

行政院國家科學委員會專題研究計畫 期中進度報告

高三酸甘油酯／低高密度脂蛋白膽固醇之家族遺傳研究

(2/3)

計畫類別：個別型計畫

計畫編號：NSC91-2314-B-002-202-

執行期間：91年08月01日至92年07月31日

執行單位：國立臺灣大學醫學院內科

計畫主持人：簡國龍

計畫參與人員：許秀卿 李源德

報告類型：精簡報告

處理方式：本計畫可公開查詢

中 華 民 國 92 年 5 月 29 日

行政院國家科學委員會專題研究計畫成果報告

高三酸甘油酯／低高密度脂蛋白膽固醇之家族遺傳研究

(II)

Family genetic studies of hypertriglyceridemia and low

HDL-cholesterol (II)

計畫編號：91-2314-B-002-202

執行期限：91年8月1日至92年7月31日

主持人：簡國龍 臺大醫院 Email: klchien@ha.mc.ntu.edu.tw

共同主持人：許秀卿 臺大醫院 計畫參與人員：李源德 臺大醫院

一、中文摘要

背景及目的： Apo AI-CIII-AIV (ApoA1/C3/A4) 基因簇集是位在染色體第 11 對上，超過 10 個以上的基因多型性被報告與 Triglyceride 及 HDL cholesterol 有關。**材料與方法：**以金山社區一般族群作為研究對象，在總共男性 388 人，女性 435 人，平均 38 歲，由共 116 個家族組成的成員，包括 ApoA1 (-75) MspI G/A substitution 及 Apo A1 (+83) C->T 及 Apo C3 (+3175) SacI C->G，Apo C3 (+3206) T->G 及 Apo A4 (+347) A->T 共五個基因型。在分析有家族聚集的案例，由於考慮家族成員彼此有相關存在，並非為獨立的樣本，因此不能以傳統的統計分析，而非利用考量資料相關的統計方法，如一般化估計方程式 (Generalized Estimation Equation, GEE) 來化解資料彼此不獨立的難題。根據以兩種分析方法 (連續性變數及極端族群變項) 不同的遺傳模式 (無母數、顯性、隱性及累加性遺傳模式)。

結果：在基因型分佈上 Apo A1 (+83) 的 T allele 多型性最少 (2.9%)，其他多型性分佈均為 0.3~0.7 之間。根據估計的方法推算其分佈頻率與檢定這五個基因型的單股體是否為彼此獨立或有相關性的統計量呈現統計上的差異 (卡方檢定值為 678.82，自由度為 26， $P < 0.00001$)，推論此五個標識有連鎖不平衡 (linkage disequilibrium)。其中 3 種 haplotypes 超過 20%，分別是 (ACCCGA)，(GCGGT) 及 (GCCTA)，分別有 27.8%、25.5% 及 20.3%。進一步我們比較兩兩之間連鎖不平衡 (pair-wise linkage disequilibrium) 的狀況，此基因簇集的 5 個基因型彼此的連鎖不平衡有明顯的差距，其中 Apo C3 (+3206) 其他的基因型連鎖係數最低 (介於 0.04~0.33 之間)。與血脂值相關研究結果顯示，以極端組別分析之下，Apo A4 (+347) 與 TG/HDL，Apo A1 (+83) 與 HDL-cholesterol，ApoA1 (-75) 與 TG 的濃度有相關。而以連續變數分析之下，Apo A4 (+347) 與 log TG/HDL，Apo

A1 (+83)與 log TG/HDL 值，Apo A1 (+83) 與 HDL-cholesterol，Apo A4(+347)與 log TG 濃度有相關，而 Apo C3 (+3175)僅在 Nonparametric(非母數模型)之下與 log TG 有相關。以 Apo AI/CIII/AIV 基因簇集上的五個基因座的連鎖不平衡分析，進而了解其單股體分佈的情形，對族群中單股體的分佈頻率及連鎖不平衡的狀況提供實際的資料。

結論：以 Apo AI-CIII-AIV 基因簇集上的五個基因座的連鎖不平衡分析，進而了解其單股體分佈的情形，對族群中單股體的分佈頻率及連鎖不平衡的狀況提供華人的資料。

關鍵詞：脂蛋白元 AI-CIII-AIV 基因簇集，三酸甘油酯，高密度脂蛋白膽固醇，連鎖不平衡，單股體

Abstract

Background & Objectives: Individual polymorphism in apolipoprotein AI-CIII-AIV gene clusters was associated with dyslipidemia. But there were scanty data on linkage disequilibrium, haplotypes of multiple loci and quantitative trait loci (QTL) studies on triglyceride (TG) and HDL cholesterol traits among ethnic Chinese. We investigated genetic association studies on 5 polymorphisms, including ApoA1(-75)G->A, ApoA1(+83)C->T, C3(+3175)C->G, ApoC3(+3206)T->G and ApoA4(+347)A->T and TG/HDL in Taiwan.

Materials & Methods: Total 388 males and 435 females (average 38 years old) from 116 families in the Chin-Shan Community were sampled. Allelic association and haplotype frequency were estimated and tested if linkage disequilibrium existed. Pair-wise disequilibrium coefficients were estimated by EM algorithm. For QTL, we used the quintile extreme group analyses; i.e. above the 80th percentile or below the 20th percentile of lipid profiles to estimate odds ratio (OR) and 95% confidence interval (CI). We applied the generalized estimation equation to handle non-independence problems among family members, and tested the significance level under various inheritance modes, including nonparametric, dominant, recessive or additive modes. Also, we used quantitative trait analyses to evaluate adjusted mean

values of three genotypes in each locus.

