Linkage Analysis between Apo AI-CIII-AIV Cluster and Plasma Lipid Levels in Cardiovascular Disease Family

Thesis by

Eun Kyoung Im

Brain Korea 21 Project for Medical Sciences

The Graduate School of Yonsei University

Linkage Analysis between Apo AI-CIII-AIV Cluster and Plasma Lipid Levels in Cardiovascular Disease Family

Directed by Professor Yangsoo Jang

A Dissertation Submitted to
the Graduate School of Yonsei University

December, 2001

by

Eun Kyoung Im

Brain Korea 21 Project for Medical Sciences
The Graduate School of Yonsei University

A Dissertation for the Degree of Master in Medical Sciences by Eun kyoung Im has been approved by

(Supervisory committee, Chairman)

(Supervisory committee)

(Supervisory committee)

Brain Korea 21 Project for Medical Sciences
The Graduate School of Yonsei University

December, 2001

감사의 글

논문이라는 작은 결실 앞에서 홀가분한 마음보다는 아쉬움이 앞서는 걸 보면 2년의 시간이 저에게 결코 길지 만은 않은 시간이었던 것 같습니다. 이 논문을 완성하면서 작은 지면을 빌어 부족하나마 감사의 마음을 전하고자 합니다

먼저 이 논문을 완성하기까지 지도와 격려를 아끼지 않으셨던 장양수 교수님께 깊은 감사를 드립니다. 그리고 지난 2년 동안 늘 가까이에서 자상한 가르침과 조언을 해주신 박현영 교수님께도 진심으로 감사를 드립니다. 또한 바쁘신 와중에도 논문이 완성될 수 있도록 자문을 아끼지 않으시고 이해의 폭을 넓혀주신 지선하 교수님, 이경률 교수님께 깊은 감사를 드립니다. 항상 인자한 모습으로 격려해주신 정광회 교수님, 양현원 교수님과 황기철 교수님께 감사 드립니다. 본 연구를 진행함에 있어 마지막까지 지도와 도움을 주신 조은영 선생님, 따뜻한 웃음으로 격려해주신 정지형 선생님께도 지면을 빌어 감사의 마음을 전합니다. 그리고 학문의 필요성을 일깨워주시고 언제나 격려와 믿음으로 지켜봐 주신 이상섭 교수님께 감사의 마음과 더불어 자주 찾아 뵙지 못한 죄송한 마음이 앞섭니다.

지난 2년 동안 여러 가지로 부족한 저에게 자상한 가르침과 도움을 준 현정언니, 밝은 미소로 늘 격려를 아끼지 않으신 손영덕 선생님, 서정철 선생님께 감사의 마음을 전합니다 오늘을 같이하지는 못했지만 힘든 대학원 생활동안 항상 밝은 마음으로 활력소가 되어주었던 지은이, 늘 즐겁고 바쁘게 사는 모습이 예쁜 진우, 언제나 밝은 웃음으로 열심히 던 보희,수진, 경희, 명랑하고 귀여운 세아, 가까이에서 도움과 격려를 아끼지 않던 경혜, 그리고 실험이 원활히 될 수 있도록 애써준 식품영양학과 친구들에게도 고마운 마음을 전합니다. 실험실 생활의 여러 가지 면에서 쉼 없는 도움과 배려로 애써준 민영, 어리지만 든든하고 미더운 해용에게 이제서야 고마운 마음을 전합니다. 그리고 누구보다도 늘 옆에 있으면서 우리라는 힘이 되

어주고 세심한 배려로 지켜봐 준 준혜에게 진심으로 고맙다는 말을 하고 싶습니다.

10년 동안 곁에서 변함없는 마음으로 지켜봐 주고 힘들어 할 때마다 내 일처럼 나서주고 힘이 되어준 나보다 더 소중한 친구 기연이, 언제나 격려를 아끼지 않던 늘 행복한 웃음이 보기 좋은 복경이, 언니처럼 자상한 배려로 애써준 현주에게도 고마운 마음을 전합니다.

손녀의 늦은 귀가를 늘 걱정해주셨던 할머니, 바쁘다는 핑계로 서운하게 했던 동생을 이해해주고 격려해준 언니들과 형부에게 미안한 마음과 함께 깊은 감사를 드립니다. 그리고 철없는 언니의 짜증까지도 웃음으로 받아준 은진이, 은혜에게 고맙다는 말을 전합니다.

마지막으로 대학원 생활의 굴곡을 늘 묵묵히 지켜봐 주시고 믿음으로 일관해주신 부모님의 소중한 사랑에 머리 숙여 깊이 감사 드리며 이 작은 결실을 드리고자 합니다.

감사합니다.

2001년 12월

임 은 경

CONTENTS

List of Figures

List of Tables

ABSTRACT

Linkage Analysis between Apo AI-CIII-AIV Cluster
and Plasma Lipid Levels in Cardiovascular Disease Family

Eun Kyoung Im

Brain Korea 21 Project for Medical Sciences
The Graduate School of Yonsei University

(Directed by Professor Yangsoo Jang)

Background Cardiovascular disease (CVD) is a complex disorder, which the genetic contributions have significant roles with environmental factors. In the many studies, increased plasma lipid levels except HDL-cholesterol were directly correlated with cardiovascular risk. The aim of this study was to determine whether Apo AI-CIII-AIV gene cluster region (chromosome 11q23) contains specific loci that affect plasma lipid concentration or not.

Methods and Results The 701 individuals from 93 CVD families were recruited. We collected genealogical informations for all participants and measured plasma lipid levels. Segregation analysis showed that the LDL-cholesterol follows the codominant Mendelian model with 61.2% of the variation and the equal transmission model. For HDL-cholesterol, the segregation analysis showed a dominant Mendelian model accounted for 51.8% of the variation. For linkage analysis, we selected 30 families (305

individuals) those have a relatively large number of subjects and multi-generation comparatively. In the analysis for the HDL-cholesterol, none of genetic markers at Apo AI-CIII-AIV gene cluster showed LOD score as evidence of linkage. In the analysis for the LDL-cholesterol, the highest LOD score was found at D11S912 of 130.9 cM (LOD score =1.18, p=0.099). To confirm the linkage between a putative gene that affects LDL-cholesterol level and D11S912, we reanalyzed the linkage with 21 families that favored a codominant Mendelian model, but there was no significant difference in LOD score.

Conclusion The segregation analysis showed both LDL-cholesterol and HDL-cholesterol are under the genetic influences, following the Mendelian model. However, HDL-cholesterol showed no linkage to the Apo AI-CIII-AIV gene cluster, indicating the major gene effect influence in HDL-cholesterol is not explained by these loci. For LDL-cholesterol, we found the tentative linked loci at 11q23.

Key words: segregation analysis**,** linkage analysis, plasma cholesterol, cardiovascular disease, Apo AI-CIII-AIV gene cluster,

Linkage Analysis between Apo AI-CIII-AIV Cluster and Plasma Lipid Levels in Cardiovascular Disease Family

Eun Kyoung Im
Brain Korea 21 Project for Medical Sciences
The Graduate School of Yonsei University

1. INTRODUCTION

Cardiovascular disease (CVD) is one of the major causes of morbidity and mortality in most developed countries. CVD is a multifactorial disease influenced by a large number of environmental and hereditary factors; neither the environment alone nor a single gene cause disease. Plasma lipoprotein levels have been known to have a major role in progressing of atherosclerosis. In the many studies, increased plasma low density lipoprotein-cholesterol (LDL-cholesterol), total cholesterol (TC) and triglyceride (TG) levels are directly correlated with cardiovascular risk, but high density lipoprotein (HDL-cholesterol) level is inversely correlated with cardiovascular risk[1,2,3,4]. The major factors which determining the LDL-cholesterol concentration are

obesity and fat absorption. Recently, family and twin study have shown to have genetic effect for ~50% of the interindividual variations in plasma LDL-cholesterol concentrations[5,6]. Many genetic factors involved in lipid abnormalities have been identified mostly for monogenic diseases, also, these accounts only for a partial cause of coronary artery disease. For elucidation of genetic effect on lipid levels, association studies using genetic variations within candidate genes have been performed[7,8,9]. However, the candidate gene approach has limitations to understand the individual differences in lipid levels. The Apo AI-CIII-AIV gene cluster have been suggested as candidate for determining genetic factors in plasma lipid level in many studies[10,11,12]. Apo AI, Apo CIII and Apo AIV are clustered within a 15kb DNA segment on the chromosome 11q23 and the several genetic markers in this gene cluster were reported to be associated with familial combined hyperlipidemia (FCHL). The rare allele (X^+) of the *Xmn*I polymorphism 5' of the Apo AI gene has been reported to be associated with FCHL[13]. The *Sst*I polymorphism in the 3' non-coding region of the Apo CIII gene was related with hypertriglyceridemia[14,15]. These findings strongly suggested that the Apo AI-CIII-AIV gene cluster has an effect on lipid levels.

The purpose of the present study was to determine a segregation model of lipid profiles and to elucidate the effect of Apo AI-CIII-AIV gene cluster on individual variation for plasma lipid levels in Korean CVD families.

