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Apolipoprotein E2 & E4 Alleles Influence on the Distribution of the Human Plasma Lipid Profiles in Normolipidemic Korean Women

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ABSTRACT

Apo E polymorphism (e2, e3, e4) was among the first reported genetic polymorphism that explained part of the normal variation in plasma cholesterol concentrations. Both alleles E2 and E4 are significantly more frequent in patients with mixed forms of hyperlipidemia and contribute on the observed differences in CHD risk among different populations. Effects of apo E polymorphism on the distribution of plasma lipid profiles were studied in 105 Normolipidemic healthy women. The relative frequencies of common alleles for gene locus of apo E in this study were that E3 allele was 0.848, E4 allele was 0.087, and E2 allele was 0.067. SBP and DBP were slightly more elevated in E2 allele than those in E3 and E4. The pulsation was also significantly ($p < 0.016$) increased by E2 allele with excess body fat % in E2 allele. There were no differences in total-, total HDL-, VLDL+LDL-, VLDL- and LDL cholesterol among the apo E alleles. However, apo E2 allele subjects had lower levels of total HDL and HDL2 cholesterol ($P < 0.047$) and significantly higher levels of HDL3 cholesterol ($P < 0.05$) than those in apo E3 and E4 allele subject. The plasma TG levels were significantly higher in the apo E2 allele than in the apo E3 allele, otherwise, the plasma TG level in E4 allele was significantly lower than that in E3 allele ($P < 0.049$ among apo E alleles. Atherogenic indices (AI) such as (TC-HDL)/HDL ($p < 0.04$) and HDL3/HDL2 ($p < 0.06$) were significantly increased in E2 allele than those in E3 and E4 allele. The conclusion is that first, it seems that apo E4-mediated alteration through LDL B/E receptors or E receptors in cholesterol metabolism results in lower plasma TG or remnant particles and in higher levels of VLDL+LDL or LDL. Second, apo E2 allele shows reciprocal effects of E4 on the plasma lipid metabolism, respectively. Third, apo E2 allele was more atherogenic than apo E4 because the higher levels of HDL3/HDL2 ratio and atherogenic index [(TC-HDL)/HDL] were criticized. (*Korean J Nutrition* 29(6) : 642~650, 1996)

KEY WORDS : apo E polymorphism · E3 or E4 or E2 allele · cholesterol · triglyceride · atherogenic index · LDL B/E or E receptors.

Introduction

Apo E is an important structural constituent of several plasma lipoproteins and remnant particles such as VLDL, VLDL remnants, chylomicrons , chylomicron remnants and HDL1 or HDLc. Therefore, apo E serves as the ligand for both the LDL(B/E) receptor and apo E receptors on the liver cell plasma membranes, thereby mediates uptake of lipoprotein which contains apo E[1)2)3)]. Moreover, apo E has a key roles in the function of HDL on reverse cholesterol transport system and may accelerate the turnover of this system

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because apo E facilitates the acquisition of cholesterol by HDL and apo E itself actively accepts cholesterol from cholesterol loaded cells[4)5)].

The common isoforms of arginine-riched apolipoprotein E(E2, E3 and E4) are encoded by three alleles($\varepsilon 2$, $\varepsilon 3$, $\varepsilon 4$) which exist in humans and give up to six apo E phenotypes in plasma. Apo E4(cys 112 - arg) and apo E2(arg 158 - cys) are derived by point mutation from the parent E3 isoform during human evolution but occur with various frequencies in differnet ethnic, cultural and geographical groups[6)7)]. Apo E2 is impared by a low rate of metabolism compared to apo E3[3)4)5)]. Due to delayed catabolism of both chylomicron and VLDL remnants and the low rate of apo E-mediated conversion of VLDL to LDL, TG-riched lipoproteins can be accumulated in palsma. Finally, the low rate of transport of remnant particles and decreased rate of LDL formation lead to up-regulation of LDL receptors. Therefore, these apo E2-mediated alteration result in higher plasma concentrations of triglyceride or remnant particles and in lower levels of LDL[5)6)]. Most of results are consistant with apo E4, the effect opposite to that of apo E2. Chylomicron and VLDL remnants will be converted to LDL at a higher rate, with delivery of cholesterol and triglycerides to liver. Also, VLDL will be converted to LDL at a higher rate, resulting in a higher LDL production rate. The higher rate of conversion of VLDL to LDL will lead to down-regulation of LDL receptor. This will result in higher LDL and lower remnant particles[2)5)6)8)].