Results: Among the 5 loci, Apo A1(+83)T allele was the least allele frequency (2.9%). There were strong linkage disequilibrium among these 5 loci (chi-square=678.82, df=26, P<0.00001). There were 3 haplotype frequencies more than 20%, i.e., (ACCGA), (GCGGT) and (GCCTA). Pair-wise linkage disequilibrium coefficients showed that Apo C3(+3206) had lowest coefficients (from 0.04 to 0.33), indicating Apo C3 polymorphism had longer evolution history than other loci. In extreme group analyses, Apo A1(-75)G polymorphism was associated with TG concentration (OR=2.72, CI=1.10-6.69, under recessive mode); Apo A1 (+83) with HDL (OR=2.65, CI=1.08-6.49, under additive mode); Apo A4(+347) with TG/HDL (OR=0.45, CI=0.22-0.92, under recessive mode).

Conclusion: These findings established that significant allelic association of 5 loci in the Apo AI/CIII/AIV gene cluster, and there were 3 haplotype frequencies more than 20% in ethnic Chinese. Also the evidence supported the contribution of common genetic variation on this gene cluster to quantitative TG and HDL phenotypes in the general population.

Keywords : Apolipoprotein AI-CIII-AIV gene cluster, triglyceride, HDL-cholesterol, linkage disequilibrium, haplotype

二、緣由與目的

Apo A-I/C-III/A-IV 基因簇集是位於染色體第 11 對上，已有很多文獻報告與三酸甘油酯及高密度膽固醇的濃度有關。在 1985 年，Karathanasis S.K. 利用 DNA fragment 的 Hybridization studies，證實此一基因簇集 Apo A1/C3/A4 基因簇集 (gene cluster)(1)。其中 Apo C-III 基因佔 3.1kb 長度，在 Apo A-I(長度 1.8kb)的 3'末端之下 2.5kb 處，而在 Apo A-IV(長度 2.6kb) 5'端上游約 5kb 的位置。Apo A-I 及 A-IV 轉譯是由相同的方向，而 Apo C-III 基因則是相反方向。超過 10 個以上的基因多型性已在 A-I/C-III/A-IV 基因簇集上發現(2)。傳統的案例對照方法以健康控制組的研究提供線索。以 G(-75)A 的多型性與 HDL-C 及 ApoA1 有關(3)。在 ApoA1 的+83bp 處有-(C->T)的多型性 M2+/+及 M2+/-表示，也是被

報告與 HDL-C 有關(4-6)。在 ApoC III 基因上的 3' untranslated 部分有 -Sst I RFLP 及在 ApoC3 有 -PuvII，分別在 3175 及 3206 位置的核苷酸序列的多型性表現。有一為 triglyceride (TG) 的變化 Apo CIII 中 3175 位置上為 Sac I C→G 的差異，另一個變異為 3206 位置上為 T→G 的變異，為另一新的變異，上述兩種變異均是在 exon4 位置上，均與三酸甘油酯有關，因此本研究即以此兩種多型性為研究對象(7)。在 Apo AIV 的突變最常見是 A-IV-1，由 A→T 取代，造成蛋白質位置 347 由 Thr 變成 Ser(8)。此 Apo A1(-75), Apo A1(+83), Apo C3 (+3175), Apo C3(+3206) 與 Apo A4(+347) 為本研究的興趣基因。

三、結果與討論

以金山社區一般族群作為研究對象，在總共男性 388 人，女性 435 人，平均 38 歲，由共 116 個家族組成的成員，以 ApoA-I/C-III/A-IV 此一基因簇集常見的 5 個位址上基因多型性的研究，包括 ApoA1 (-75) MspI G/A substitution 及 Apo A1 (+83) C→T 及 Apo C3 (+3175) SacI C→G, Apo C3 (+3206) T→G 及 Apo A4 (+347) A→T 共五個基因型。在基因型分佈上，在此研究族群中以 Apo A1 (+83) 的多型性最少，並無 homogenous genotype (M2, -/-)。其他則是有 3 個基因型分佈。其 allele frequency 估計如表一，其中以 Apo A1 (+83) T allele frequency 最低(0.029)，其他多型性分佈均為 0.3~0.7 之間。

在一共有 32 個可能的單股體(haplotypes)，根據估計的方法推算其分佈頻率為表二(9;10)。而檢定這五個基因型的單股體是否為彼此獨立或有相關性的統計量呈現統計上的差異，(卡方檢定值為 678.82，自由度為 26， $P < 0.00001$)，因此我們可以推論此五個標識有連鎖不平衡(linkage disequilibrium)。而其中有 8 種 haplotypes 頻率超過 1%，有 3 種 haplotypes 超過 20%，分別是(ACCCGA), (GCGGT) 及 (GCCTA)，分別有 27.8%、25.5% 及 20.3%。進一步我們比較兩兩之間連鎖不平衡(pair-wise linkage disequilibrium)的狀況，分別以連鎖不平衡係數，連鎖不平衡指數(即係數與最大値之比)與 P11 的 haplotypes 頻

率表示(表三之一)，在此基因簇集的 5 個基因型彼此的連鎖不平衡有明顯的差距，其中 Apo C3 (+3206) 其他的基因型連鎖係數最低(介於 0.04~0.33 之間)，其相對應的 P 值則在表三之二。

