

한국인에서 Lipoprotein Lipase Gene Polymorphism의 빈도와 관동맥질환의 정도와의 관계분석

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Incidence of Lipoprotein Lipase Gene Polymorphism and Correlation with Severity of Coronary Artery Disease in Korean

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ABSTRACT

Background : Lipoprotein lipase (LPL) is a key enzyme in the metabolism of serum triglyceride (TG) which is utilized in the peripheral tissue as free fatty acid and stored in adipose tissue. LPL gene consists of 10 exons which encode 475 amino acids and more than 9 LPL gene polymorphisms have been reported. LPL gene polymorphism is related to lipids level and the severity of atherosclerosis in coronary artery disease. In Korea, LPL polymorphism has not been reported yet. The purpose of this study is to know the incidences of LPL gene polymorphism and it's relationship with blood lipids level and the severity of atherosclerosis. **Methods :** Subjects were divided into three groups ; normal controls (n=50), coronary artery disease (CAD, n=51) and cerebrovascular disease (CVD, n=52). The PCR-amplified genomic DNA from peripheral white blood cell was analyzed with restriction fragment length polymorphism (RFLP) by two different restriction enzymes (Pvu¹, Hind¹). **Results :** Total cholesterol (TC) was higher in CVD than in controls and CAD (203 ±60 mg/dl vs 188 ±37, 167 ±42, p<0.01). Triglyceride (TG) was also elevated in CAD (166 ±65 mg/dl vs 122 ±62 in controls, p<0.05). HDL cholesterol (HDL-C) was higher in controls than in CVD and CAD (49 ±9 mg/dl vs 36 ±10, 44 ±9, p<0.05). The incidence of Hind¹ RFLP and Pvu¹ RFLP was not different among groups. There was no correlation between LPL gene RFLP and lipid profile. There was no correlation between LPL gene RFLP and severity of coronary arterial stenosis. The incidence of Hind¹ RFLP (-/-) homozygotes was lower in Korean than in other country (5% vs 7-10%). The incidence of Pvu¹ RFLP (-/-) homozygotes was lower in Korean than in other country (10.3% vs 18-29%). **Conclusions :** The LPL gene mutations in intron 6 and 8 have no direct effects on the lipid profiles and the severity of coronary artery disease. Although LPL is a key enzyme in TG metabolism, two mutations in this study could not change the activity of LPL, nor were a marker linked to other site of mutation (s). The mutation (s) in exon which encode

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amino acid for enzyme activity should be detected to dissect the pathphysiologic mechanism in the atherogenesis. (Korean Circulation J 1999;29(1):6-13)

KEY WORDS : Lipoprotein lipase (LPL) · PL polymorphism · Coronary artery disease.

lipoprotein(a), apoprotein A - 1, B

서 론

LPL

glycoprotein (chylomi - cron) (VLDL)

3)

(lipoprotein) 475 encode 10 exon 30 Kb 9

Pvu intron 6 Hind intron 8 (Fig. 1).⁴⁻⁹⁾

(very low density lipoprotein, VLDL) (triglyceride, TG) 가

lipo - 가 (Table 1).

protein lipase(LPL)

VLDL 가 , 5 6 가 , 10 가

가

1)

(premature atherosclerosis)

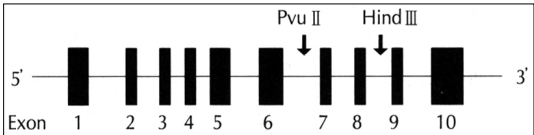


Fig. 1. Map of Lipoprotein lipase gene.

Table 1. Incidences of lipoprotein lipase gene polymorphism

Restriction site	Hind RFLP			Pvu RFLP		
	(- / -)	(- / +)	(+ / +)	(- / -)	(- / +)	(+ / +)
Gerdes ⁵⁾	7%	43%	50%	29%	49%	22%
Wang ¹⁰⁾	CAD (+)	11%	38%	51%	20%	51%
	CAD (-)	8%	38%	54%	23%	47%
Mattu ¹¹⁾	CAD (+)	7%	38%	55%	22%	47%
	CAD (-)	10%	49%	41%	14%	57%

CAD : coronary artery disease RFLP : restriction fragment length polymorphism

방포

LPL
Hind restriction fragment length poly -
morphism (RFLP) Pvu RFLP

	14	heparinized tube
	10 ml	1
coll	(buffy coat)	1500 rpm
15		- 20

대상 및 방법

대 상

1996	11	1997	6
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50%

51

5 7

1996	11	1997	6
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(brain CT)

(brain MRI)

52 .
1997 3 6

(complete

blood count),

50

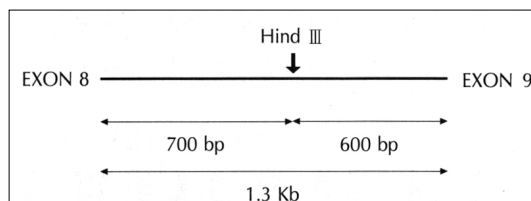


Fig. 2. Restriction site of Hind RFLP on intron 8.