Moreover, alleles E2 and E4 are significantly more frequent in patients with mixed forms of hyperlipidemia. Over 20% of these patients have one phenotypes 4/4, 4/2 or 2/2 compared to 5.3% in the general population[5)9)]. Most commonly, type Ⅲ hyperlipoproteinemia occurs in association with the E2/2 phenotype. Nontheless, apo E from hyper-, normo-, or hypocholesterolemic subjects with E2/2 has equally defective binding to the apo B/E LDL receptor. Since 8% of the variation in the concentration of the LDL cholesterol in serum may be associated with apo E gene locus, there is a high correlation between the apo E phenotypes and increased risk of myocardial infarction.

In a world review of relative allele frequencies, south American Indians, Chinese, Japanese and Caucasians populations are estimated(presented in Table 2) [10)11)12)13)14)]. Each of three alleles are presented in all of the population groups with the exception of South American Indians[6)]. The relative frequency of E2 is lowest in Japan whereas that of the E4 is lowest in Chinese[15)]. A much higher frequency of E4 allele occurs in Finnish populaiton whose risk of CHD (coronary heart disease) is quite high compared to Japanese[16)]. It seems that the modest differences in allele frequencies will explain the difference in prevalence of CHD between high and low risk populations[17)18)19)20)].

Therefore, the aim of this study was to inform on the effects of different apo E isoforms on the plasma distribution of lipid profiles including all types of cholesterol, TG, β-lipoprotein in 105 females. The two major objectives of this study were the following : 1) to report, firstly, the relative frequencies of apo E2, E3, E4 alleles in Korean population with modification of the isoelectricfocusing method of apo E phenotyping. 2) to evaluate the variation of plasma cholesterols and apolipoproteins according to apo E isoforms.

Material and Methodology

1. Research design

Subjects were recruited from among Sungshin Women's University females from September to October, 1995. Among volunteers, 105 healthy females, ages 19 up to 26 years, screened on the basis of information from a health questionnaire for the checking drug, alcohol, smoking, coffee & exercise, and physical examination for anthropometic parameters. Subjects were not known history of metabolic diseases and had not taken any medication, specially thyroids or steroids, that affects plasma lipid levels. Blood was collected after 12 hours fast at the one of three days of bleeding period, Oct 3/4/5, 1995 for the consideration of menstrual stage. Plasma was separated promptly at 4 degree C by cetrifugation at 3000 rpm for the blood chemistry as followings : (plasma for separation of lipoprotein should be used freshly.

2. Apo E phenotyping

Apo E phenotypes were separated by an isoelectricfocusing(IEF) technique using urea polyacrylamide gel. This method was modified by Drs. Kamboh and Ferrell, Department of Human Genetics, University of Pittsburgh, using a differnt method of sample preparation with dithiothreitol(DTT) solution[21)22)]. The IEF gel, 5% PAGE-3M urea gel, freshly prepared, was placed on an IEF machine and prefocused for 20 min at 1000 volts and 150 mA. After the gel was run at

1500V, 150mA, 10 W for 30 min or 1 hr, sample wicks were removed and IEF was continued for 2 or 2 and half hours. IEF gel were transferred overnight to a nitrocellulose membrane with blotting paper. Immunoblotting was performed by steps of blocking with 5% nonfat milk and incubation of 1st(Atlantic apo E antigoat) and 2nd antibody(Atlantic rabbit antigoat antibody). Boric acid solution with β-naphyl phosphate, Fast Blue BB salt and magnesium was used for staining until the bands were dark enough.

3. Anthropometric parameters

Weight(Kg)and height(m)were measured at the period of bleeding days and BMI(body mass index) was calculated by the weight/height[23]. Fat% was measured by the Bioelectrotrical Impedence Assay (BIA) using the analyzer GIF-891(Gil-Woo Trading Company) which BIA equation derived from Japanese (Tanaka's study) can be applied for the estimation of Korean, not for Caucasian[24].

The blood pressure & pulsation were measured by the automatic blood pressure monitor and the pulsimeter.

4. Total-, free-, esterified, total HDL-, HDL3-, HDL2-, VLDL+LDL-, LDL-, and VLDL cholesterol

Total and free cholesterol were analyzed by enzymatic procedures modified from the total cholesterol procedure[25]. Ester form of cholesterol were determined by difference. Using the spectrophotometer, total and free cholesterol were determined by reading the solutions at 500 nm after incubation at 37 degree C for 5 minutes. Total HDL cholesterol were measured after precipitation of VLDL and LDL cholesterol with dextransulfate-1.0/1 mg solution[26]. HDL3 cholesterol were determined after HDL2 particles were removed by precipitation with dextransulfate-3.0 /1 mg solution. HDL2 particles was calculated by substraction of total HDL and HDL3 cholesterol. For the separation of VLDL plus LDL and HDL fraction, density gradient ultracentrifugation with potassium bromide was modified becasuse of insufficient amounts of plasma[27]. Two different density solutions of potassium bromide 1.063 kg/l(stock A) for VLDL plus LDL and 1.35 kg/l(Stock B) for the HDL fraction were used. LDL cholesterol was calculated by Friedewarld's equation : LDL cholesterol= total choleserol- total HDL cholesterol- 0.2*TG[28]. VLDL cholesterol was calculated by difference from VLDL plus LDL and LDL cholesterol.