基因型與連續變數的表現型之相關研究，我們可以採取四種不同的模型，即假設為(1)自體顯性(autosomal dominant)模型;(2)自體隱性(autosomal recessive)模型，(3)累加性(additive)模型，(4)無母數(nonparametric)遺傳模式。以(A 及 B)兩種 allele 而言 基因型(AA, AB, BB)，若是 A 是帶病基因，則顯性遺傳模式的 penetrance(穿透率)即為(1, 1, 0)，而隱性遺傳為(1, 0, 0)，而累加模型則為(2, 1, 0)，另外若不假設遺傳模型，則是以 BB 為基礎，分別估計 AB 及 AA 比上 BB 對於疾病的危險，即分別估計 AA vs. BB, AB vs. BB 及 AA vs. AB 的估計值及相對應的信賴區間。根據以兩種分析方法(連續性變數及極端族群變項)不同的遺傳模式(無母數、顯性、隱性及累加性遺傳模式)，對血脂值，特別是 log TG HDL, log TG 及 HDL, TG 四項變數作表格分析。性狀的順序為: log TG/HDL ratio → TG/HDL → log TG → HDL → TG; 依無母數遺傳模式，顯性、隱性及累加性模式分析。

結果顯示以各種脂質濃度為表現型。首先，各種在男女性別的差異上，明顯在女性有較高的總膽固醇，HDL-C，而男性有較高的三酸甘油酯，TG/HDL 比率，取對數後的 TG 及 TG/HDL 值。在年齡，LDL，Apolipoprotein A1 及 Apolipoprotein B 值則無明顯的差異。(Table 4)

再用連續性及極端組別分析比較，結果顯示以極端組別分析之下，Apo A4(+347)與 TG/HDL，Apo A1 (+83)與 HDL，ApoA1 (-75)與 TG 的濃度有相關。而以連續變數分析之下，Apo A4(+347)與 log TG/HDL，Apo A1 (+83)與 log TG/HDL 值，Apo A1 (+83)與 HDL-cholesterol，Apo A4(+347)與 log TG 濃度有相關，而 Apo C3 (+3175)僅在 Nonparametric(非母數模型)之下與 log TG 有相關。(Table 5-Table 6) 在 ApoA1 MspI G/A substitution(取代)的基因多型性，在本研究族群並無明顯的相關係，與

之前的文獻報告有類似的情形，可能的原因可能是族群異質性。

在分析有家族聚集的案例，由於考慮家族成員彼此有相關存在，並非為獨立的樣本，因此不能以傳統的統計分析，而非利用考量資料相關的統計方法，如一般化估計方程式(Generalized Estimation Equation, GEE)來化解資料彼此不獨立的難題(11;12)，同時在計量 95th 信賴區間時，使用 profile likelihood confidence interval 會比傳統的 Wald 信賴區間來的正確，特別是在小樣本的狀況之下，因此我們均採用此一信賴區間表示(13)。在家族資料樣本中，Tregoutet et al 建議使用 GEE 的方法，研究發現在相同成員數目的核心家庭之下，利用 GEE 計算的效力與最大概似值的效力是相等，與相同數目的無相關性樣本有相同的效力。但是假如家族成員數目不太大 ($n < 50$)，則 type I error 可能有增大的危險(14)。

相關分析可以連續變數值當作表現型，即利用類似 ANOVA 的方法亦可將資料分成五等分，其極端族群(extreme group)作列聯表分析，此種極端族群分析的方法在手足配對(affected sibpair)連鎖分析(linkage analysis)證明有用，且有較高 power (15)，而在相關分析中也能適用(16)，效力(power)及可行性上均是有較好的優點，特別是樣本較多的情況下。使用極端族群的分析要注意偵測的目的是找控制極端性狀，而非原先的連續變數，因此在解釋上必須與連續變數性狀的結果作區別，此種以極端族群值方法，

類似為 truncated measured genotype (TMG) 檢定，此種檢定被證明比 ANOVA 及一般 linear 線性迴歸來得有效力，值得一試。但文獻上對於以表現型性狀是二項式及連續性變數的結果並非一致，主要的理由是因為在複雜性狀的基因影響效力較少，很難明顯偵測到它的作用。系統分析(meta-analysis)可以提供一綜合性的分析(17)，但也會受限在其出版偏差(publication bias)的難題上。

四、計畫成果自評

以 Apo AI-CIII-AIV 基因簇集上的五個基因座的連鎖不平衡分析，進而了解其單股

體分佈的情形，對族群中單股體的分佈頻率及連鎖不平衡的狀況提供實際的資料。進一步以家族資料作此五個基因座的多型性與 TG/HDL 值的相關研究中，一方面考慮家族成員的互相關連性的現象，以一般性估計方程式來處理統計上的難題。另一方面在不同的遺傳模型的假設之下，如自體顯性遺傳、隱性遺傳、累加性遺傳以及非母數遺傳模式下作分析，最後則在性狀上以連續變數及極端族群分組方式來處理，得到的結果也確實看到此五個基因座與 TG/HDL 有統計上相關的結果。在新的一年計畫，則是進一步利用連續分析，特別注著在 QTL(Quantitative trait loci)的研究，期望提供有關此 Apo AI-CIII-AIV 基因座國人與血脂質之間相關的證據。