II. MATERIALS AND METHODS

1. Subjects

The 701 individuals from 93 families who visited the cardiovascular genome center were recruited with written informed contents. All probands had one of the cardiovascular diseases; angiographically confirmed angina pectoris, unstable angina, myocardial infarction and hypertension. Genealogical information has been collected and pedigrees for family were constructed for genetic study. Figure 1 shows an example of pedigree used in this study. Demographic data including current age, sex, history of hypertension, diabetes mellitus (DM), smoking, drinking, cardiovascular events, life style variables were collected at the time of enrolment. LDL-cholesterol, HDL-cholesterol and TG were measured. Blood samples for DNA analyses have also been collected. For linkage analysis, we selected 30 families (305 individuals) those have a relatively large family size and multigeneration comparatively. Unrelated 61 men who had no evidence of coronary artery disease in the medical history were selected to determine the informativeness of genetic markers in Korean. Table 1 shows heterozygosity of markers obtained in the present study with reference data from CEPH (Centre d' Etude du polymorphisme Humain) database.

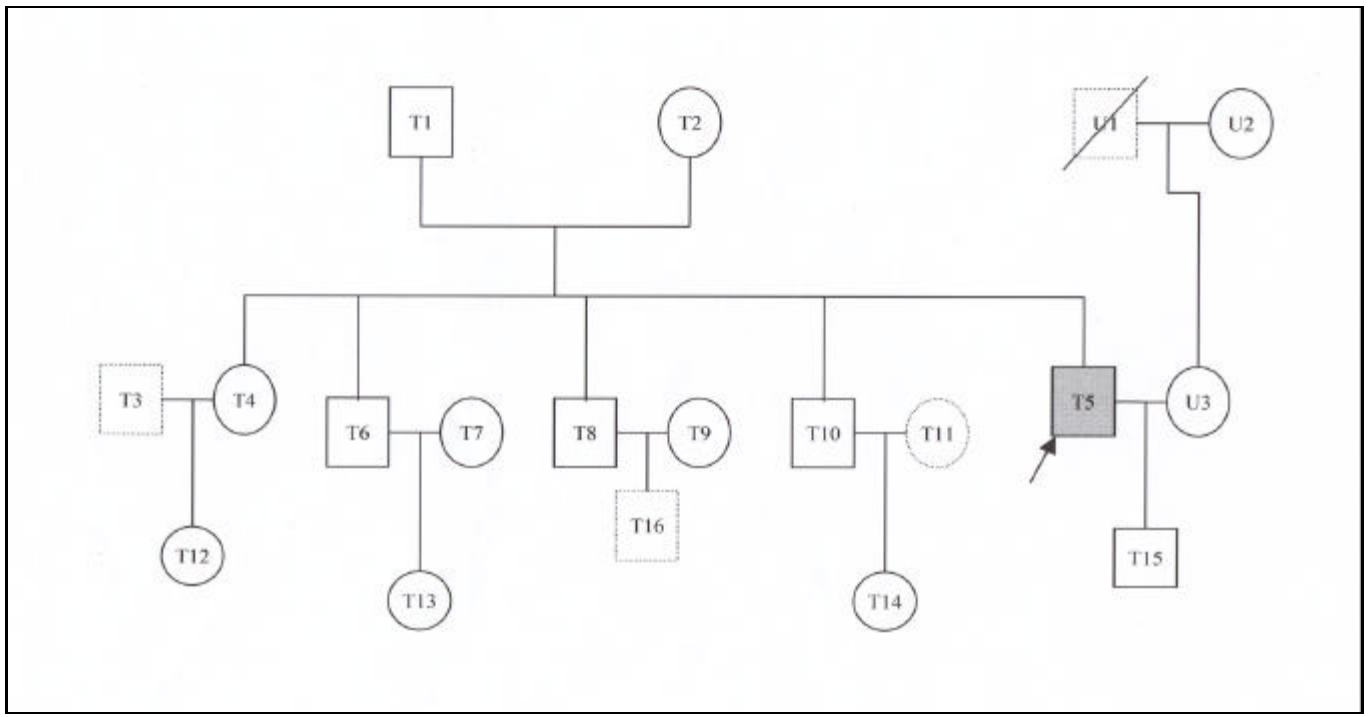


Figure 1. An example of pedigree used in the study. Black symbol (an arrow points to it) indicates a proband and other symbols indicate relative individuals of the proband. The diagrams described by dot and slant line mean the missing samples.

Table 1. Observed heterozygosity for microsatellite markers in the Korean population.

markers (locus)	heterozygosity (%)	
	observed	CEPH*
D11S1338	63.9	78.6
D11S4191	96.7	78.6
D11S908	42.6	77.8
D11S4127	73.8	85.7
D11S925	83.6	85.2
D11S4094	77.0	71.4
D11S4151	55.7	64.3
D11S912	82.0	74.8
D11S4126	47.5	64.3
D11S1320	57.4	55.6
D11S968	45.9	82.1

*Centre d' Etude du polymorphisme Humain (CEPH); genotype database

2. Measurement

All subjects were asked to fast for 12 hours before their visit. Blood samples were centrifuged at 3000g for 10 minutes at 4℃ and then stored at -70℃ before measurement. Plasma TC, LDL-cholesterol and TG levels were measured by enzymatic assay on the Hitachi Autoanalyzer 7170 (Hitachi Ltd. Tokyo. Japan). HDL-cholesterol was measured in the supernatant after precipitation of other lipoprotein fractions by enzymatic method. Intraassay variation was less than 3 % for TC and TG, and less than 5% for HDL-cholesterol. Plasma LDL-cholesterol concentration was calculated by subtracting the HDL-cholesterol concentration and TG from TC. Plasma Apo B and Apo AI were determined by immunoturbidimetric assay[16].

3. Segregation analysis

Statistical analysis was performed with the variance components method for quantitative traits[17]. Analysis was done with the unified mixed model. These models were assumed that variation among individuals for a quantitative trait is the result of a major gene effect and residual variation that may influence both individual variation and familial correlations[18]. A general model emanates from that the major effect is assumed to result from the segregation at a single locus with 2 alleles (L and H), resulting in three possible types of

individuals (LL, LH, HH). The mean LDL-cholesterol and HDL-cholesterol level value associated with each type is indicated μLL, μLH and μHH, with a variance σ^2 assumed equal among all three types. We defined that H allele has high trait value. In addition, τLL, τLH and τHH are the genotype-specific probabilities to transmit allele L from parents to offspring. Under the Mendelian assumption, these probabilities are equal to 1.0, 0.5 and 0.0, respectively. The model gave interindividual correlations in 3 familial classes; spouse (sp), parent-offspring (po), sib-sib correlations (ss). In this study, we tested by using regressive models described by Bonney[19], as implemented in the SAGE (statistical analysis for genetic epidemiology) program (release 3.1). The SAGE program fitted the model directly to the family data under the assumption that founder individuals come from a population in Hardy-Weinberg equilibrium. First, the certain parameters were restricted with several specific values, a probability of correlation between interindividual (sp, po, ss). These restricted models were compared with the general model by using the likelihood ratio test, which is the difference in minus twice the log-likelihoods (-2In L) obtained under the two different models. Mendelian models constrain these segregation probabilities τLL, τLH and τHH to be 1.0, 0.5 and 0.0 respectively. One of the Mendelian model was a `codominant' model that each type has a separate mean and `recessive' model restricts to the same value of μLH and μHH. On the contrary, a `dominant' models restrict to the same value of μLL and μLH.

Equal transmission models are that the prior probability of an offspring having a certain type is completely independent of parental types. In the likelihood ratio test, Akaike's Information Criterion (AIC) was calculated. These values were used to decide the fit of unfixed models. The one with the smallest value is the most parsimonious model[20]. We performed a segregation analysis for LDL-cholesterol adjusted for age, sex, alcohol drinking status, cigarette smoking status, exercise, body mass index (BMI), lipid lowering drug used and DM in 93 families (701 individuals) and 30 selected families (305 individuals) respectively. For HDL-cholesterol, the values were adjusted to additional parameters, body fat and TG.

4. Genotyping

A genomic scan to detect loci related to lipid concentration has been conducted in 305 individual in 30 extended pedigrees. We selected eleven polymorphic microsatellite markers on chromosome 11. Used markers were eight microsatellite markers approximately equally spaced every 5 cM adjacent to ApoAI-CIII-AIV gene cluster on the chromosome 11 and two microsatellite markers included gap of 46cM on the chromosome 11. The locations of genetic markers are indicated in Figure 2. Informations about the primers of markers used for genotyping, heterozygosity, allele size and sequences were obtained from CEPH database (http://www.cephb.fr/cephdb).

