24. Wozniak MA, Itzhaki RF, Faragher EB, James MW, Ryder SD, Irving WL: Apolipoprotein e-epsilon 4 protects against severe liver disease caused by hepatitis c virus. *Hepatology* 36:456-63,2002
25. Wu AL, Windmueller HG: Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. *J Biol Chem* 254:7316-22,1979

- 국문 요약 -

B형간염 바이러스 감염 환자에서 질병의 경과와 아포지단백 E 유전형과의 관련성

아주대학교 대학원 의학과

안 선 주

(지도교수: 정 재 연)

목적: 아포 지단백 E (Apo E) 는 체내의 지방과 콜레스테롤의 이동 및 대사를 조절하는데 중요한 역할을 담당하고 있는 단백질로 알려져 있다. 본 연구에서는 한국인 간세포암 환자와 간경변증 환자 및 정상 대조군들을 대상으로 ApoE 유전형 및 대립형질의 분포를 조사하여 ApoE 유전적 다형성이 B형 간염 바이러스 감염 환자들의 질병의 경과에 영향을 미치는지 알아보고자 하였다.

방법: 2007년도부터 2009년도 사이에 수원 아주대 병원에 내원하여 간세포암과 간경변증으로 치료를 받았던 환자들과 정상 대조군들의 혈액을 채혈하여 Apo E 유전형 검사 및 혈청내 ApoE 치를 측정하였다. ApoE 유전형 검사는 PCR 법을 이용하였고 혈청내 ApoE치는 ELISA kit을 이용하여 측정하였다.

결과: 정상 대조군과 간경변증 및 간세포암군 모두에서 Apo E 유전자의 세가지 대립형질 중 $\varepsilon 3$ 대립형질의 빈도가84.7% 로 가장 높았으며 그 다음으로 $\varepsilon 4$가 9.6%, $\varepsilon 2$가 5.7% 의 빈도로 나타났다. 또한 ApoE 유전자형 중 가장 빈도가 높았던 것은 $\varepsilon 3/3$ 유전자형이었다. $\varepsilon 3/3$ 유전자형을 가지거나 $\varepsilon 4$ 대립형질이 없는 경우 간경변증으로 진행할 가능성이 높았다. 간세포암의 발생에는 ApoE 유전자형이 영향을 미치지 않는 것으로 나타났다. 혈청내 ApoE 수치는 정상 대조군에 비해 간경변증 및 간세포암 환자에서 통계학적으로 유의하게 높게 측정되었으나 ApoE 유전자형에 따른 혈청 ApoE치는 차이가 없었다.

결론: Apo E 유전자형이 만성 B형 간염 바이러스 감염자들의 질병의 경과에 영

향을 미치는 유전적 요인 중 하나라고 생각할 수 있다.

핵심어: 아포 지단백 E, B형 간염 바이러스, 유전자, 간경변증