5. Total triglycerides

Total TG were analyzed by enzymatic method using TG kits from Sigma Chemical Co.(#339). [29] Using the spectrophotometer, total TG were measured at 540 nm for 5 minutes at 37 degree C.

6. Plasma β-lipoprotein

After the precipitation of β-lipoprotein with the Heparin-Na^{++} solution, the levels of cholesterol in plasma β-lipoprotein was analyzed by the modified enzymatic procedure with the spectrophotometer at 505 nm(National Chemicals #15110, Japan). Using the standard solution of β-lipoprotein(900 mg/dl) including cholesterol(300 mg/dl), β-lipoprotein was calculated by the proportion of cholesterol in plasma β-lipoprotein reciprocally.

7. Data analysis

After normality test by univariate probability using the all subjects and each different apo E isoforms, student t-test, correlation and regression analysis for the comparison, one-way analysis of variance and multiple regression for the differences among the apo E alleles were used for the data analysis.

Results and Discussion

1. Apo E phenotypes

Among the one hundred and five health females, eighty nine were apo E3/3(r.f.=0.848), seven were E 4/2(r.f.=0.067) and E3/2(r.f.=0.067), one was E4/3(r.f.=0.009) and E4/4(r.f.=0.009). There were no found E2/2. Immunolocalized isoelectricfocusing patterns of these five different apo E phenoytpes of subjects in this study were shown in Fig. 1. Fig. 1 presented the three major bands of the apo E 3/3 phenotype and the six bands of the heterozygote except for five bands of E3/2 types. Urea gel for the phenotyping of apo E polymorphism are most useful to identify of rare apo E alleles so far. Moreover, this IEF method has several advantages : a) simple, rapid and reproducible mono-, dimensional IEF as compared to other methods, b) ultracentrifugation for the separaton of VLDL was not necessary, c) very small amounts of plasma were needed[21)22)30].

The relative frequencies of common alleles for gene locus of apo E in this study were that E3 allele was 0.848, E4 allele was 0.087, and E2 allele was 0.067(Table 1). Comparing the others for apo E polymorphism, the

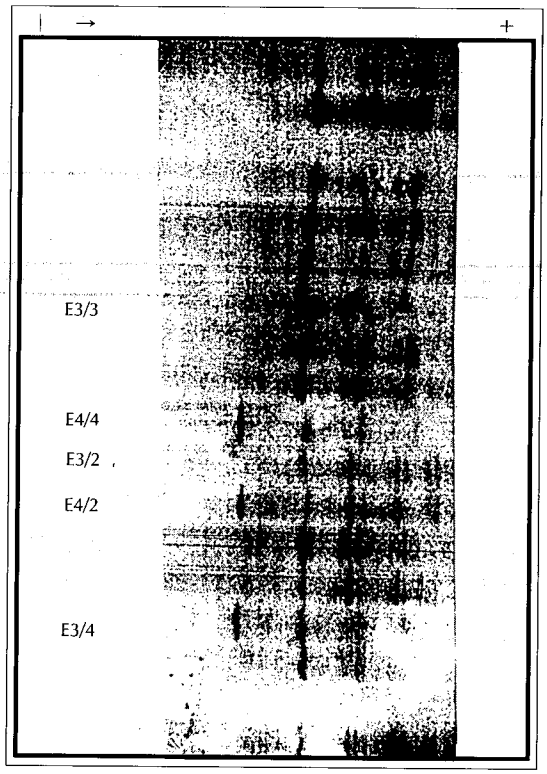

Fig. 1. The five common apo E phenotypes of subjects in this study determined by isoelectric focusing(IEF) on 5% PAGE-3M urea gel.

relative frequencies of E2 and E4 alleles were lower than those in study for Caucasians such as following studies for Asian. The relative frequency of apo E2 allele was 0.05 in the Chinese of K. Wang's study[7] and 0.023 in Japanese of S. Taushiya's study[12]. C.F. Sing[14] reported that the relative frequency of apo E2 was 0.07 in Caucasians. The results of lower frequencies of E2 and E4 alleles in Korean in this study and Kim's study explained that risk of the CHD is quite low compared to Caucasians. However, even though the sample size in this study was not much large for the population study in Korean, the relative frequency of apo E2 allele in these subjects was slightly higher than that in Japanese in spite of E2/2 were not found.