(total ch -
 olesterol, TC) Liebermann - Buchardt ,
 heparin precipitate ,
 (triglyceride, TG) glycerol - 3 - phosphate ox -
 idase . (LDL -
 cholesterol) Friedwald formula

Genomic DNA(gDNA)
(buffy coat) QIAamp blood kit(Qiagen, USA)
gDNA
(Polymerase chain reaction, PCR)
- 70 . , 1.5
ml microfuge tube 200 μ L, protase K 25 μ L,
Buffer AL solution 200 μ L , 10 70
ethanol(100%) DNA
. QIAamp spin column attach
buffer(AW solution) 2 70
buffer(AE solution 50 μ L)

DNA

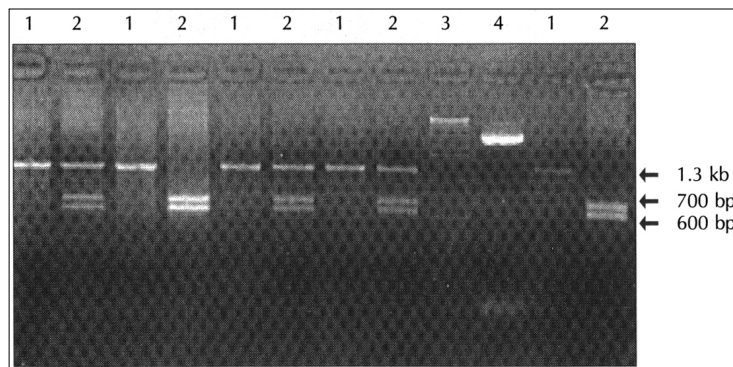


Fig. 3. Hind Restriction analysis of PCR product of intron 8 fragment (1. before enzyme digestion, 2. after enzyme digestion, 3. molecular weight marker, 4. positive control). First number 2 lane shows (- / +) heterozygote and second number 2 lane shows (+ / +) homozygote.

Hind primer (PCR)
gDNA Hind primer PCR
, primer .

Hind sense primer :
5' - TTAGGCCTGAAGTTTCCAC - 3'
Hind antisense primer :
5' - CTCCTAGAACAGAAGATC - 3'

PCR denaturation 95 1 , annealing 60 2 , extension 72 2
30 , Hind (restriction site)가 1.3 Kb intron 8 , 1.5% agarose gel (Fig. 2).

Restriction analysis of Hind PCR product
PCR product 5 µL Hind enzyme 1 µL
L Hind buffer solution 4 µL 37 2
(digestion) . Ethidium bromide
1.5% agarose gel PCR product enzyme digestion product (side by side)
UV transilluminator .

Fig. 3 PCR product Hind
, 1 PCR intron 8 1.3 kb
, 2 1 enzyme digestion . 1, 2 1.3 kb, 700 bp, 600 bp Hind 가 (- / +) heterozygote , 1, 2 700 bp, 600 bp가 (+ / +) homozygote .

Pvu primer
gDNA Pvu primers PCR
primer .

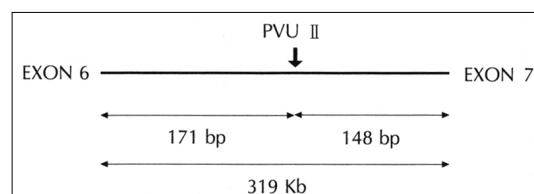


Fig. 4. Restriction site of Pvu RFLP on intron 6.

Pvu sense primer :
5' - AGGCTTCACTCATCCGTCCTCC - 3'
Pvu anti - sense primer :
5' - TTATGCTGCTTTAGACTCTTGTC - 3'

PCR denaturation 94 1 , annealing 62 2 , extension 72 2 30
, Pvu (restriction site)가 316bp intron 6 , 1.5% agarose gel (Fig. 4).

Restriction analysis of Pvu PCR product
PCR product 5 µL Pvu enzyme 1 µL
Pvu buffer solution 4 µL 37 2 (digestion) . Ethidium bromide
1.5% agarose gel PCR product enzyme digestion product (side by side)
UV transilluminator . Fig. 5 PCR product Pvu enzyme digestion . 2 PCR
319 bp intron 8 , 3 PCR product . 3
2 319 bp, 171 bp, 148 bp 가 (- / +) heterozygote , 3 1
319 bp 가 (- / -) homozygote .