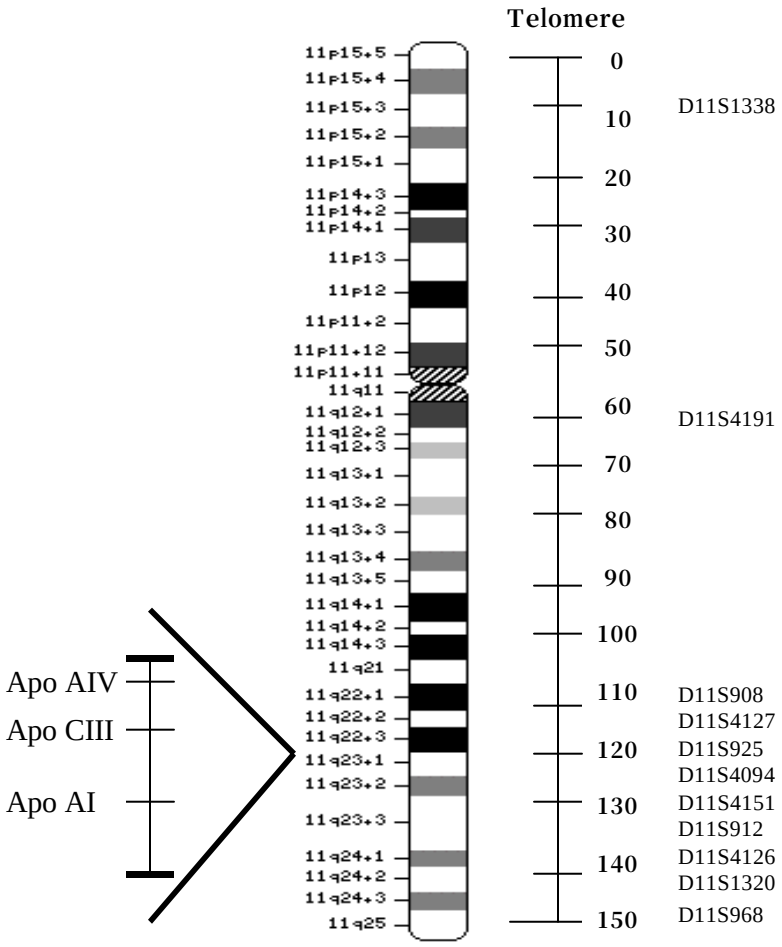


Figure 2. Ideogram of human chromosome 11. Markers are listed to the right, with their respective chromosomal locations. Apo AI-CIII-AIV gene cluster are indicated to the left. Apo AI-CIII-AIV gene cluster was located between the marker D11S925 and D11S4094

Genomic DNA was isolated from 500 μℓ of whole blood samples with a Wizard® Genomic DNA Purification kit (Promega, Madison, USA). PCR was performed in a final volume of 15 μℓ, which contained 60 ng of genomic DNA, 1× GeneAmp PCR Buffer II, 0.75mM dNTP, 2.5 mM $MgCl_2$, and 0.6 U of AmpliTaq Gold ™ (Perkin-Elmer, Norwalk, CT). Each tube contained fluorescent labeled primers combined at a 10 mM primer mix. Amplification was performed with a PTC-200™ Peltier Thermal Cycler (MJ Research, Watertown, MA). Samples were held for 12 minutes at 95°C and performed as followed by 10 cycles of 15 seconds at 94°C, 15 seconds at 55°C, and 30 seconds at 72°C and then cycled through as followed by 20 times of 15 seconds at 89°C, 15 seconds at 55°C, and 30 seconds at 72°C. The final step was performed for 10 minutes at 72°C. An example of the fragment size obtained after PCR is shown in Figure 3. After PCR amplification, we pooled PCR products according to panel-set category. The pooled DNA fragments was run under denaturing conditions on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and analyzed with the GENESCAN version 3.1 (Figure 4). The genotype was determined by GENOTYPER version 2.0 software (Applied Biosystems) (Figure 5).

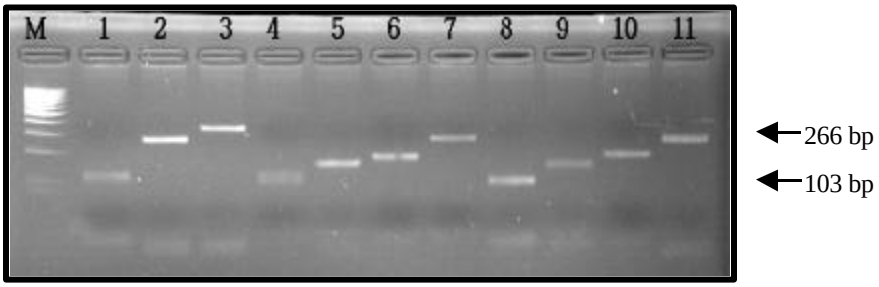


Figure 3. The amplified DNA fragments using primers of genetic markers. M : DNA size marker(ΦX174DNA/*Bsu*RI(*Hae*III) Marker (MBI Fermentas, Vilnius, Lithuania)). Labeled numbers; 1:D11S912, 2:D11S1320, 3: D11S4151, 4: D11S4191, 5: D11S968, 6: D11S908, 7: D11S1338, 8: D11S4127, 9: D11S4126, 10: D11S4094, 11: D11S925.

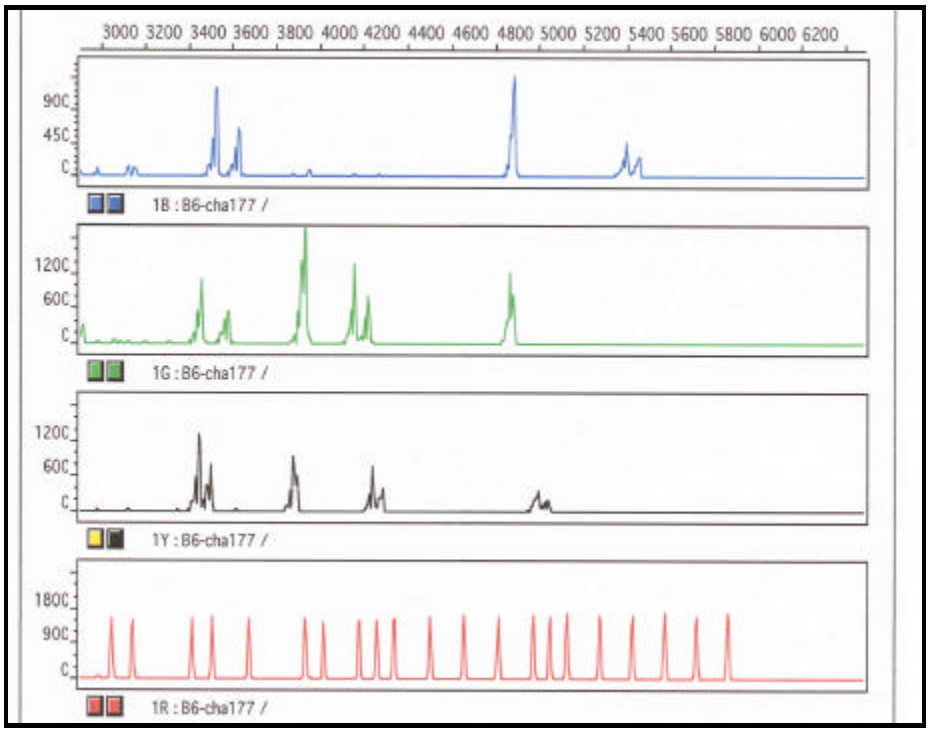


Figure 4. GeneScan electropherogram of ABI PRISM Linkage Mapping Sets. Dinucleotide repeat loci are labeled with three dyes (FAM/blue color, HEX/green color, NED/black color), except red color, which is used for the size standard. DNA fragments of one individual run under denaturing conditions on the ABI PRISM 310 Genetic Analyzer using POP-4 polymer at 60℃

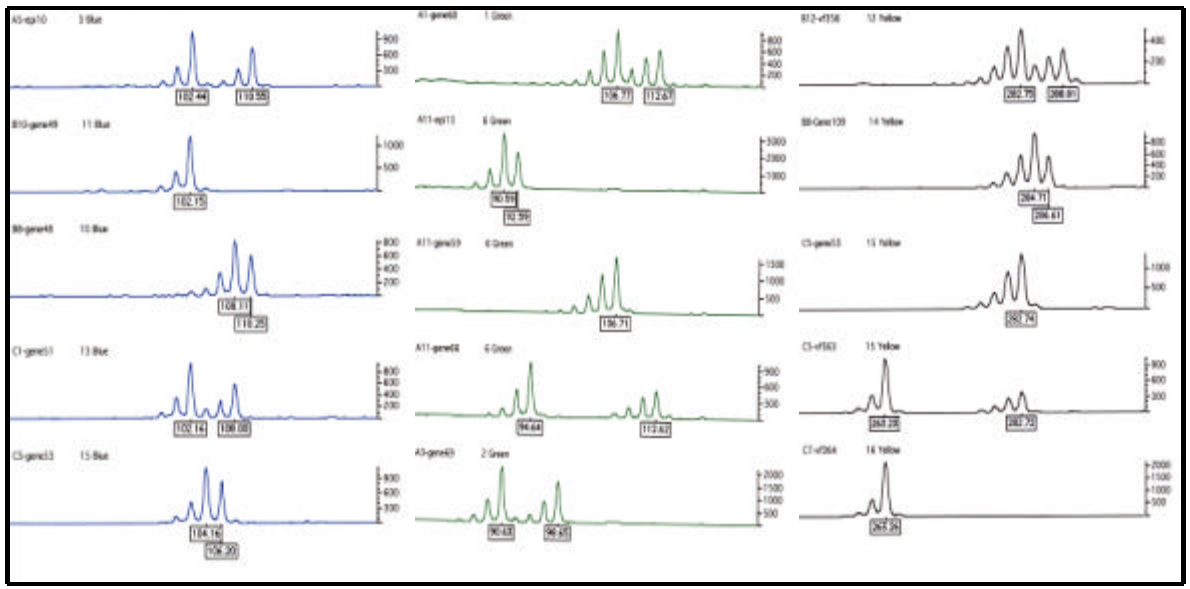


Figure 5. An example of Genotyping using Genotyper 2.0 electropherogram plot. The genotype of five individuals from the CVD families amplified with microsatellite markers. Vertical range shows the fluorescence intensities of labeled dye signal and horizontal range shows the data point of microsatellite markers. Calling of each peak indicated the size of amplified DNA fragment