五、參考文獻

- (1) Talmud PJ, Xu CF. Molecular biology of apolipoproteins: control of gene expression and structure-function relationship. In: Betteridge DJ, Illingworth DR, Shepherd J, editors. Lipoproteins in health and disease. London: Arnold Publisher, 1999: 181-197.
- (2) Kessling AM, Rajput-Wiliams J, Bainton D, Scott J, Miller NE, Baker I, Humphries SE. DNA polymorphisms of the apolipoprotein AII and AI-CIII-AIV genes: a study in men selected for differences in high-density-lipoprotein cholesterol concentration. *Am J Hum Genet* 1988; 42(3):458-467.
- (3) Mata P, Lopez-Miranda J, Pocovi M, Alonso R, Lahoz C, Marin C, Garces C, Cenario A, Perez-Jimenez F, de Oya M, Ordovas JM. Human apolipoprotein A-I gene promoter mutation influences plasma low density lipoprotein cholesterol response to dietary fat saturation. *Atherosclerosis* 1998; 137(2):367-376.
- (4) Larson IA, Ordovas JM, Barnard JR, Hoffmann MM, Feussner G, Lamou-Fava S, Schaefer EJ. Effects of apolipoprotein A-I genetic variations on plasma apolipoprotein, serum lipoprotein and glucose levels. *Clin Genet* 2002; 61(3):176-184.
- (5) Pulkkinen A, Viitanen L, Kareinen A, Lehto S, Laakso M. MspI polymorphism at +83 bp in intron 1 of the human apolipoprotein A1 gene is associated with elevated levels of HDL cholesterol and apolipoprotein A1 in nondiabetic subjects but not in type 2 diabetic patients with coronary heart disease. *Diabetes Care* 2000; 23(6):791-795.
- (6) Tsai MY, Hanson NQ, Copeland KR, Beheshti I, Garg U. Determination of a T/G polymorphism at nucleotide 3206 of the apolipoprotein C III gene by amplification refractory mutation system. *Clin Chem* 1994; 40(12):2235-2239.
- (7) Lopez-Miranda J, Jansen S, Ordovas JM, Salas J, Marin C, Castro P, Ostos MA, Cruz G, Lopez-Segura F, Blanco A, Jimenez-Perez J, Perez-Jimenez F. Influence of the SstI polymorphism

at the apolipoprotein C-III gene locus on the plasma low-density-lipoprotein-cholesterol response to dietary monounsaturated fat. *Am J Clin Nutr* 1997; 66(1):97-103.

(8) Jansen S, Lopez-Miranda J, Salas J, Ordovas JM, Castro P, Marin C, Ostos MA, Lopez-Segura F, Jimenez-Perez JA, Blanco A, Perez-Jimenez F. Effect of 347-serine mutation in apoprotein A-IV on plasma LDL cholesterol response to dietary fat. *Arteriosclerosis, Thrombosis & Vascular Biology* 1997; 17(8):1532-1538.

(9) Hartl DL, Clark AG. Darwinian evolution in Mendelian population. *Principles of population genetics*. Sunderland, Massachusetts: Sinauer Associates, Inc., 1989: 1-60.

(10) Lewontin RC. On measures of gametic disequilibrium. *Genetics* 1988; 120(3):849-852.

(11) Liang KY, Beaty TH, Cohen BH. Application of odds ratio regression models for assessing familial aggregation from case-control studies. *Am J Epidemiol* 1986; 124(4):678-683.

(12) Ziegler A, Kastner C, Brunner D, Blettner M. Familial associations of lipid profiles: a generalized estimating equations approach. *Stat Med* 2000; 19(24):3345-3357.

(13) SAS Institute Inc. *SAS/STAT User's guide*, Version 8. Cary, NC: SAS Institute Inc., 1999.

(14) Tregouet DA, Ducimetiere P, Tiret L. Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *Am J Hum Genet* 1997; 61(1):189-199.

(15) Risch N, Zhang H. Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science* 1995; 268:1584-1589.

(16) Schork NJ, Nath SK, Fallin D, Chakravarti A. Linkage disequilibrium analysis of biallelic DNA markers, human quantitative trait loci, and threshold-defined case and control subjects. *Am J Hum Genet* 2000; 67(5):1208-1218.

(17) Juo SH, Wyszynski DF, Beaty TH, Huang HY, Bailey-Wilson JE. Mild association between the A/G polymorphism in the promoter of the apolipoprotein A-I gene and apolipoprotein A-I levels: a meta-analysis. *American Journal of Medical Genetics* 1999; 82(3):235-241.

Appendix

Table 1. Comparison of genotype frequencies of 5 polymorphisms of Apo A1/C3/A4 gene clusters

Locus/marker	Genotype	Frequency	Percent (%)
ApoA1 (-75)	GG	366	45.13
MspI G/A substitution	GA	351	43.28
	AA	94	11.59
Apo A1 (+83)	CC	765	94.21
C->T	CT	47	5.79
	TT	0	0
Apo C3 (+3175)	CC	416	50.61
SacI C->G	CG	347	42.21
	GG	59	7.18
Apo C3 (+3206)	TT	34	4.14
T->G	TG	282	34.31
	GG	506	61.56
Apo A4(+347)	AA	340	43.53
A->T	AT	358	45.84
Protein 347 Thr->Ser	TT	83	10.63

Table 2. Estimates of Gene Frequencies (Assuming Independence) by Haplotype analyses by PM and EH program among study population

locus	Allele 1	Allele 1 frequency	Allele 2	Allele 2 frequency
ApoA1 (-75)	G	0.662	A	0.338
Apo A1 (+83)	C	0.972	T	0.029
Apo C3 (+3175)	C	0.717	G	0.283
Apo C3 (+3206)	T	0.215	G	0.785
Apo A4(+347)	A	0.665	T	0.335