Judkin's met -

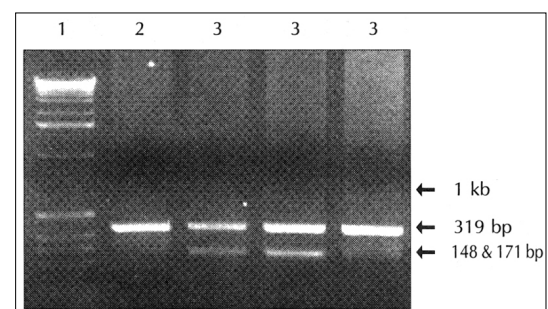


Fig. 5. Pvu Restriction analysis of PCR product of intron 6 fragment (1. molecular weight marker, 2. before enzyme digestion, 3. after enzyme digestion). First and second lanes of number 3 show (- / +) heterozygotes and third lane of number 3 shows (- / -) homozygote.

hod 가

50%

LPL

통계 및 분석

± (mean ± SEM)

Student t - test ANOVA ,

chi - square test SPSS for window 7.5

program , p 0.05

결 과

정상 대조군, 관동맥질환자 및 뇌혈관질환자와 혈중 지질치

(n=50), (n=51)

(n=52)

62 ± 8 , 62 ± 8 ,

64 ± 7 ,

(Table 2).

(203 ±

60 mg /dl vs 167 ± 42 mg/dl, p<0.01).

(166 ± 65 mg/dl vs 122 ± 62 mg/dl, p<0.05).

HDL - C

(49

± 9 mg/dl vs 36 ± 10 mg/dl, 44 ± 9 mg/dl, p<0.05).

LDL - C

(127 ± 51 mg/dl vs 103 ± 36 mg/dl, p<0.05,

Table 2).

LPL 유전자 다형성과 혈관질환의 관계

Hind RFLP (- / -) homozygote가 가

(7/151, 4.5%), (+ / +) homozygote

가 가 (100/151, 66.2%, Table 3).

Hind RFLP 가

Pvu RFLP (- / -) homozy -

gote가 가 Pvu RFLP

가 (Table 3).

Table 2. Comparisons of Lipid Profiles between Controls and Patients with Atherosclerosis

	Control	CAD	CVD
Number	50	51	52
Age (years)	62 ± 8	62 ± 8	64 ± 7
Sex (M/F)	24/26	33/18	31/21
TG (mg/dl)	122 ± 62	142 ± 106	166 ± 65*
TC (mg/dl)	188 ± 37	167 ± 42	203 ± 60**
LDL-C (mg/dl)	115 ± 35	103 ± 36	127 ± 51*
HDL-C (mg/dl)	49 ± 9	36 ± 10*	44 ± 9*

* p<0.05 compared with controls, + p<0.05 compared with CAD Data are mean ± SEM

CAD : coronary artery disease, CVD : cerebrovascular disease, TG : triglyceride, TC : total cholesterol, LDL-C : low density lipoprotein-cholesterol, HDL-C : high density lipoprotein-cholesterol

Table 3. Incidence of lipoprotein lipase gene RFLP among groups

	Normal (n=50)	CAD (n=51)	CVD (n=52)
Hind restriction site			
Number	50	51	50
(- / -)(n=7)	1 (2%)	2 (4%)	4 (8%)
(- / +)(n=44)	14 (28%)	16 (31%)	14 (28%)
(+ / +)(n=100)	35 (70%)	33 (65%)	32 (64%)
Pvu restriction site			
Number	48	51	35
(- / -)(n=14)	5 (8%)	7 (14%)	2 (6%)
(- / +)(n=59)	18 (31%)	23 (45%)	18 (51%)
(+ / +)(n=71)	35 (60%)	21 (41%)	15 (43%)

CAD : coronary artery disease, CVD : cerebrovascular disease

Table 4. Comparisons of Lipid Profiles with Hind RFLP

Hind restriction	(- / -)	(- / +)	(+ / +)
Number	7	44	100
Age	70 ± 5	63 ± 8	62 ± 8
Sex (M/F)	4/3	23/21	60/40
TG (mg/dl)	152 ± 55	135 ± 85	146 ± 83
TC (mg/dl)	197 ± 41	183 ± 45	186 ± 52
LDL-C (mg/dl)	115 ± 34	113 ± 36	115 ± 45
HDL-C (mg/dl)	47.3 ± 10.4	43.6 ± 10.6	42.0 ± 10.4
Normal Inc.	4/7 (57%)	24/44 (32%)	32/100 (34%)
CAD Inc.	1/7 (14%)	14/44 (32%)	35/100 (32%)
CVD Inc.	2/7 (29%)	16/44 (36%)	33/100 (34%)