5. Linkage analysis

LOD score was a likelihood (model)-based parametric linkage approach to estimate the recombination fraction and significance of the evidence for linkage. The score was computed by using LODLINK program version 3.1 within the SAGE package. The linkage analyses were calculated in a Mendelian model of inheritance for LDL-cholesterol and HDL-cholesterol, respectively. To assess the cosegregation of the eleven markers on the near Apo AI-CIII-AIV gene cluster with a putative gene that affects lipid levels, these markers reflecting a several region, around the Apo AI-CIII-AIV gene cluster were tested for linkage individually. The genetic-map construction was based on the Marshfield linkage maps[21], these baseline data were consistent with the maps used other linkage studies[22,23,24]. LOD score was calculated by the likelihood ratio test for linkage by $2\log_e(10)$ on 388 individuals of 30 families, although 83 individuals had missing data. The LOD score was represented $\log_{10}$ of the ratio for two likelihoods, the likelihood of observing a particular configuration of a trait and a marker locus in a set of families assuming linkage (θ <0.5), and the likelihood of observing the same configuration of the two loci within the same set of families assuming linkage ($\theta = 0.5$)[25]. We have used a LOD score of ≥ 3.00 to indicate adequate evidence of linkage and a LOD score of ≥ 2.00 as suggestive[26]. We assigned a LOD score of ≥ 1.00 as tentative evidence of linkage.

III. RESULTS

1. Study Population

The clinical characteristics of 701 individuals in 93 families were shown in Table 2. Individuals of families were recruited from 74 probands with coronary artery disease and 19 probands with hypertension. 27 individuals of subjects with coronary artery disease were diagnosed as hypertension at a time. The mean age of probands and remaining relatives was 55.2 years and 35.2 years respectively. In both groups, there were no significant differences in BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP). The frequencies of family according to family size are exhibited in Figure 6. The sizes of pedigrees range from 3 to 29, representing complex at over two generations.

2. Segregation Analysis

For LDL-cholesterol, data were divided into two different models had the lowest AIC. One is a codominant Mendelian model that has effect of major gene in lipid levels and the other is an equal transmission model that shows a superiority of an environmental factor (Table 3). The inferred major gene in this codominant Mendelian model accounted for 61.2% of the variation in LDL-cholesterol. Estimated mean LDL-cholesterol levels for the three supposed

Table 2. Clinical characteristics of study population

Characteristics	Probands (n= 93) Mean ± SD	Remaining relatives (n= 608) Mean ± SD	Total (n= 701) Mean ± SD
Age (years)	55.2±9.2	35.2±16.4	38.5±17.1
Sex (% male)	76.1	43.4	47.7
BMI (kg/m^2)	24.8±2.4	22.1±3.3	22.5±3.4
Current Drinking (%)	38.9	28.0	29.9
Current Smoking (%)	22.5	24.3	24.0
SBP (mmHg)	124.3±18.0	117.5±16.7	118.6±17.1
DBP (mmHg)	78.7±9.9	71.5±12.2	72.7±12.1
DM (%)	12.1	0.85	2.4
HDL-cholesterol (mg/dl)	51.0±10.3	60.1±14.2	58.6±14.0
LDL-cholesterol (mg/dl)	122.2±33.3	116.0±35.1	116.9±34.9
TG (mg/dl)	171.8±89.2	123.1±94.9	130.8±95.6
TC (mg/dl)	206.3±38.4	200.0±39.3	201.0±7.9
Apo AI (mg/dl)	126.2±22.8	133.9±27.6	132.6±27.0
Apo B (mg/dl)	92.2±21.8	82.1±24.3	83.8±24.2

values are mean ± SD.

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, DM; diabetes mellitus, HDL-cholesterol; high density lipoprotein cholesterol, LDL-cholesterol; low density lipoprotein cholesterol, TG; triglyceride, TC; total cholesterol, Apo AI; apolipoprotein AI, Apo B; apolipoprotein B

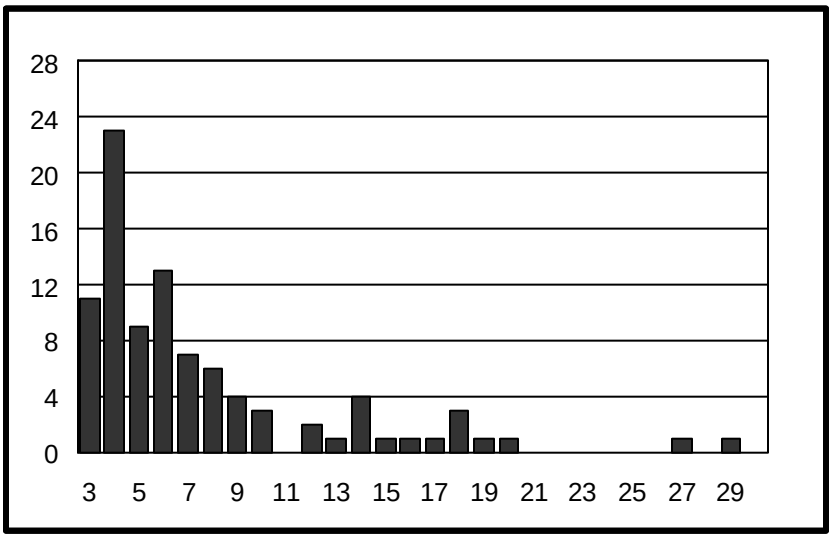


Figure 6. The frequencies of family according to family size. Vertical range shows the number of families and horizontal range shows the constituent of families.

Table 3. Segregation analysis of adjusted LDL-cholesterol* in 701 individuals of 93 families ascertained to the cardiovascular disease

	q_L	μ_{LL} (s.d)	μ_{LH} (s.d)	μ_{HH} (s.d)	σ^2 (s.d)	τ_{LL}	τ_{LH}	τ_{HH}	ρ_{sp}	ρ_{po}	ρ_{ss}	-2LnL	X^2	df	*P*	AIC
1. Sporadic	[1]	115.7 (1.3)			953.6 (57.7)				[0]	[0]	[0]	5295.2	45.3	8	<0.001	5299.2
2. Familial correlation																
a. Unimodel, sp	[1]	115.7 (1.3)			953.6 (57.7)				0.01	[0]	[0]	5295.1	45.2	7	<0.001	5301.1
b. Unimodel, sp, po	[1]	115.7 (1.5)			949.5 (58.5)				0.08	0.17	[0]	5280.1	30.2	6	<0.001	5288.1
c. Unimodel, sp, po, ss	[1]	115.7 (1.7)			959.0 (63.1)				0.01	0.22	0.31	5257.4	7.5	5	>0.1	5267.4
3. Mendelian																
a. Dominant	0.84	114.3 (2.3)	114.3 (2.3)	169.3 (28.6)	889.3 (82.6)	[1]	[0.5]	[0]	0.05	0.24	0.34	5255.1	5.2	3	>0.1	5269.1
b. recessive	0.89	108.9 (6.0)	142.1 (10.3)	142.1 (10.3)	774.4 (129.1)	[1]	[0.5]	[0]	0.03	0.16	0.24	5254.5	4.7	3	>0.1	5268.5
c. Codominant	0.63	96.4 (7.0)	121.5 (8.7)	154.0 (9.1)	583.4 (127.1)	[1]	[0.5]	[0]	0.04	0.05	0.08	5252.3	2.4	3	>0.25	5266.3 ‡
4. Equal transmission	0.99	120.3 (2.7)	85.0 (7.4)	204.2 (30.0)	797.5 (88.1)	q_H	q_H	q_H	0.01	0.28	0.45	5252.6	2.7	3	>0.25	5266.6 ‡
5. General (τ free)	0.98	122.7 (2.7)	89.5 (6.1)	170.0 (15.8)	691.8 (15.8)	1.00	0.6	1.0	0.03	0.26	0.41	5249.9				

*adjusted for age, sex, alcohol drinking status, cigarette smoking status, exercise, BMI, lipid lowering drug used and DM.