Table 3. Distribution of haplotype frequency of 5 markers and disease status

Haplotype	Frequency	ApoA1 (-75)	Apo A1 (+83)	Apo C3 (+3175)	Apo C3 (+3206)	Apo A4 (+347)
1	27.83%	A	C	C	G	A
2	25.49%	G	C	G	G	T
3	20.28%	G	C	C	T	A

4	13.86%	G	C	C	G	A
5	4.09%	A	C	C	G	T
6	2.54%	G	T	C	G	A
7	2.03%	G	C	C	G	T
8	1.37%	G	C	G	G	A

Table 3. Coefficients of linkage disequilibrium in paired genotypes

Pairs 1	Pairs 2	P11, allele frequency	D*	Z	D.max	D'	P
ApoA1 (-75)	Apo A1 (+83)	0.6499	-0.0063	-2.3366	0.0191	-0.3295	0.0097
ApoA1 (-75)	Apo C3 (+3175)	0.4000	-0.0786	-10.5409	0.1887	-0.4166	0.0000
ApoA1 (-75)	Apo C3 (+3206)	0.2378	0.0695	9.3353	0.0753	0.9227	0.0000
ApoA1 (-75)	Apo A4 (+347)	0.3782	-0.0624	-7.7759	0.2222	-0.2807	0.0000
Apo A1 (+83)	Apo C3 (+3175)	0.6965	-0.0010	-0.3699	0.0208	-0.0471	0.3557
Apo A1 (+83)	Apo C3 (+3206)	0.2139	0.0062	2.5664	0.0062	1.0000	0.0051
Apo A1 (+83)	Apo A4 (+347)	0.6438	-0.0031	-1.1018	0.0189	-0.1640	0.1353
Apo C3 (+3175)	Apo C3 (+3206)	0.2129	0.0602	9.3642	0.0602	1.0000	0.0000
Apo C3 (+3175)	Apo A4 (+347)	0.6487	0.1717	22.5836	0.1876	0.9154	0.0000
Apo C3 (+3206)	Apo A4 (+347)	0.2091	0.0658	9.4633	0.0724	0.9087	0.0000

Table 3-2. Pair-wise Linkage disequilibrium values (right upper triangle) and responsive P values (left lower triangle) in the 5 genotypes.

	ApoA1 (-75)	Apo A1 (+83)	Apo C3 (+3175)	Apo C3 (+3206)	Apo A4
ApoA1 (-75)		-0.32946	-0.41657	0.92271	-0.28071
Apo A1 (+83)	0.0097		-0.04709	1	-0.16404
Apo C3 (+3175)	0.0000	0.3557		1	0.915416
Apo C3 (+3206)	0.0000	0.0051	0.0000		0.908663
Apo A4 (+347)	0.0000	0.1353	0.0000	0.0000	

Table 4. Characteristics of age and various lipid profiles among study population.

	Men (N=388)		Women (N=435)		P
	Mean	SD	Mean	SD	
Age (years)	38.03	20.00	37.66	19.97	0.793
Total cholesterol (mg/dl)	184.14	41.31	189.40	40.58	0.066
Triglyceride (mg/dl)	140.14	138.31	118.86	106.35	0.015
HDL cholesterol(mg/dl)	42.10	11.45	44.32	10.95	0.005

LDL cholesterol(mg/dl)	107.88	44.10	112.36	40.76	0.130
Apolipoprotein A1(mg/dl)	1.19	0.24	1.20	0.23	0.487
Apolipoprotein B(mg/dl)	0.58	0.21	0.56	0.18	0.254
Triglyceride/HDL cholesterol	3.98	5.85	3.21	4.83	0.042
Logarithm triglyceride	4.69	0.64	4.59	0.55	0.014
Logarithm TG/HDL cholesterol ratio	0.99	0.79	0.83	0.71	0.002

Table 5. Extreme distribution values of various lipid profiles by quintiles in the study population

Variable	Total Number	20th percentile	Number	80th percentile	Number
Total cholesterol (mg/dl)	838	152	165	220	168
Triglyceride (mg/dl)	823	63	161	161	168
HDL cholesterol(mg/dl)	823	34	168	52	168
LDL cholesterol(mg/dl)	823	76	164	143	168
Apolipoprotein A1(mg/dl)	823	1.01	169	1.35	168
Apolipoprotein B(mg/dl)	823	0.43	168	0.67	168
Triglyceride/HDL cholesterol	823	1.343	164	4.149	168
Logarithm triglyceride	823	4.13	161	5.082	168
Logarithm TG/HDL cholesterol ratio	823	0.295	163	1.43	168

Table 6. Extreme group analysis :

(1) Dominant mode of inheritance

The odds ratios, 95% partial profile confidence interval, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Stratified group	Odds ratio	Lower 95% CI	Upper 95% CI
(Logarithm) Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	0 vs. 2, 1	1.159	0.670	2.005
Apo A1 (+83)	0 vs. 2, 1	3.248	0.544	19.392
Apo C3 (+3175)	0 vs. 2, 1	0.744	0.411	1.347
Apo C3 (+3206)	0 vs. 2, 1	0.680	0.213	2.174
Apo A4 (+347)	0 vs. 2, 1	0.753	0.447	1.268

Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	0 vs. 2, 1	1.043	0.647	1.681
Apo A1 (+83)	0 vs. 2, 1	0.378	0.154	0.927
Apo C3 (+3175)	0 vs. 2, 1	1.036	0.641	1.674
Apo C3 (+3206)	0 vs. 2, 1	1.036	0.292	3.674
Apo A4 (+347)	0 vs. 2, 1	1.025	0.644	1.633
(Logarithm) Triglyceride				
ApoA1 (-75)	0 vs. 2, 1	1.520	0.881	2.623
Apo A1 (+83)	0 vs. 2, 1	3.926	0.724	21.304
Apo C3 (+3175)	0 vs. 2, 1	0.696	0.391	1.237
Apo C3 (+3206)	0 vs. 2, 1	0.685	0.194	2.422
Apo A4 (+347)	0 vs. 2, 1	0.660	0.358	1.215