Data are mean ± SEM

CAD : coronary artery disease, CVD : cerebrovascular disease, TG : triglyceride, TC : total cholesterol, LDL-C : low density lipoprotein-cholesterol, HDL-C : high density lipoprotein-cholesterol, Inc. : incidences

Table 5. Comparisons of Lipid Profiles with Pvu RFLP

Pvu restriction	(- / -)	(- / +)	(+ / +)
Number	14	59	71
Age	60 ± 8	62 ± 8	63 ± 7
Sex (M/F)	6/8	35/24	36/27
TG (mg/dl)	128 ± 56	130 ± 62	157 ± 104
TC (mg/dl)	192 ± 41	180 ± 41	186 ± 52
LDL-C (mg/dl)	120 ± 32	111 ± 34	114 ± 47
HDL-C (mg/dl)	46.3 ± 11.9	43.4 ± 10.5	40.8 ± 10.2
Normal Inc.	5/14 (36%)	18/59 (31%)	35/71 (49%)
CAD Inc.	7/14 (50%)	23/59 (39%)	21/71 (30%)
CVD Inc.	2/14 (14%)	18/59 (31%)	15/71 (21%)

Data are mean \pm SEM

CAD : coronary artery disease, CVD : cerebrovascular disease, TG : triglyceride, TC : total cholesterol, LDL-C : low density lipoprotein-cholesterol, HDL-C : high density lipoprotein-cholesterol, Inc. : incidence

Table 6. Comparisons of LPL Polymorphism and Severity of Coronary Arterial Stenosis

Stenosis of coronary artery		1 vessel	more than 2 vessel
Hind	(- / -)	1	1
	(- / +)	9	7
	(+ / +)	19	14
Pvu	(- / -)	3	4
	(- / +)	15	8
	(+ / +)	11	10

LPL 유전자 다형성과 혈중 지질치의 관계

Hind RFLP (-/-) HDL -
C 가 (47.3 ±
10.4 mg/dl vs 43.6 ± 10.6 mg/dl, 42.0 ± 10.4 mg/dl,
(-/-) momozygote vs (-/+) heterozygote, (+/+) homozygote, respectively, p>0.05, Table 4).

Pvu RFLP (+/+) TG γ
, (-/-)
HDL-C, (Ta-
ble 5).

LPL 유전자 다형성과 관동맥질환 정도와의 관계

Pvu RFLP Hind RFLP

(Table 6).

고찰

가

가 가

Lipoprotein lipase(LPL)
glycoprotein

가 . LPL
(chylomicron)
(VLDL)

3) (lipoprotein)

. . . LPL

LDL.

VLDL particle

13)

LPL

LPL 8p22 . 475

30 Kb

encode 10 exon

,¹²⁾ exon intron

9

. Exon 6 Cys239 stop ,⁴⁾ exon 6

Asn291 - Ser, ¹⁴⁾ intron 6 Pvu
restriction fragment length polymorphism(RFLP),¹⁵⁾

intron 8	Hind	RFLP, ¹⁶⁾	Exon 9	Ser
447	Ter	¹⁷⁾		

LPL

5)

Hind RFLP
Mattu 11)

Thorn ¹⁸⁾ Hind (+)

19) Wang

intron 6 LPL Hind RFLP가 lipoprotein(a), apolipo - protein가 HDL - C가

결론

LPL, Wang¹⁰⁾ Pvu RF - LP Pvu (-/-) Pvu (+/+)가 LPL Pvu RFLP Hind RFLP apoprotein, HDL - C, TG¹⁰⁾ LPL Pvu RFLP intron 6 RFLP intron Hind RFLP가 LPL가 LPL가 LPL LDL, VLDL part - icle¹³⁾ Hind & Pvu RFLP⁵⁾¹⁰⁾¹¹⁾ Table 1 LPL Gerdes⁵⁾ HDL - C Hi - nd (-/-)가 Hind (+/+) HDL - C가 Hind (+/+)가 Heizmann²⁰⁾ Hind (-/-) HDL - C Hind RFLP^{10)14)18)19)21 - 23)} HDL - C가 Hind가 Pvu RFLP가

중심 단어 : Lipoprotein lipase(LPL) · LPL

감사문

1997

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