‡ The most parsimonious models, AIC (Akaike's Information Criterion)

q_L indicates the prevalence of the L allele; μ, mean with each genotype(LL,LH,HH); σ^2, within-type variance; τ, probability of transmitting L allele; and ρ_{sp}, ρ_{po} and ρ_{ss}, spouse,parent-offspring, and sibling correlations, respectively.

genotypes LL,LH, and HH were 96.4, 121.5, and 154.0 mg/dl, with relative frequencies of 39.7%, 46.6%, and 13.7%, respectively. The same analysis was performed on 30 families, but data was not found parsimonious model significantly (Table 4). The ratio, $-2\ln(L_C/L_E)$ was calculated to determine whether the family favored a codominant Mendelian model ($-2\ln(L_C/L_E)$ ratio< 0) or an environmental model ($-2\ln(L_C/L_E) > 0$). The results are shown in Figure 7. For HDL-cholesterol, the results of segregation analysis are presented in Table 5 and 6, respectively. Models with no major gene effect, and environmental non-transmission were also rejected. The result for HDL-cholesterol levels was best explained by a major gene effect inherited by a dominant Mendelian model ($p>0.99$). Estimated mean HDL-cholesterol levels were 53.0, 53.0, and 71.2 mg/dl, with relative frequencies of 21.2%, 49.6%, and 29.2%, respectively. This best fitting dominant Mendelian model describes a dominant major gene effect for HDL-cholesterol level to 53.0 (0.8 S.D. from the mean). The inferred major gene in this dominant Mendelian model also accounted for 51.8% of the variation in HDL-cholesterol.

3. Linkage analysis

The genomic scan to detect loci related to lipid concentration has been conducted in 305 individual in 30 extended pedigrees. The clinical parameters and plasma lipid levels of 30 selected families are shown in Table 7. The allele frequencies of selected markers for 305 individual in 30 extended

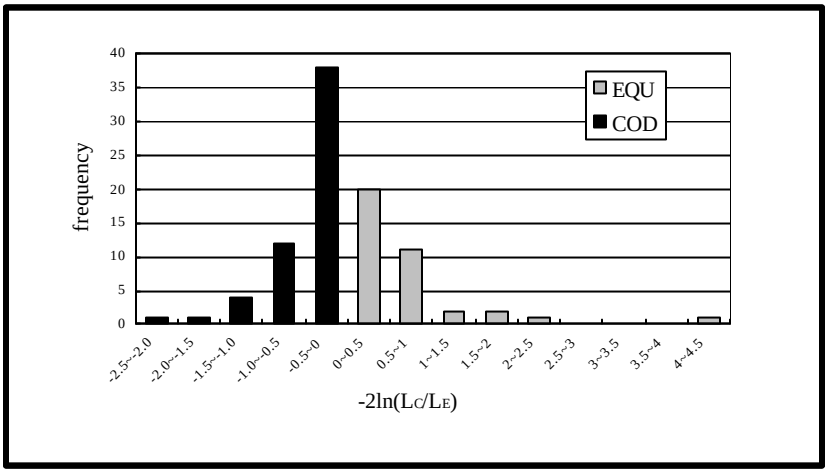
Table 4. Segregation analysis of adjusted LDL-cholesterol* in 305 individuals of 30 families ascertained to the cardiovascular disease**

	q_L	μ_{LL} (s.d)	μ_{LH} (s.d)	μ_{HH} (s.d)	σ^2 (s.d)	τ_{LL}	τ_{LH}	τ_{HH}	ρ_{sp}	ρ_{po}	ρ_{ss}	-2LnL	X^2	df	*P*	AIC
1. Sporadic	[1]	115.0 (2.1)			1272.4 (104.4)				[0]	[0]	[0]	2966.0	63.9	8	<0.001	2970.0
2. Familial correlation																
a. Unimodel, sp	[1]	114.9 (2.1)			1272.4 (104.5)				0.38	[0]	[0]	2965.9	63.8	7	<0.001	2971.9
b. Unimodel, sp, po	[1]	114.4 (2.4)			1265.6 (105.7)				0.66	0.17	[0]	2957.1	55.0	6	<0.001	2965.1
c. Unimodel, sp, po, ss	[1]	115.9 (3.0)			1300.0 (122.5)				0.65	0.28	0.38	2936.1	34.0	5	<0.001	2946.1
3. Mendelian																
a. Dominant	0.64	107.5 (3.2)	107.5 (3.2)	171.4 (9.7)	835.7 (111.7)	[1]	[0.5]	[0]	0.85	0.18	0.29	2922.4	20.3	3	<0.001	2936.4
b. recessive	0.94	108.7 (3.5)	172.3 (11.4)	172.3 (11.4)	898.8 (126.8)	[1]	[0.5]	[0]	0.77	0.16	0.32	2924.3	22.2	3	<0.001	2938.3
c. Codominant	0.75	97.5 (4.6)	132.6 (9.1)	196.9 (14.7)	677.2 (140.9)	[1]	[0.5]	[0]	0.26	0.15	0.33	2919.9	17.8	3	<0.001	2933.9
4. Equal transmission	0.75	92.6 (3.6)	134.8 (4.6)	191.9 (10.5)	532.7 (107.5)	q_H	q_H	q_H	0.31	0.45	0.49	2927.1	25.0	3	<0.001	2941.1
5. General (τ free)	0.48	90.9 (3.6)	119.3 (4.5)	172.8 (5.5)	546.7 (87.4)	1.0	0	1.0	0	0.02	0	2902.1				

*adjusted for age, sex, alcohol drinking status, cigarette smoking status, exercise, BMI, lipid lowering drug used ,DM

**For parameters definition see Table 3

(a)



(b)

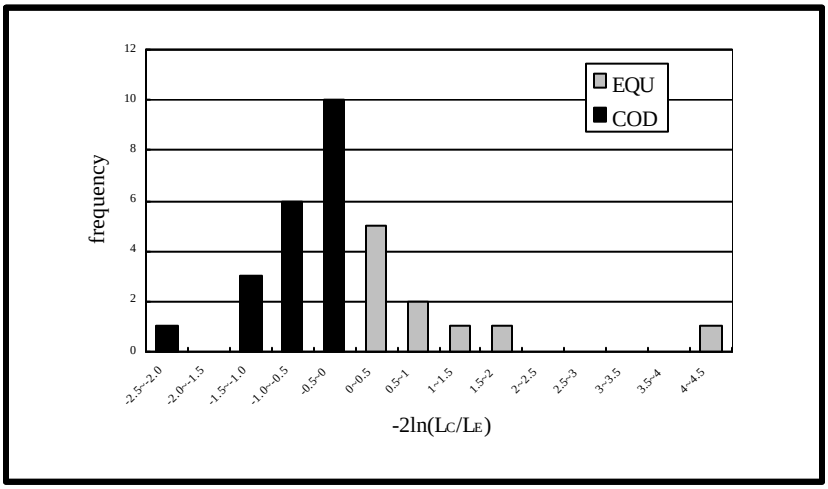


Figure 7. Relative likelihood of the equal transmission model $-2\ln[L]_E$ and the codominant Mendelian model $-2\ln[L]_C$. The ratio $2\ln(L_C/L_E)$ measures the extent to which each family supported one model over the other. (a) Likelihood ratio for LDL-cholesterol in 93 full families (b) Likelihood ratio for LDL-cholesterol in 30 selected families. Equal transmission model (EQU) Codominant Mendelian model (COD)

Table 5. Segregation analysis of adjusted HDL-cholesterol* in 701 members of 93 families ascertained to the cardiovascular disease**

	q_L	$_{LL}$ (s.d)	$_{LH}$ (s.d)	$_{HH}$ (s.d)	σ^2 (s.d)	τ_{LL}	τ_{LH}	τ_{HH}	ρ_{sp}	ρ_{po}	ρ_{ss}	-2LnL	X^2	df	*P*	AIC
1. Sporadic	[1]	58.3 (0.5)			140.9 (8.5)				[0]	[0]	[0]	4305.7	73.2	8	<0.001	4309.7
2. Familial correlation																
a. Unimodel, sp	[1]	58.4 (0.5)			141.2 (8.6)				0.24	[0]	[0]	4299.0	66.5	7	<0.001	4305.0
b. Unimodel, sp, po	[1]	58.4 (0.6)			138.4 (8.5)				0.20	0.19	[0]			6	<0.001	4283.2
c. Unimodel, sp, po, ss	[1]	58.5 (0.7)			140.4 (9.3)				0.20	0.28	0.29	4275.2	42.7	5	<0.001	4263.8
3. Mendelian																
a. Dominant	0.46	53.0 (0.8)	53.0 (0.8)	71.2 (1.3)	73.0 (7.5)	[1]	[0.5]	[0]	0.30	0.24	0.27	4232.6	0.1	3	>0.99	4246.6 ‡
b. recessive	0.84	54.0 (1.4)	69.1 (1.8)	69.1 (1.8)	93.2 (13.2)	[1]	[0.5]	[0]	0.27	0.18	0.21	4248.4	15.9	3	<0.005	4262.4
c. Codominant	0.81	53.4 (2.0)	67.4 (4.2)	75.5 (6.3)	89.6 (15.6)	[1]	[0.5]	[0]	0.29	0.15	0.18	4248.2	15.7	3	<0.005	4262.2
4.Equal transmission	0.71	51.1 (2.1)	63.6 (4.0)	77.7 (2.9)	76.7 (10.6)	q_H	q_H	q_H	0.28	0.41	0.44	4238.2	5.7	3	>0.1	4252.2
5.General (τ free)	0.84	52.7 (1.2)	69.4 (2.6)	77.7 (5.5)	72.7 (10.2)	1.0	0	1.0	0.28	0.29	0.38	4232.5				

* adjusted for age, sex, , alcohol drinking status, cigarette smoking status, exercise, BMI, lipid lowering drug used ,DM, body fat and TG.