(2) Recessive mode of inheritance

The odds ratios, 95% partial profile confidence interval, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Startified group	Odds ratio	Lower 95% CI	Upper 95% CI
(Logarithm) Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	0, 1 vs. 2	1.890	0.887	4.030
Apo A1 (+83)	0, 1 vs. 2			
Apo C3 (+3175)	0, 1 vs. 2	0.663	0.220	1.994
Apo C3 (+3206)	0, 1 vs. 2	0.874	0.494	1.546
Apo A4 (+347)	0, 1 vs. 2	0.451	0.221	0.918
HDL cholesterol ratio				
ApoA1 (-75)	0, 1 vs. 2	0.538	0.188	1.539
Apo A1 (+83)	0, 1 vs. 2			
Apo C3 (+3175)	0, 1 vs. 2	1.056	0.444	2.511
Apo C3 (+3206)	0, 1 vs. 2	0.903	0.566	1.442
Apo A4 (+347)	0, 1 vs. 2	1.297	0.629	2.674
(Logarithm) Triglyceride				
ApoA1 (-75)	0, 1 vs. 2	2.716	1.103	6.689
Apo A1 (+83)	0, 1 vs. 2			
Apo C3 (+3175)	0, 1 vs. 2	0.803	0.282	2.282
Apo C3 (+3206)	0, 1 vs. 2	1.074	0.580	1.989
Apo A4(+347)	0, 1 vs. 2	0.661	0.268	1.629

(3) Additive mode of inheritance

The odds ratios, 95% partial profile confidence interval, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Startified group	Odds ratio	Lower 95% CI	Upper 95% C
(Logarithm) Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	One unit increase from 0,1,2	0.798	0.556	1.143
Apo A1 (+83)	One unit increase from 0,1,2	0.308	0.052	1.838
Apo C3 (+3175)	One unit increase from 0,1,2	1.302	0.812	2.088
Apo C3 (+3206)	One unit increase from 0,1,2	1.158	0.721	1.859
Apo A4 (+347)	One unit increase from 0,1,2	1.405	0.966	2.043
HDL cholesterol				
ApoA1 (-75)	One unit increase from 0,1,2	1.112	0.757	1.633
Apo A1 (+83)	One unit increase from 0,1,2	2.646	1.079	6.486
Apo C3 (+3175)	One unit increase from 0,1,2	0.968	0.657	1.428
Apo C3 (+3206)	One unit increase from 0,1,2	1.075	0.718	1.611
Apo A4 (+347)	One unit increase from 0,1,2	0.931	0.660	1.312
(Logarithm) Triglyceride				
ApoA1 (-75)	One unit increase from 0,1,2	0.631	0.435	0.915
Apo A1 (+83)	One unit increase from 0,1,2	0.255	0.047	1.382
Apo C3 (+3175)	One unit increase from 0,1,2	1.309	0.828	2.068
Apo C3 (+3206)	One unit increase from 0,1,2	1.007	0.603	1.683
Apo A4 (+347)	One unit increase from 0,1,2	1.385	0.872	2.199

(4) Non-parametric mode of inheritance

The odds ratios, 95% partial profile confidence interval, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Startified group	Odds ratio	Lower 95% CI	Upper 95%
(Logarithm) Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	0 vs. 2	1.911	0.881	4.142
	1 vs. 2	1.872	0.795	4.407
	0 vs. 1	1.021	0.551	1.892
Apo A1 (+83)	0 vs. 2			
	1 vs. 2			
	0 vs. 1	3.248	0.544	19.392
Apo C3 (+3175)	0 vs. 2	0.589	0.189	1.831
	1 vs. 2	0.767	0.240	2.450
	0 vs. 1	0.768	0.411	1.438
Apo C3 (+3206)	0 vs. 2	0.656	0.197	2.180
	1 vs. 2	0.909	0.508	1.627
	0 vs. 1	0.721	0.221	2.357
Apo A4(+347)	0 vs. 2	0.421	0.192	0.923
	1 vs. 2	0.480	0.229	1.006
	0 vs. 1	0.878	0.510	1.511
HDL cholesterol				
ApoA1 (-75)	0 vs. 2	0.584	0.208	1.639
	1 vs. 2	0.510	0.169	1.534
	0 vs. 1	1.145	0.684	1.917
Apo A1 (+83)	0 vs. 2			
	1 vs. 2			
	0 vs. 1	0.378	0.154	0.927
Apo C3 (+3175)	0 vs. 2	1.071	0.430	2.668
	1 vs. 2	1.039	0.429	2.519
	0 vs. 1	1.030	0.630	1.686
Apo C3 (+3206)	0 vs. 2	0.995	0.280	3.537
	1 vs. 2	0.894	0.549	1.457
	0 vs. 1	1.113	0.298	4.163
Apo A4 (+347)	0 vs. 2	1.282	0.599	2.745
	1 vs. 2	1.310	0.610	2.813