2. Subject information

Weight, height, BMI, and Fat% were not different among the apo E alleles(Table 2). However, weight and height were slightly larger in E2 allele than those in E3 and E4. Seventy percents of E2 allele belong to the range 21 to 25 in BMI compared to the 50% of E3 and E4 in that area. Also, 57% of E2 allele were in the range of 26 to 30% of the total fat% even 33%,

Table 1. The relative frequencies of the common alleles for the gene locus coding for apo E in normolipidemic population of this study comparing the others

Populations	Sample	E3	E4	E2	References*
Korean					
1. M.S.Lee	105	0.848	0.087	0.067	a)
2. J.K.Kim	100	0.790	0.140	0.070	b)
Amerindians	95	0.816	0.184	0.000	c)
Chinese	196	0.852	0.064	0.084	d),e)
Japanese	880	0.851	0.112	0.035	f),g)
Caucasians	5805	0.769	0.150	0.080	h),i),j),k)
Overall**	7181	0.820	0.123	0.056	

a) 1st study for the relative frewuency of apo E polymorphism in normolipidemic Korean Women.
b) J.K. Kim, Korean J. of Lipidology vol 3, No1, 1993
c) Asakawa J. et al, Hum. Genet, 70 : 222-230, 1985.
d) Wang K. et al, Acta Acad Med Sinicae 8 : 198, 1986.
e) Brennimeijer BJ et al, Arteriosclerosis, 6 : 5461, 1986
f) Taushiya et al, Jpn J. Genet, 30 : 271-278, 1985.
g) Cumming AM et al, Clin Genet 25 : 310-313, 1984.
h) Eto M, Cli Genet, 29 : 477-484, 1986.
i) Sing C. et al, Am J. Hum Genet, 1985
j) Ehnholm et al, J. Lipid Res, 27 : 227-235, 1986.
k) Utermann et al, Hum. Genet, 60 : 344-351, 1982.
* Calculated by weighted mean value from sugested reports.
** Calculated by weighed mean value form all reports according to apo E alleles

22% of E3 and E4 alleles were belonged to. These mean that the people had a E2 allele is heavier, higher, fatter than those of E3 and E4 alleles even the statistic significance was not found. The relationship between a fat% and apo E polymorphism could not be concluded because of no comparing data, but the abnormality of plasma lipid metabolism in a apo E mutant condition might be accelerated with a higher percentage of fat in the body.

3. Blood pressure and Pulsation

Table 3 presented blood pressure and pulsation in apo E alleles. Systolic and diastolic blood pressure were slightly elevated in E2 allele more than those in E3 and E4. The pulsation was also increased by E2 allele with significance of $p < 0.016$. These results were consistent with elevated total body fat% in E2 allele. Although the absolute body fat corresponding to significant risk factor for high blood pressure is not known, excess upper-body fat has been asssociated with elevated blood pressure, serum lipid, and lipoprotein fractions[31][32]. The reason for the body fat and blood pressure increased in E2 allele might be due to delayed TG removal from plasma in E2 allele compared to E3 and E4.

Table 2. Mean(±SD) Values of age, height, body mass index(BMI) and fat % of subjects according to apo E isoforms in this study(n=105)

Variables	E3 allele(n=89)	E4 allele(n=9)	E2 allele(n=7)	P-value*(apo E isoforms)
Weight(Kg)	53.86±6.32	53.61±5.52	55.36±4.44	0.815
Height(cm)	160.65±5.09	161.22±5.55	165.21±9.80	0.114
BMI(Kg/M^2)	20.85±2.16	20.69±1.42	21.01±1.61	0.956
< 20(n)	(37)	(4)	(2)	
21 – 25(n)	(49)	(5)	(5)	
> 26(n)	(3)	(0)	(0)	
Fat %	23.55±3.41	23.69±2.60	23.80±3.91	0.977
< 20%(n)	(13)	(0)	(1)	
20 – 25%(n)	(44)	(7)	(2)	
26 – 30%(n)	(27)	(2)	(4)	
> 30%(n)	(4)	(0)	(0)	

*Significance among the apo E3, E4, E2 allele

Table 3. Mean(±SD) Values of Systolic Blood Pressure(SBP), Diastolic Blood Pressure(DBP) and Pulsation according to apo E isoforms in this study(n=105)

Variables	E3 allele(n=89)	E4 allele(n=9)	E2 allele(n=7)	P-value*(E isoforms)
SBP(mmHg)	114.99±11.44	116.33±11.00	122.43± 9.31	0.244
DBP(mmHg)	73.91± 9.60	73.67± 6.39	78.89±11.07	0.058
Pulsation	78.62±11.06	69.44± 7.30	85.14±13.51	0.016*

*Significance among the apo E3, E4, E2 allele at $p < 0.05$.