**For parameters definition see Table 3

‡ The most parsimonious model

Table 6. Segregation analysis of adjusted HDL-cholesterol* in 305 members of 30 families ascertained to the cardiovascular disease**

	q_L	μ_{LL} (s.d)	μ_{LH} (s.d)	μ_{HH} (s.d)	σ^2 (s.d)	τ_{LL}	τ_{LH}	τ_{HH}	ρ_{sp}	ρ_{po}	ρ_{ss}	-2LnL	X^2	df	*P*	AIC
1. Sporadic	[1]	58.4 (0.7)			143.9 (11.8)				[0]	[0]	[0]	2318.6	45.0	8	<0.001	2322.6
2. Familial correlation																
a. Unimodel, sp	[1]	58.7 (0.7)			143.9 (11.9)				0.15	[0]	[0]	2317.1	43.5	7	<0.001	2323.1
b. Unimodel, sp, po	[1]	58.4 (0.8)			141.7 (11.8)				0.12	0.16	[0]	2306.8	33.2	6	<0.001	2314.8
c. Unimodel, sp, po, ss	[1]	58.7 (1.0)			144.0 (12.9)				0.13	0.25	0.28	2294.5	20.8	5	<0.001	2304.5
3. Mendelian																
a. Dominant	0.44	52.7 (1.0)	52.7 (1.0)	71.9 (1.5)	67.2 (8.2)	[1]	[0.5]	[0]	0.20	0.17	0.22	2275.5	1.9	3	>0.5	2289.5‡
b. recessive	0.81	53.1 (1.4)	69.8 (1.8)	69.8 (1.8)	82.5 (12.9)	[1]	[0.5]	[0]	0.22	0.10	0.16	2288.5	14.6	3	<0.005	2302.5
c. Codominant	0.77	52.1 (2.0)	67.4 (3.7)	76.3 (4.3)	75.7 (15.3)	[1]	[0.5]	[0]	0.21	0.04	0.01	2287.5	13.8	3	<0.005	2301.5
4. Equal transmission	0.73	50.7 (1.6)	64.8 (2.8)	79.3 (2.7)	71.0 (12.6)	q_H	q_H	q_H	0.12	0.46	0.59	2279.4	5.7	3	>0.1	2293.4
5. General (τ free)	0.47	49.8 (3.8)	53.9 (2.3)	71.9 (1.8)	65.7 (10.8)	1.0	0	1.0	0.18	0.15	0.32	2273.6				

* adjusted for age, sex, alcohol drinking status, cigarette smoking status, exercise, BMI, lipid lowering drug used ,DM, body fat and TG.

**For parameters definition see Table 3

‡ The most parsimonious model

Table 7. Clinical characteristics of 30 families selected for segregation and linkage analysis

Characteristics	Probands (n= 30) Mean ± SD	Remaining relatives (n= 275) Mean ± SD	Total (n= 305) Mean ± SD
Age (years)	53.7±9.3	35.9±17.4	37.6±17.5
Sex (% male)	76.7	43.6	46.9
BMI (kg/m^2)	25.0±2.3	21.8±3.4	22.1±3.4
Current Drinking (%)	20.0	16.4	16.7
Current Smoking (%)	20.0	22.2	22.0
SBP (mmHg)	120.7±27.0	119.2±17.4	119.4±18.5
DBP (mmHg)	78.0±12.7	70.8±13.0	71.5±13.1
DM (%)	6.7	1.8	2.3
HDL cholesterol (mg/dl)	49.5±10.9	60.1±14.5	59.1±14.5
LDL cholesterol (mg/dl)	114.3±27.6	115.2±35.5	115.1±34.8
TG (mg/dl)	143.9±59.1	125.9±98.1	127.6±95.2
TC (mg/dl)	192.6±33.0	199.6±38.7	199.0±38.2
Apo AI (mg/dl)	116.9±21.0	132.5±28.7	131.1±28.5
Apo B (mg/dl)	88.0±16.0	85.1±24.8	85.3±24.2

All values except those for sex, alcohol drinking status, cigarette smoking status and DM are mean ± SD.

For abbreviations definition see Table 2

pedigrees are shown in Table 8. These data were used for linkage analysis. The linkage analysis was performed using the data derived segregation analysis and genotyping. Figure 8 shows the result of linkage analysis between markers and a putative gene that affects lipid levels. In the analysis for HDL-cholesterol, none of these markers show as evidence of linkage. For LDL-cholesterol, the significant highest LOD score was found at D11S912, located 130.9cM (LOD score =1.18, p=0.099). Markers on other region were not found the significant evidence of linkage to LDL-cholesterol (Table 9). To confirm the tentative evidence of linkage in D11S912, we analyzed the LOD score in 21 families that favored the codominant Mendelian model. The D11S912 was significant linked in selected samples, while the LOD score was not changed (Table 10).

Table 8. Allele frequencies for microsatellite markers near Apo AI-CIII-AIV gene cluster from 305 individuals in 30 extended pedigrees.

	Allele frequency										
(n)	D11S912	D11S1320	D11S4151	D11S4191	D11S968	D11S908	D11S1338	D11S4127	D11S4126	D11S4094	D11S925
1	0.2351	0.0083	0.0099	0.1794	0.0033	0.0116	0.3129	0.0083	0.0414	0.0166	0.3990
2	0.1159	0.1761	0.0248	0.1279	0.0911	0.0017	0.0232	0.2781	0.0066	0.1920	0.0049
3	0.3692	0.6811	0.6209	0.0930	0.0728	0.7152	0.0083	0.0911	0.5315	0.2318	0.0530
4	0.1209	0.1296	0.0977	0.1096	0.6341	0.2368	0.2020	0.0778	0.3444	0.1507	0.1970
5	0.0281	0.0033	0.1772	0.1163	0.1474	0.0330	0.4420	0.4404	0.0364	0.3543	0.1341
6	0.0416	0.0016	0.0315	0.0033	0.0414	0.0017	0.0099	0.0629	0.0248	0.0464	0.0728
7	0.0762	-	0.0380	0.0199	0.0099	-	0.0017	0.0348	0.0149	0.0082	0.0762
8	0.0050	-	-	0.0332	-	-	-	0.0000	-	-	0.0199
9	0.0080	-	-	0.0797	-	-	-	0.0066	-	-	0.0215
10	-	-	-	0.0980	-	-	-	-	-	-	0.0033
11	-	-	-	0.0914	-	-	-	-	-	-	0.0017
12	-	-	-	0.0349	-	-	-	-	-	-	0.0066
13	-	-	-	0.0049	-	-	-	-	-	-	0.0050
14	-	-	-	0.0085	-	-	-	-	-	-	0.0050

Number (n) means the number of dinucleotide repeats and allows from the most little repeat number.

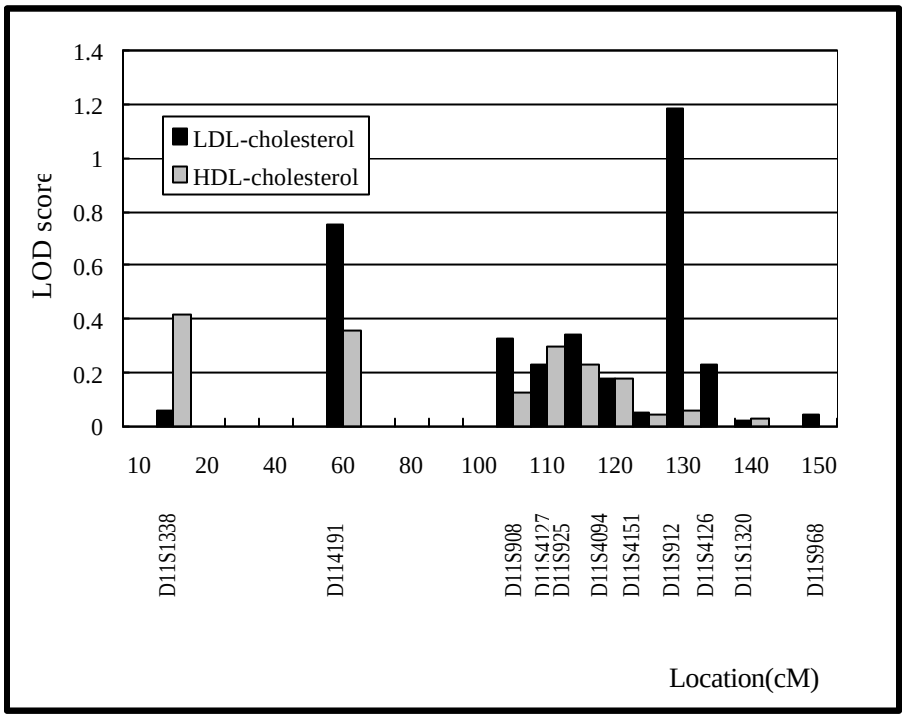


Figure 8. Linkage analyses between markers and a putative gene that affects lipid levels on chromosome11. A blue and red bars indicate LOD score of following name marker which results of linkage analysis using LODLINK program. The horizontal axis values indicate distance (in cM) from telomere and the names below horizontal axis indicate marker on the location.