	0 vs. 1	0.979	0.601	1.594
(Logarithm) Triglyceride				
ApoA1 (-75)	0 vs. 2	3.146	1.294	7.650
	1 vs. 2	2.426	0.861	6.841
	0 vs. 1	1.297	0.699	2.405
Apo A1 (+83)	0 vs. 2			
	1 vs. 2			
	0 vs. 1	3.926	0.724	21.304
Apo C3 (+3175)	0 vs. 2	0.673	0.227	2.000
	1 vs. 2	0.963	0.322	2.879
	0 vs. 1	0.699	0.383	1.276
Apo C3 (+3206)	0 vs. 2	0.728	0.197	2.697
	1 vs. 2	1.132	0.603	2.128
	0 vs. 1	0.643	0.174	2.374
Apo A4(+347)	0 vs. 2	0.531	0.197	1.429
	1 vs. 2	0.758	0.306	1.877
	0 vs. 1	0.701	0.376	1.304

Table 7. Quantitative trait analysis :

(1) Dominant mode of inheritance (2,1) vs. 0

The adjusted mean values standard error of mean, difference between three genotypes, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Grouping	Mean	SEM	Difference baseline : vs.
Logarithm Triglyceride vs. HDL cholesterol ratio				
ApoA1 (-75)	0	0.936	0.041	0.
	1,2	0.871	0.041	Bas
Apo A1 (+83)	0	0.914	0.033	0.
	1,2	0.679	0.112	
Apo C3 (+3175)	0	0.865	0.045	-0.
	1,2	0.936	0.042	
Apo C3 (+3206)	0	0.750	0.157	-0.
	1,2	0.907	0.033	

Apo A4(+347)		0	0.852	0.046	-0.
		1,2	0.931	0.043	
Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)		0	3.435	0.197	-0.
		1,2	3.691	0.320	
Apo A1 (+83)		0	3.626	0.220	0.
		1,2	2.731	0.490	
Apo C3 (+3175)		0	3.526	0.285	-0.
		1,2	3.601	0.263	
Apo C3 (+3206)		0	2.768	0.566	-0.
		1,2	3.598	0.216	
Apo A4(+347)		0	3.357	0.240	-0.
		1,2	3.739	0.301	
HDL cholesterol					
ApoA1 (-75)		0	42.570	0.636	-1.
		1,2	43.601	0.699	
Apo A1 (+83)		0	42.880	0.564	-4
		1,2	47.153	1.601	
Apo C3 (+3175)		0	43.679	0.746	0.
		1,2	42.712	0.682	
Apo C3 (+3206)		0	43.251	1.768	0.
		1,2	43.200	0.573	
Apo A4(+347)		0	43.700	0.708	0.
		1,2	42.966	0.690	
Triglyceride					
ApoA1 (-75)		0	127.371	4.998	-2.
		1,2	130.170	6.802	
Apo A1 (+83)		0	129.546	4.770	12
		1,2	117.398	20.720	
Apo C3 (+3175)		0	127.441	6.537	-2.
		1,2	130.155	5.815	
Apo C3 (+3206)		0	105.389	16.685	-24
		1,2	129.811	4.676	
Apo A4(+347)		0	125.302	6.716	-5.
		1,2	131.095	5.974	
Logarithm Triglyceride					
ApoA1 (-75)		0	4.658	0.031	0.

		1,2	4.608	0.031	
Apo A1 (+83)		0	4.638	0.026	0.
		1,2	4.502	0.096	
Apo C3 (+3175)		0	4.602	0.034	-0.
		1,2	4.662	0.033	
Apo C3 (+3206)		0	4.489	0.124	-0.
		1,2	4.638	0.025	
Apo A4(+347)		0	4.595	0.036	-0.
		1,2	4.657	0.033	

(2) Recessive mode of inheritance 2 vs. (1,0)

The adjusted mean values standard error of mean, difference between three genotypes, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

		Group	Mean	SEM	Difference from baseline genotype (0,1) vs. 2
Logarithm Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)		0, 1	0.916	0.034	0.143
		2	0.774	0.082	
Apo A1 (+83)		0, 1	0.900	0.032	
		2			
Apo C3 (+3175)		0, 1	0.891	0.032	-0.130
		2	1.020	0.084	
Apo C3 (+3206)		0, 1	0.873	0.046	-0.044
		2	0.917	0.039	
Apo A4(+347)		0, 1	0.879	0.034	-0.171
		2	1.050	0.075	
Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)		0, 1	3.609	0.217	0.304
		2	3.305	0.585	
Apo A1 (+83)		0, 1	3.572	0.211	
		2			
Apo C3 (+3175)		0, 1	3.554	0.218	-0.119
		2	3.673	0.390	
Apo C3 (+3206)		0, 1	3.239	0.221	-0.519

		2	3.758	0.275	
Apo A4(+347)		0, 1	3.538	0.231	-0.309
		2	3.847	0.309	
HDL cholesterol					
ApoA1 (-75)		0, 1	42.873	0.569	-2.362
		2	45.235	1.551	
Apo A1 (+83)		0, 1	43.131	0.547	
		2			
Apo C3 (+3175)		0, 1	43.265	0.578	0.848
		2	42.418	1.153	
Apo C3 (+3206)		0, 1	42.896	0.780	-0.487
		2	43.384	0.632	
Apo A4(+347)		0, 1	43.378	0.594	0.869
		2	42.510	1.213	
Triglyceride					
ApoA1 (-75)		0, 1	129.854	4.829	8.359
		2	121.494	11.435	
Apo A1 (+83)		0, 1	128.816	4.564	
		2			
Apo C3 (+3175)		0, 1	128.328	4.702	-6.152
		2	134.480	9.902	
Apo C3 (+3206)		0, 1	121.104	5.980	-12.316
		2	133.420	5.667	
Apo A4(+347)		0, 1	127.119	4.986	-13.612
		2	140.731	8.582	
Logarithm Triglyceride					
ApoA1 (-75)		0, 1	4.640	0.026	0.085
		2	4.555	0.058	
Apo A1 (+83)		0, 1	4.630	0.025	
		2			
Apo C3 (+3175)		0, 1	4.623	0.025	-0.117
		2	4.740	0.066	
Apo C3 (+3206)		0, 1	4.597	0.036	-0.055
		2	4.652	0.030	
Apo A4(+347)		0, 1	4.614	0.026	-0.152
		2	4.766	0.060	