Table 4. Mean(±SD) Values of plasma distribution of cholesterol, triglycerides and Artherogenic index(AI) in the different apo E alleles(mmol/l)

Variables	E3 allele(n=89)	E4 allele(n=9)	E2 allele(n=7)	P-value(E isoforms)
Cholesterol				
Total-	4.42± 0.67	4.47± 0.42	4.66± 0.38	0.523
LDL -1)	2.33± 0.73^a	2.62± 0.57^{ab}	2.57± 0.48^{ab}	0.215
HDL cholesterol				
Total-	1.30± 0.10	1.29± 0.14	1.27± 0.07	0.298
HDL3-	0.96± 0.14^a	0.98± 0.13^a	1.06± 0.18^b	0.050*
HDL2-	0.33± 0.16^b	0.31± 0.16^b	0.19± 0.20^a	0.047*
TG-riched lipoprotein cholesterol				
VLDL+LDL-2)	3.26± 0.83^a	3.54± 0.69^{ab}	3.70± 1.09^{ab}	0.214
VLDL-3)	0.92± 0.73^a	1.00± 0.75^a	1.15± 1.11^b	0.072
Triglyceride				
Total-	2.28± 0.44^b	1.98± 0.26^a	2.36± 0.75^b	0.049*
Artherogenic index				
LDL/HDL-4)	1.98± 0.64^a	2.28± 0.68^{ab}	2.23± 0.39^a	0.240
(TC-HDL)/HDL-4)	2.77± 0.62^a	2.85± 0.51^{ab}	3.05± 0.27^b	0.040*
HDL3/HDL2- 4)	2.91± 0.87^a	3.16± 0.82^b	5.58± 0.90^c	0.006*
β-lipoprotein(mg/dl)	374.1 ±93.9^a	396.9 ±83.5^a	424.3 ±129.6^b	0.098

1) Calculated by Friedwald's equation
2) Measured by VLDL plus LDL fraction obtained by density gradient ultracentrifugation.
3) Calculated by the differences from VLDL+LDL(2) and LDL(1)
4) Calculated by the ratio of the each cholesterol.
* Significance among the apo E isoforms at $p < 0.05$.
a,b,c values within the apo E3, E4 and E2 alleles with different superscripts are significantly at $p < 0.05$

4. Lipid profiles of subjects between apo E3 and E2 isoforms

The mean plasma total-, HDL-, TG-riched lipoprotein-, and TG as a lipid profiles in subjects with apo E alleles were shown in Table 4. Total cholesterol was not different among the E alleles in this study. C.F. Sing[14], et al reported that the total cholesterol levels of E2/2, E3/2, E3/3, E4/2, E4/3, E4/4 phenotypes were 136, 161, 174, 178, 184, 180 mg /dl, respectively, in populations of caucasians. S. Taushiya,

et al[12] reported the mean total cholesterol levels of E 2/2, E3/2, E3/3, E4/2. E4/3, E4/4 phenotypes were 142, 164, 179, 214, 178, 188 mg/dl, respectively, in Japanese population. G. Assmann, et al[14] agreed to the phenotypes mean rank as E2/2 < E3/2 < E3/3 < E4/3 for the average total cholesterol of populations. These means that plasma cholesterol levels were a lower in E2 and higher in E4 than that in E3 allele.

The plasma concentrations of LDL cholesterol in apo E2 and E4 allele were 99.04±18.56 mg/dl(2.57±0.48 mmol/l) and 101.09±22.13 mg/dl(2.62±0.57 mmol/l). A higher LDL levels of E2 and E4 were shown than that in the apo E3 [89.99±28.08 mg/dl(2.33±0.73 mmol/l)].

H.J Lenzen, et al found that the alleles E2 and E4 had major effects on LDL cholesterol[33]. The same stepwise decreasing gradient in plasma LDL cholesterol, going from E4/3 to E 3/3 to E3/2 to E2/2, occurred both in patients with coronary artery disease and myocardial infraction, and in the normal group. M. Eto and G. Uterman, et al[5][13][19] agreed there was a highering effect of apo E4 allele on LDL cholesterol as was suggested by the results of this study. Plasma cholesterol of VLDL cholesterol in apo E2 allele was significantly higher than that in apo E3 allele at the levels of $p=0.017$(not shown in table). These results were consisted to the resuls of the plasma TG levels.

The plasma TG levels were significantly higher in the apo E2 allele(2.36±0.75 mmol/l) than in the apo E3 allele(2.28±0.44 mmol/l) in this study. Otherwise, the plasma TG levels in E4 allele(1.98±0.26 mmol/l) was significantly lower than that in E3 allele. This result tended to agreeing with reports of C. F. Sing[14] and G. Utermann[19] but, not with the report of Lenzen that TG did not vary with apo E phenotypes[33].