Table 9. Linkage analyses of markers on chromosome 11 with a putative gene that affects lipid levels for 30 selected families.

markers	cM	LDL-cholesterol			HDL-cholesterol		
		Rf (θ [a])	LOD score	*P* value	Rf (θ [a])	LOD score	*P* value
D11S1338	15.0	0.67	0.06	0.255	0.20	0.42	0.083
D11S4191	61.3	0.12	0.75	0.032	0.21	0.36	0.100
D11S908	107.5	0.12	0.32	0.111	0.33	0.12	0.226
D11S4127	110.1	0.20	0.23	0.151	0.26	0.30	0.121
D11S925	116.9	0.18	0.34	0.104	0.31	0.23	0.153
D11S4094	121.8	0.32	0.18	0.182	0.30	0.18	0.183
D11S4151	126.0	0.39	0.05	0.310	0.43	0.04	0.329
D11S912	130.9	0.09	**1.18**	**0.099** [†]	0.38	0.06	0.305
D11S4126	136.2	0.13	0.23	0.151	0.52	-0.07	1.000
D11S1320	139.6	0.27	0.02	0.382	0.41	0.03	0.368
D11S968	147.6	0.39	0.05	0.321	0.52	-0.07	1.000

Bold writing values are the highest score in the result

[a] Recombination fraction, [†] $P < 0.1$

Table 10. Comparison of LOD scores for LDL-cholesterol concentrations with D11S912.

families (n)	number of individuals	cM	Rf (θ [a])	LOD score	*P* value
30	388	130.9	0.089	1.18	0.099 †
21	262	139.6	0.125	1.06	0.014 ‡

[a] Recombination fraction

† $P < 0.1$

‡ $P < 0.05$

IV. DISCUSSION

Not only elevated levels of LDL-cholesterol but low levels of HDL-cholesterol are risk factors in the cardiovascular disease. These lipid levels are influenced by both genetic determinants and environment factors. However, the genetic determinants on lipid levels remain poorly understood. A few studies showed that common alleles in ApoE and CYP7 are associated with plasma LDL-cholesterol levels, but only accounts for 5~10% of the population variance suggesting the existence of other gene[27,28]. Susanne et al have shown that ABCA1 heterozygotes have an approximate 50% decrease in HDL-cholesterol and increase in TG[7]. Recently, association studies have attempted to examine the linkage between apolipoprotein AI-CIII-AIV gene cluster and dyslipidamia, they reported the association between genetic markers in this gene cluster and lipid levels to this region[13,29,30,31,32].

The purpose of the present study was to identify genomic region, which determine the interindividual variations for lipid levels in Koreans. We hypothesized that genetic variation in the Apo AI-CIII-AIV gene complex might be associated with plasma lipid levels. We performed segregation analysis to define the segregation patterns of LDL-cholesterol and HDL-cholesterol. We found that segregation pattern of codominant Mendelian model with equal transmission model for adjusted LDL-cholesterol, and a dominant Mendelian model for adjusted HDL-cholesterol. These results previewed that lipid level is under major gene effect. The observed Mendelian

mechanism with a codominant Mendelian model of inheritance for LDL-cholesterol levels in this study is similar to with the reported in previous study[33]. The other study, however, reported a recessive model, which restricts to high trait value (H allele)[34]. We also found results that segregation pattern indicates a dominant Mendelian model for adjusted HDL-cholesterol. This finding is similar to the analyses presented by Michael et al[35] and Amos et al[36]. But a few studies reported on contrary opinions that could not found a single major factor controlling HDL-cholesterol levels[37,38]. The dissimilarities in the results may be explained by the ethnic difference or the small size of the pedigrees in our sample. We selected 30 families (305 individuals) those have a relatively large family number to analyze the linkage between the genetic markers in Apo AI-CIII-AIV cluster and a putative gene that affects lipid levels. Although Kort et al[22] reported a LOD score of 2.91 at D11S912, which has indicated linkages on 11q23.3 in 105 large Utah pedigrees related clusters of early CHD and dyslipidemia, our data provided a tentative evidence of linkage (LOD score $\geq$ 1.0) at D11S912 suggesting the existence of candidate genes in this genomic region. Also, we could not found any linkage between HDL-cholesterol and Apo AI-CIII-AIV gene cluster. All scores of markers are showed these less than score 0.5 (The highest LOD score=0.42 at D11S1338) (Figure 8). This result agrees with the study that reported by Almasy et al[39]. They reported that the chromosome 8 and 15 appear to influence HDL-cholesterol concentration. In this study, we also examined the

extent to which genetic linkage in selected segregation model. We selected 21 families that favored a codominant Mendelian model for a second linkage analysis. Average of LDL-cholesterol concentrations of 21 codominant Mendelian model families and 9 equal transmission model families was measured 119±34.8 and 107±34.0 respectively (data not shown). We supposed that these codominant Mendelian model families have influenced as more effective model on increased LOD scores. However, LOD score in 21 families was not significantly different, while the significance was increased (Table 9). There are several limitations in the present study. The generation of pedigrees was not sufficient to find the evidence of linkage between genetic markers and a putative gene that affects LDL-cholesterol. Also, we couldn't evaluate other regions because this study was limited at the region of chromosome 11. Therefore, additional studies with large sample sizes as well as other regions of genome may be required to help clarify the evidence for linkage.

V. CONCLUSIONS

In the analyses of inheritance for plasma lipid concentration in Korean population, segregation model for LDL-cholesterol provided both a codominant Mendelian model and an equal transmission model. The analysis of HDL-cholesterol showed superiority of a dominant Mendelian model. In the linkage analysis, tentative evidence for linkage with a gene on chromosome 11q23 (130.9 cM) influencing plasma LDL-cholesterol level was observed. The gene in the region close by D11S912 (130.9 cM) may effect on lipid metabolism. Therefore, further studies are required to identify the genetic elements responsible for the observed linkage with LDL-cholesterol level.

REFERENCES

1.Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. Arteriosclerosis 1988;8:737-41.

2. Grundy SM. Cholesterol and coronary heart disease. The 21st century. Arch Intern Med 1997;157:1177-84.

3. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. Ann Intern Med 1971;74:1-12.

4. Assmann G, Schulte H. Relation of high density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease: the PROCAM experience. Am J Cardiol 1992;70:733-7.

5. Perusse L, Despres JP, Tremblay A, Leblanc C, Talbot J, et al. Genetic and environmental determinants of serum lipids and lipoproteins in French Canadian families. Arteriosclerosis 1989;9:308-18.

6. Rice T, Vogler GP, Laskarzewish PM, Perry TS, Rao DC. Familial aggregation of lipids and lipoproteins in families ascertained through random and nonrandom probands in the Iowa Lipid Research Clinics

Family study. Hum Hered 1991;41:107-21.

7. Susanne MC, John JPK, Marjel VD, Michel M, Kirsten R, Karin YZ, et al. Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. J Clin Invest 2000;106:1263-70.

8. Jansen S, Lopez-Miranda J, Ordovas JM, Zambrana JL, Marin C, Ostos MA, et al. Effect of 360His mutation in apolipoprotein A-IV on plasma HDL-Cholesterol response to dietary fat. J Lipid Res 1997; 38(10): 1995-2002.

9. Ordovas JM, Schaefer EJ, Salem D, Ward RH, Glueck CJ, Vergani C, et al. Apolipoprotein A-I gene polymorphosm associated with premature coronary artery disease and familial hypoalphalipoproteinaemia 1986;314:671-7.

10. Jeenah M, Kessling A, Miller N, Humphries S. G to A suubstitution in the promoter region of the apolipoprotein A-I gene is associated with elevated A-I and high density lipoprotein cholesterol concentrations. Mol Biol Med 1990;7:233-41.

11. Sigurdsson G Jr, Gudnason V Sigurdsson G, Humphries SE. Interaction between a polymorphism of the apo A-I promoter region and smoking determines plasma levels of HDL and apo A-I. Arterioscler Thromb 1992;12:1017-22.

