

한국인 남자에 있어서 아포 E 지단백의 유전형 다형성이 관상동맥질환 발생 및 혈청지질에 미치는 영향

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= Abstract =

Influences of the Apolipoprotein E Polymorphism on the Development of Coronary Artery Disease and on Serum Lipids in the Korean Males

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Background : Apo E lipoprotein is polymorphic and exists in three common isoforms (E2, E3 and E4), which are the gene products of three apoE alleles, 2, 3 and 4. Apo E lipoprotein plays an important role in the regulation of the lipid metabolism through its ability to bind to receptors. Depending on the genotypes apo E polymorphism is either protective or increases risk for atherosclerosis and coronary artery disease. The purpose of this study is to evaluate i) the association between apo E allele and the development of coronary artery disease, ii) the association between apoE alleles and dyslipidemia in Korean males.

Methods : We studied 241 patients with angiographically verified coronary artery disease and 257 male subjects without evidence of coronary artery disease. Apo E genotyping was determined with the INNO-LiPA Apo E kit (Innogenetics, Belgium), which is based on reverse hybridization.

Results : There was a higher frequency of the apo 4 allele in subjects with coronary artery disease than in normal controls. The frequencies of apo E genotype were not significantly associated with the extents of coronary artery disease. Compared to the subjects with apo 3, the subjects with apo 2 were associated with higher levels of triglyceride and lower LDL, and the subjects with apo

4 had lower levels of HDL cholesterol.

Conclusion : ApoE polymorphism is a genetic marker for risk of the development of coronary artery disease and an important determinant of dyslipidemia.

KEY WORDS : Apo E polymorphism · Coronary artery disease · Lipid.

가

서 론

5). E

6). E

34. E (E)

2kD (VLDL) E

1). E

cDNA

2). 가

가

연구대상 및 방법

1.연구대상

50%

241

(reverse hybridiza -

tion)

E (VLDL),

(chylomicron), (chylomicron

remnants), (IDL)

(HDL)

LDL E

(LDL - receptor related protein, LRP) li -

gand

3). E 3

2, 3, 4가 3가 wild type

112 158 cysteine arg -

inine . 2 112 158

cysteine , 4 arginine .

2 4)

4

가

가,

2

4

cholesterol oxidase

glycerolphosphate oxidase

257

SPSS(Stati -

stical Package for Social Sciences)

Chi - square , t , ANOVA

p<0.05 .

2. 방 법

12

EDTA 4cc 4

20

dextran sulfate MgCl₂ 2
Hitachi 747(Hitachi Co.,
Japan)
400mg/dl Friedewald
equation Lp(a) Im -
munozyme Lp(a) kit(IMMUNO GmbH, Germany)
1 cell lysis buffer
DNA DNA phenol /chloro -
form PCR(polymerase chain
reaction) 10 µl amplification buffer 10 µl
E 5',3' - primer , 10 µl MgCl₂ solution, 10 µl
glycerol (50%), 1 unit Taq polymerase
DNA 0.1 0.5 µg 가
50 µl PCR 95
30 , 60 20 ,
72 20 30 DNA
1% agarose gel 10 µl load 100 140V
288bp band E
Reverse hybridization
line probe assay INNO - LiPA Apo E kit (Inno -
genetics, Belgium) trough
trough denaturation solution
amplified DNA 10 µl 20 25 5
50 hybridization solution 1ml
가 forceps strip 45
30
(Allele Specific Oligonucleotide ;
ASO)가 strip biotinylated DNA
1ml stringent wash solution
2 stringent wash solu -
tion 1 ml 45 10
alkaline
phosphatase labelled streptavidin 가
biotinylated hybrid
1 : 100 BCIP/NBT(Bromochlo -
roindolyl phosphate and nitroblue tetrazolium in
dimethylformamide) chromogen shaker 20
25 incubation /
1 probe 112
arginine, 2 probe 112 cysteine, 3

probe 158 arginine, 4 probe 158
cysteine

결 과

E 3/3 ;
70.1%, 4/3 ; 18.7%, 3/2 ; 8.2%, 4/4 ; 1.4%,
2/4 ; 1.0%, 2/2 ; 0.6%,
2 ; 0.052, 3 ; 0.835, 4 ; 0.112
(Table 1).

가 (Table 2).

가 (Table 3).

(Table 4).

(Table

5).

Table 1. Apolipoprotein E isoforms and allele frequencies(n=498)

Apo E genotype							
	2/2	2/3	2/4	3/3	3/4	4/4	Total
Number	3	41	5	349	93	7	498
(%)	(0.6)	(8.2)	(1.0)	(70.1)	(18.7)	(1.4)	(100)

Alleles				
	2	3	4	Total
Number	52	832	112	996
(%)	(5.2)	(83.5)	(11.2)	(100)

$$\epsilon 2=2 \times \epsilon 2/2 + \epsilon 2/3 + \epsilon 2/4 \quad \epsilon 3=2 \times \epsilon 3/3 + \epsilon 3/4 + \epsilon 3/2$$

$$\epsilon 4=2 \times \epsilon 4/4 + \epsilon 3/4 + \epsilon 2/4$$

Table 2. Relative allele frequencies among different populations

Normal		CAD	
Koreans		Americans ¹⁾	
n	257	5444	241
2	0.053	0.070	0.052
3	0.860	0.798	0.809
4	0.87	0.132	0.139
p-value*	0.095		0.720