Since the apo E2 allele had a binding default of LDL receptor and accumulation of TG rich lipoproteins, the higher levels of TG in apo E2 allele was affected from clearance of VLDL plus LDL lipoproteins as expected. Moreover, the higher catabolism of plasma TG riched lipoproteins in apo E4 associated with the lower plasma level of TG. Secondly, higher rate of LDL formation leads to down-regulation of LDL receptors. Therefore, these E4-mediated alteration result in lower level of TG and higher level of LDL. Most of effect of E4 had an opposite to that of E2 allele[1][19].

It was interested in that the results of lipid profile in apo E alleles was the alteration in the cholesterol of HDL fraction. Total HDL cholesterol was not different among apo E alleles. However, apo E2 allele subjects had lower levels of total HDL and HDL2 cholesterol and significantly higher levels of HDL3 cholesterol than those in apo E3 and E4 allele subjects(Fig. 2). H.J. Lenzen[33] mentioned that the mean plasma concentrations of total HDL cholesterol did not vary significantly within the apo E phenotypes. However, many researchers did not expect a difference between apo E phenotypes in the levels of HDL3 and HDL2 cholesterol, this relationship was examined in this study such as in M. Lee's study.[4][34] In this study, apo E2 allele subjects had lower levels of HDL2 and significantly higher levels of HDL3 cholesterol than those in apo E3 allele. However, plasma cholesterol levels in HDL fraction was not affected by apo E4 allele. Consistently, higher ratio of HDL3 vs HDL2- in apo E2 allele than that in apo E3 in E4 suggested the higher risk of CHD. Therefore, atherogenic indices(AI) such as(TC-HDL)/HDL and HDL3/HDL2 were significantly increased in E2 allele than those in E4 allele(Fig. 3). These results are related to the common reports for apo E polymorphism to be mentioned that all people with type III hyperlipidemia

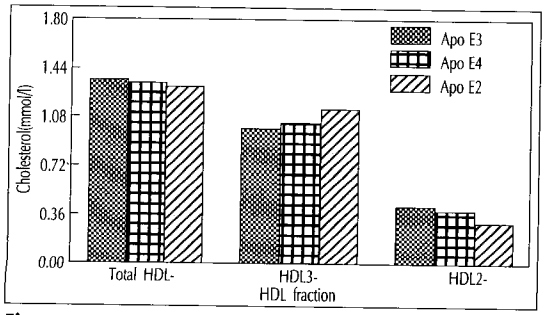

Fig. 2. Mean(±SD) values of plasma cholesterol distibution in each HDL fraction according to different Apo E alleles(mmol/l).

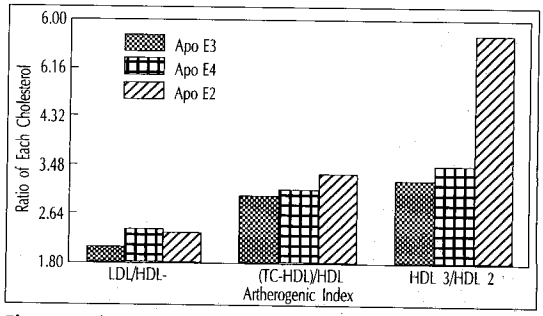

Fig. 3. Artherogenic indexes such as LDL/HDL(TC-HDL)/HDL, HDL3/HDL2 accoding to different Apo E alleles(ratio).

have E2/2, otherwise, fewer than 5% of E2/2 have the type III. Even though E2/2 was not found in this study, it is important with higher levels of AI in E2 allele than that in E3 and E4[35)36)37)].

This study suggested more important two things for apo E polymorphism study. First, the E2 allele is a stronger risk factor of CHD than in E4 allele by the lower levels of HDL2 cholesterol. Second, the HDL3 and HDL2 fraction should be considered in parts of lipid metabolism study as the influencing factors.

Conclusion

The effects of apo E allele on the distribution of plasma lipid profiles with artherogenic indexes were investigated in 105 Normolipidemic female subjects. The summary of results are as followings :

1) The relative frequencies of common alleles for gene locus of apo E in this study were that E3 allele was 0.848, E4 allele was 0.087, and E2 allele was 0. 067. Comparing the others for apo E polymorphism, the relative frequency of E2 and E4 alleles were lower in Asian than those in study for Caucasians.

2) SBP and DBP were slightly elevated in E2 allele more than those in E3 and E4. The pulsation was also significantly increased by E2 allele with excess body fat% in E2 allele.

3) There are no difference in total-, total HDL-, VLDL+LDL-, VLDL-, LDL cholesterol among the apo E alleles. However, the plasma TG levels were significantly higher in the apo E2 allele than in the apo E3 allele, otherwise, the plasma TG levels in E4 allele was significantly lower than that in E3 allele. Morever, apo E2 allele subjects had lower levels of total HDL and HDL2 cholesterol and significantly higher levels of HDL3 cholesterol than those in apo E3 and E4 allele subjects.