12. Saha N, Tay JS, Low PS, Humphries SE. Guanidine to adeine(G/A) substitution in the promoter region of the apolipoprotein AI gene is associated with elevated serum apolipoprotein AI levels in Chinese non-smokers. Genet Epidemiol 1994;11:255-64.

13. Wojciechowski AP, Farrall M, Cullen P, Wilson TM, Bayliss JD, Farren B, et al. Familial combined hyperlipidaemia linked to the apolipoprotein AI-CIII-AIV gene cluster on chromosome 11q23-q24. Nature 1991;349:161-4.

14. Shoulders CC, Ball MJ, Mann JI, Baralle FE. Genetic marker in apolipoprotein AI/CIII gene complex associated with hypercholesterolaemia[letter]. Lancet 1986;2:1286-7.

15. Aalto-Setala K, konttula, K, Sane T, Nieminen M, Nikkila E. DNA polymorphisms of apolipoprotein A-I/C-III and insulin genes in familial hypertriglyceridemia and coronary heart disease. Atherosclerosis 1987;66:145-52.

16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

17. Lange K, Westlake J, Spence MA. Extensions to pedigree analysis III:

variance components by the scoring method. Ann Hum Genet 1976;46:373-83.

18. Coresh J, Beaty TH, Kwiterovich PO Jr. Inheritance of plasma apolipoprotein B levels in families of patients undergoing coronary arteriography at an early age. Genet Epidemiol 1993;10:159-76.

19. Bonney GE. On the statistical determination of major gene mechanisms in continuous human traits: regressive models. Am J Med Genet 1984;18:731-49.

20. Akaike H. A new look at the statistical model identification. IEEE Trans Automat Control 1974;19:719-23.

21. Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 1998;63:861-9.

22. Kort EN, Ballinger DG, Ding W, Hunt SC, Bowen BR, Abkevich V et al. Evidence of Linkage of Familial Hypoalphalipoproteinemia to a Novel Locus on Chromosome 11q23. Am J Hum Genet 2000;66:1845-56.

23. Klos KL, Kardia SL, Ferrell RE, Turner ST, Boerwinkle E, Sing CF. Genome-wide linkage analysis reveals evidence of multiple regions that influence variation in plasma lipid and apolipoprotein levels associated with risk of coronary heart disease. Arterioscler Thromb Vasc Biol 2001;21:971-8.

24. Perola M, Ohman M, Hiekkalinna T, Leppavori J, Pajukanta P, Wessman M, et al. Quantitative-Trait-Locus analysis of body-mass index and of stature, by combined analysis of genome scans of five finnish study groups. Am J Hum Genet 2001;69:117-23.

25. Jonathan LH, Margaret AP. Approaches to Gene Mapping in Complex Human Diseases: WILEY-LISS;1998

26. Morton NE. Significance levels in complex inheritance. Am J Hum Genet 1998;62:690-7.

27. Boerwinkle E, Sing CF. The use of measured genotype information in the analysis of quantitative phenotypes in man. III. Simultaneous estimation of the frequencies and effects of the apolipoprotein E polymorphism and residual polygenetic effects on cholesterol, betalipoprotein and triglyceride levels. Ann Hum Genet 1987;51:211-26.

28. Wang J, Freeman DJ, Grundy SM, Levine DM, Guerra R, Cohen JC. Linkage Between Cholesterol 7-Hydroxylase and High Plasma Low-density Lipoprotein Cholesterol Concentrations. J Clin Invest 1998;101(6):1283-91.

29. Peacock RE, Hamsten A, Johansson J, Nilsson-Ehle P, Humphries SE. Association of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis and high density lipoprotein subclasses. Clin Genet 1994;46:273-82.

30. Cohen JC, Wang Z, Grundy SM, Stoesz MR, Guerra R. Variation at the hepatic lipase and apolipoprotein AI/CIII/AIV loci is a major cause of genetically determined variation in plasa HDL cholesterol levels. J Clin Invest 1994; 94:2377-84.

31. Dammerman M, Sandkuijl LA, Halaas JL, Chung W, Breslow JL. An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3 untranslated region polymorphisms. Proc Natl Acad Sci USA 1993;90:4562-6.

32. Kaprio J, Ferrell RE, Kottke BA, Kamboh MI, Sing CF. Effects of polymorphisms in apolipoproteins E, A-IV, and H on quantitative traits

related to risk for cardiovascular disease. Arterioscler and Thromb 1991;11:1330-48.

33. **박정용**. **한국인 관상동맥 환자 가족의** LDL- **콜레스테롤 수준의 유전역학적 연구-유전자 분리모형**(segregation analysis). **연세대 학교 보건대학원 역학 및 질병관리학과**(**석사논문**); 1999.

34. Friedlander Y, Kark JD. Complex segregation analysis of plasma lipid and lipoprotein variables in a Jerusalem sample of nuclear families.Hum Hered 1987;37(1):7-19.

35. Michael CM, John B, David LR, Anthony GC, John LV, Michael PS, et al. A major locus influencing plasma high-density lipoprotein cholesterol levels in the San Antonio family Heart Study. Segregation and linkage analyses. Arterioscler Thromb Vasc Biol 1995 Oct; 15(10):1730-9.

36. Amos CI, Elston RC, Srinivasan SR, Wilson AF, Cresanta JL, Ward LJ, et al. Linkage and segregation analyses of apolipoproteins AI and B, and lipoprotein cholesterol levels in a large pedigree with excess coronary heart disease: the Bogalusa Heart Study. Genet Epidemiol 1987;4(2):115-28.

37. Friedlander Y, Leitersdorf E. Influence of apolipoprotein E genotypes on plasma lipid and lipoprotein concentrations: results from a segregation analysis in pedigrees with molecularly defined familial hypercholesterolemia. Genet Epidemiol 1996;13(2):159-77.

38. Borecki IB, Rao DC, Third JL, Laskarzewski PM, Glueck CJ. A major gene for primary hypoalphalipoproteinemia. Am J Hum Genet 1986;38:373-81.

39. Almasy L, Hixson JE, Rainwater DL, Cole S, Williams JT, Mahaney MC, et al. Human pedigree-based quantitative-trait-locus mapping: localization of two genes influencing HDL-cholesterol metabolism. Am J Hum Genet 1999;64:1686-93.

국문요약

심혈관계 질환 가족에서 혈중 지질농도와 Apo AI-CIII-AIV Cluster와의 연관분석

심혈관계 질환은 여러 요인들에 의해 발생되는 다 인자성 질환이다. 최근 유전체 관련 연구가 활성화되면서 심혈관계 질환 및 그 위험요인과 유전형과의 관련성에 대해 활발한 연구가 진행되어오고 있으나 혈중지질대사에 관여하는 유전자들의 분리모형 및 관련성에 대한 연구는 그 결과들이 아직 논란이 많은 실정이다. 본 연구에서는 심혈관계 질환자들과 그 가족을 중심으로 혈중지질 치에 대한 분리모형을 설정하고 혈중 지질 치와 관련이 있다고 알려져 있는 Apo AI-CIII-AIV 유전자 근접한 유전체 부위에 대해 microsatellite marker를 이용한 연관분석을 시행하였다. 심혈관계질환으로 진단 받은 환자와 그 가족 (93가족, 701구성원)을 대상으로 저밀도 지단백 콜레스테롤에 대한 분리분석결과 codominant Mendelian 모형과 equal transmission 모형을 따랐다. 고밀도 지단백 콜레스테롤에 대한 분리분석에서는 dominant Mendelian 모형이 우세하게 나타났다. 대상가족 중 다세대이며 가족구성원이 많은 가족 30 가족을 선정하여 이를 대상으로 연관분석을 시행한 결과 HDL-콜레스테롤에 대한 분석에서는 모든 marker에서 연관성이 없었으며 LDL-콜레스테롤에 대한 분석에서는 marker들 중 D11S912 (130.9cM)에서 비교적 높은 관련성을 나타냈으나 유의성은 가지지 못하였다 (LOD score

=1.18, p=0.099). 분석된 30가족 중 codominant Mendelian 모형을 따르는 21 가족을 대상으로 연관분석을 시행한 결과에서는 유의성은 비교적 높아졌으나 LOD score의 유의한 차이는 없었다(LOD score =1.06, p=0.014). 이상의 결과로 심혈관계 질환 가족에서 LDL-콜레스테롤에 대한 분리모형은 codominant Mendelian 및 equal transmission 모형을 따르고 HDL-콜레스테롤에 대한 분리모형은 dominant Mendelian 모형을 우세하게 따르는 것을 알 수 있었다. 또한 Apo AI-CIII-AIV 유전자 근접 부위에서 LDL-콜레스테롤 농도에 영향을 주는 유전자가 있을 가능성을 시사하며 이들 유전자에 대한 연구에 이용될 수 있을 것이다.

핵심 되는 말 : 유전자 분리분석, 연관분석, 혈중 콜레스테롤, 심혈관계 질환, Apo AI-CIII-AIV 유전자