1) : Ordovas et al, 1987⁴⁰⁾ 2) : Marshall et al, 1994⁴¹⁾

CAD : coronary artery disease

*Probabilities by Chi-square test

Table 3. ApoE genotype vs mean levels of clinical variables and presence of risk factors

	2/2(n=3)	2/3(n=41)	2/4(n=5)	3/3(n=349)	3/4(n=93)	4/4(n=7)	*P-value
Age(yrs)	50.3±1.5	55.2±1.3	58.2±7.0	52.6±0.5	53.1±8.8	53.6±2.7	0.401
BMI	20.2±1.5	21.1±1.9	21.4±1.0	20.4±0.1	20.4±0.2	19.9±0.6	0.376
Smoking (+)	0.8	8.1	1.6	68.2	19.8	1.5	
% (-)	0.4	8.4	0.4	71.8	17.6	1.4	0.785
DM (+)	0	8.5	0	67.8	22	1.7	
% (-)	0.7	8.1	1.2	71	17.6	1.4	0.881
HBP (+)	0	10.4	2.2	67.2	19.4	0.7	
% (-)	0.8	7.5	0.6	70.9	18.6	1.7	0.335

BMI : Body mass index(weight in kilograms divided by the square of height in meters), DM : Diabetes mellitus, HBP : Hypertension. Data are presented as mean±SE. *Probabilities for ANOVA.

Table 4. Apo E allele frequencies in normal control and coronary artery disease

	n	2	3	4	*P-value
Normal control	257	0.053	0.860	0.087	
CAD(+)	241	0.052	0.809	0.139	0.036
Normal control	257	0.053	0.860	0.087	
Angina	110	0.054	0.827	0.118	0.427
Normal control	257	0.053	0.860	0.087	
MI	131	0.049	0.794	0.156	0.015
Angina	110	0.054	0.827	0.118	
MI	131	0.049	0.794	0.156	0.476

CAD : coronary artery disease, MI : myocardial infarction.

*probability by Chi-square test

Table 5. Relation between ApoE genotype and extents of coronary artery disease

Number of significantly diseased vessels		
	Single vessel(0,1)	Multi-vessel(2, 3)
2(n)	11	9
3(n)	72	87
4(n)	33	24
*P-value	0.227	

$\epsilon_2 = \epsilon_2/2 + \epsilon_2/3$; $\epsilon_3 = 3/3$; $\epsilon_4 = \epsilon_3/4 + \epsilon_4/4$

*Probabilities by Chi-square test.

492

E

E Lp(a) 가

가

180.1±13.7mg/dl 3

145.9±4.2mg/dl (p=0.0

23) 2 116.8

±4.9mg/dl 3

가 가

가

12-13)

14).

2 pH

128.7±1.6mg/dl (p=0.029).

4 38.9±

0.8mg/dl 3 42.3±0.6mg/dl

(Table 6).

고 안

E 19

CI, CII, LDL receptor

3.6Kb 4 exon 3 intron

7).

E

8).

9-10).

E

E

11).

E

가 가

Table 6. Apo E genotype and mean lipid levels

	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Lp(a) (mg/dl)
2 (n= 44)	194.3 ± 5.5	180.1 ± 13.7	116.8 ± 4.9	42.2 ± 1.6	18.7 ± 3.0
3 (n=348)	199.9 ± 1.8	145.9 ± 4.2	128.7 ± 1.6	42.3 ± 0.6	23.4 ± 1.1
4 (n=100)	203.5 ± 4.6	156.7 ± 8.3	133.2 ± 4.6	38.9 ± 0.8	20.9 ± 2.1
p-value*	0.383	0.023	0.030	0.017	0.267

TC : total cholesterol, TG : triglycerides, LDL : low density lipoprotein cholesterol, HDL : high density lipoprotein cholesterol.

ϵ 2 = ϵ 2/2 + ϵ 2/3 ; ϵ 3 = ϵ 3/3 ; ϵ 4 = ϵ 3/4 + ϵ 4/4

Data are presented as mean ± SE.

*Probabilities by ANOVA.

가 . E 4 exon 가 , ,
E 80% , 112 158
E
, DNA 4 exon PCR 4 가
Allele Specific Oligonucleotide(ASO) 가 23 - 24)
,
E Hha I 가 E
가 , E2, E4 가 E3/4 가
25) 3/4
15 - 16) 40
(Reverse 4/4 가 16
hybridization) 4/4
oligonucleotide probe가 nitroc - 26 - 27) 가
cellulose strip 4 23) 4
band reading chart 28) 4 6
E
wild type 3 가 가 29)
. 4 E lipoprotein li -
17 - 18) pase
가 30) 4
50% 가 19) 가
2, 3, 4
5.3%, 86%, 8.7%
4가 가
가 . (atheromatous plaque)
E
E4/4 E3/4 20)
4 가 가 21) 가
1994 Framingham 22) E 8%, B 12%

Lp(a) 가

4

31 - 32)

2 3 E 3

2% 가 , 2 4 E

가 ,

요 약

연구배경 :

E

가

E

33 - 34)

3 2

방 법 :

4 50%

241 가 257

35) Framingham Study 2, 4

(>250mg/dl)

500mg/dl

DNA E

(reverse hybridization) INNO - Li -

PA Apo E kit(Innogenetics, Belgium)

결 과 :

4

E

36) Lenzen

37) Dallongeville

4/3 3/3

가

492 3

2

4

3

Lp(a) 2

24.8% 가 4

38)

39)

25.7% E

결 론 :

E

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