4) Atherogenic indices(AI) such as(TC-HDL)/HDL and HDL3/HDL2 significantly increase in E2 allele than those in E4 allele.

In conclusion, a) Apo E4-mediated alteration through LDL B/E receptors or E receptors in cholesterol metabolism results in lower plasma TG or remnant particles and in higher levels of VLDL+LDL or LDL. b) Apo E2 allele shows reciprocal effects of E4 on the plasma lipid metabolism, respectively. c) Apo E2 allele was more atherogenic than apo E4 because the higher levels of HDL3/HDL2 ratio and atherogenic index [(TC-HDL)/HDL] was criticized. Therefore, HDL3 and HDL2 fraction should be considered in parts of lipid profiles as the influencing factor.

Literature cited

1) Lee MS, Apo E. polymorphism and diseases. *Korean J Lipidology* 5 (1) : p11-23, 1995
2) Beisiegel U, Weber W, Herz J and Stanley KK. the LDL receptor related protein, LRP, is an apo E binding protein. *Nature* Vol 341 (14) : 162-164, 1989
3) Davingnon J, Gregg RE and Sing CF. Apo E polymorphism and atherosclerosis. *Arteriosclerosis* 8 : 1-21, 1988
4) Lee MS. Apo E isoforms influence on the distribution of Human plasma cholesterols & Apolipoproteins. *Korean J of Lipidology* 3 (2) : p221-228, 1993
5) Utermann G. Apo E polymorphism in health & disease. *Am Heart J* 113 : p433-440, 1987
6) Weisgranner KH. Abnormal lipoprotein receptor binding activity of the human apo E due to cysteine arginine interchange at a single sites. *J Biol Chem* 257 : 2518-2521, 1982
7) Kamboh M & Ferrell RE. Genetic Studies of human apo E. *J Lipid Res* 29 : 1535-1543, 1988
8) Gregg RE, et al. Abnormal in vivo metabolism of apo E4 in humans. *J Clin Invest* 78 : 815-821, 1986
9) Zannis VI. Genetic polymorphism in human apo E. *Methods Enzy* 128 : 823-851, 1986
10) Asakawa J. Takshashi N, Rosenblum BB. & Nell JV. Two dimential gel studies of genetic variation in the plasma proteins of Amerindians and Japanese. *Hum Genet* 70 : 222-230, 1985
11) Wang K. Studies apo E genetic isoforms and their phenotypes among the chinese populations, Acta. Acad. *Med Sinicae* 8 : 198, 1986
12) Taushiya S, Yamamouchi Y, and Onuki M, Frequencies of apo E5 and E7 in apparently healthy Japanese. *Jpn J Hum Genet* 30 : 271-278, 1985
13) Eto M. Reciprocal effects of apo E alleles (e2 & e4) on plasma lipid levels in notipidemic subjects. *Clin Genet* 29 : 477-484, 1986
14) Sing C, et al. Role of the apo E polymorphism on determining normal plasma lipid and lipoprotein variation. *Am J Hum Gent* 37 : 268-285, 1985
15) Brennimeijer BJ, et al. Hypertriglyceridemia and apo E polymorphism. *Arteriosclerosis* 6 : 5461, 1986
16) Cumming AM. Polymorphism at the apo E locus in relation to risk of coronary disease. *Clin Genet* 25 : 310-313, 1984
17) Steinmets, et al. Differential distribution of apo E iso-

forms in human plasma lipoprotein. *Atherosclerosis* 80 : 27-31, 1989

18) Glying, et al. Apo E phenotype and cholesterol metabolism in familial hypercholesterolemia. *Arteriosclerosis* 5 : 283-291, 1985

19) Utermann G. Genetic polymorphism in apo E, Expecta. *Medica* 1-28, 1985

20) Wardell MR. Genetic variation in human apo E. *J Lipid Res Atherosclerosis* 3 : 310-315, 1983

21) Kamboh MI, Ferrell RE and Kottke B. Genetic studies of huamn apo E. *J Lipid Res* 29 : 1535-1543, 1988

22) Kelly L, Kamboh MI and Ferrell RE. Genetic studies of human apo E and apo A-IV. *Electrophoresis* 2 : 53-57, 1986

23) Health Services and Mental Health. Administration, Skinfold, Body Girths, Biacromial diameters and Selected anthropometric Indices of Adults, National center for Health Statistics, Series 11, N0 35. 1960-1962

24) Tanaka K, et al. Body composition prediction Equations based on Bioelectrical Impedance and anthropometric Variables for Japanese Obese Women. *Am J Human Biology* 4 : 739-745, 1992

25) Allain CA, et al. Enzymatic determination of total serum cholesterol. *Clin Chem* 20 : 470, 1974

26) Warnick GR, et al. Precipitation methods of total HDL-, HDL3 & HDL2. *Clin Chem* 18 : 499-502, 1972

27) Kelley JL and Kruski AW. Density gradient ultracentrifugation of serum lipoproteins, Methods. *Enzyogy* 128 : 170-181, 1986

28) Friedwald W. Estimation of the concentrations of LDL in plasma without use of the peoparative ultracentrifugation. *Clin Chem* 18 : 499-502, 1972

29) Lowell B, et al. Stable reagents for determination of serum TG by colorimetric Hantzsch condensation method. *Clin Chem* 19 : 338-340, 1973

30) Boerwinkle E, Visvikis S, Welsh D, Steinmetz J, and Sing CF. The use of measuring genotype information in the analysis of quantitative phenotypes in man II. *Am J Med Genet* 27 : 567-582, 1987

31) William DP, et al. Body fatness and risk for elevated Blood pressure, total cholesterol, & serum lipoprotein ratios in children and aldolescents. *Am J Public Health* Vol 82 (3)358-363, 1992

32) Freedman DS, et al. Relation of body fatness to lipid and lipoprotein concentration in children and aldolescents : The Bogalusa Heart Study. *Am J Clin Nutr* Vol 50 : 930-939, 1989

33) Lenzen HJ. Genetic polymorphism in human apo E. *Methods Enzy* 128 : 823-851, 1986

34) Lee MS & Snook JT. Differential Effects of PUFA on the reverse cholesterol transport Mechanism According to the Apo E isoforms in Noripidemic Females. *Korean J of Lipidology* Vol.3 (2), p291, 1993

35) Rall SC, et al. identification of a new structural variant of human apo E_2 in type III hyperlipoproteinemic subject. *J Clin Invest* 72 : 1288-1297, 1983

36) Yamamoto A. Heredity Trait of Dyslipoproteinemia in patients with Atheroselerosis. *Korean J of lipidology* Vol3 (2), p289, 1993

37) Sing CF, Davingnon J. Role of apo E polymorphism is determining normal plasma lipid & lipoprotein variation. *Am J Hum Genet* 37 : 268-285, 1985

=국 문 초 록=

아포리포 단백질 E 유전자의 E2 와 E4 변이형이 정상 한국여성의 혈중 지질 수준 분포에 미치는 영향

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Apo E 유전자의 다형성은 인체 혈중 콜레스테롤 농도의 정상분포를 설명하는 중요한 인자이다. 특히, E2 와 E4 allele들은 고지혈증환자의 혈중에 현저히 나타나며, 각 다른 인종간의 이들의 상대빈도수가 관상 동맥 질환 유발 위험도와의 상관관계가 아주 높다. Apo E 유전자의 다형성이 혈중 지질 대사변화에 미치는 영향에 관한 실험은 105명의 건강한 한국여성을 대상으로 시행되었다. Apo E alleles의 한국인 상대빈도수는 E3가 0.848, E4가 0.087, 그리고 E2는 0.067이었다. 본 연구에서는 E2/2 표현형이 나타나지 않았지만, 중국 및 일본인과 같이 E4와 E2 allele가 아주 낮았다. 최고및 최저혈압, 맥박($p < 0.016$)에서 E2 allele를 갖는군이 E4 와 E3 allele 보다 높았는데, 이는 총 체지방 %가 E2 allele에서 증가한것과 연계적이다. 혈중의 Total-, total HDL-, VLDL+LDL-, LDL-, VLDL- cholesterol등은 apo E alleles간에 유의적이지 않았지만, E2 allele 군에서 HDL3 cholesterol)$p < 0.05$) 이 높은 반면, HDL2 cholesterol 은 유의적으로($p < 0.047$) 낮았다. 또한, TG(중성지방)은 E2 allele가 E3와 E4 alleles 보다 높게 나타났고, E4에서는 낮았다($p < 0.049$ among apo E alleles) 특히, 동맥질환의 위험인자 지수(AI)인 (TC-HDL)/HDL($p < 0.04$)과 HDL3/HDL2($p < 0.06$)등도 E2 allele에서 유의적으로 높았다. 결론적으로, 혈중 지질대사에서 E4 변이형에서는 LDL B/E or E receptors와의 결합능력 상승으로 인한 TG remnants의 혈중축적과 LDL수용체의 down- regulation에 따른 혈중 LDL cholesterol의 증가를 볼 수 있다. 둘째, E2 allele는 이론적으로 E4와 반대 현상이 나타나는데, 본 연구에서도 혈중 TG수준에서 현저하게 나타났다. 세째, 본 연구에서 Apo E2 allele가 E4 보다 관상동맥 질환 위험인자로 지적되었는데,(TC-HDL)/HDL 뿐만 아니라, 특히 HDL3/HDL2 비율이 높아서 지질대사의 혈중 지질 수준측정 인자로써 HDL fraction등이 고려되어야 하겠다.