(3) Additive mode of inheritance as 0,1,2

The estimated parameters, 95% partial profile confidence interval, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Parameter estimate	Standard error of mean	Lower 95% CI	Higher 95% CI	
Logarithm Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)	-0.069	0.036	-0.140	0.002	
Apo A1 (+83)	-0.234	0.116	-0.462	-0.006	
Apo C3 (+3175)	0.070	0.044	-0.016	0.156	
Apo C3 (+3206)	0.052	0.051	-0.049	0.153	
Apo A4(+347)	0.085	0.042	0.002	0.167	
Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)	0.071	0.247	-0.413	0.555	
Apo A1 (+83)	-0.894	0.529	-1.930	0.142	
Apo C3 (+3175)	0.070	0.253	-0.425	0.565	
Apo C3 (+3206)	0.482	0.255	-0.018	0.981	
Apo A4(+347)	0.288	0.209	-0.122	0.698	
HDL cholesterol					
ApoA1 (-75)	1.126	0.612	-0.072	2.325	
Apo A1 (+83)	4.273	1.693	0.955	7.590	
Apo C3 (+3175)	-0.786	0.662	-2.084	0.512	
Apo C3 (+3206)	0.354	0.684	-0.986	1.694	
Apo A4(+347)	-0.628	0.570	-1.744	0.489	
Triglyceride					
ApoA1 (-75)	-0.358	5.302	-10.749	10.034	
Apo A1 (+83)	-12.148	21.637	-54.556	30.260	
Apo C3 (+3175)	2.821	6.118	-9.171	14.812	
Apo C3 (+3206)	11.972	6.421	-0.614	24.557	
Apo A4(+347)	6.326	5.747	-4.938	17.590	
Logarithm Triglyceride					
ApoA1 (-75)	-0.047	0.027	-0.100	0.006	
Apo A1 (+83)	-0.136	0.100	-0.332	0.061	
Apo C3 (+3175)	0.060	0.035	-0.009	0.129	
Apo C3 (+3206)	0.059	0.040	-0.019	0.138	
Apo A4(+347)	0.070	0.035	0.002	0.139	

(4) Non-parametric mode of inheritance

The adjusted mean values standard error of mean, difference between three genotypes, and corresponding P values of five loci in the Apo AI-CIII-AIV gene clusters, after adjusting for age and gender effect, under generalized estimation equation model

	Annotation I	Annotation II	Mean	SEM	Difference
Logarithm Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)	0	11	0.936	0.041	
	1	12	0.896	0.049	
	2	22	0.772	0.082	
Apo A1 (+83)	0	11	0.914	0.033	
	1	12	0.679	0.112	
	2	22			
Apo C3 (+3175)	0	11	0.864	0.045	
	1	12	0.922	0.044	
	2	22	1.024	0.084	
Apo C3 (+3206)	0	11	0.746	0.157	
	1	12	0.887	0.047	
	2	22	0.918	0.039	
Apo A4(+347)	0	11	0.851	0.046	
	1	12	0.905	0.045	
	2	22	1.053	0.076	
Triglyceride vs. HDL cholesterol ratio					
ApoA1 (-75)	0	11	3.437	0.197	
	1	12	3.788	0.360	
	2	22	3.314	0.585	
Apo A1 (+83)	0	11	3.626	0.220	
	1	12	2.731	0.490	
	2	22			
Apo C3 (+3175)	0	11	3.525	0.285	
	1	12	3.589	0.292	
	2	22	3.676	0.390	
Apo C3 (+3206)	0	11	2.741	0.561	
	1	12	3.298	0.237	
	2	22	3.761	0.275	
Apo A4(+347)	0	11	3.356	0.241	

	1	12	3.712	0.341	
	2	22	3.860	0.312	
HDL cholesterol					
ApoA1 (-75)	0	11	42.56	0.64	
	1	12	43.19	0.79	
	2	22	45.27	1.54	
Apo A1 (+83)	0	11	42.88	0.56	
	1	12	47.15	1.60	
	2	22			
Apo C3 (+3175)	0	11	43.68	0.75	
	1	12	42.77	0.72	
	2	22	42.33	1.16	
Apo C3 (+3206)	0	11	43.19	1.77	
	1	12	42.86	0.83	
	2	22	43.38	0.63	
Apo A4(+347)	0	11	43.71	0.71	
	1	12	43.07	0.76	
	2	22	42.46	1.21	
Triglyceride					
ApoA1 (-75)	0	11	127.41	5.00	
	1	12	132.42	8.01	
	2	22	121.57	11.42	
Apo A1 (+83)	0	11	129.55	4.77	
	1	12	117.40	20.72	
	2	22			
Apo C3 (+3175)	0	11	127.41	6.54	
	1	12	129.42	6.42	
	2	22	134.53	9.91	
Apo C3 (+3206)	0	11	104.92	16.64	
	1	12	123.02	6.35	
	2	22	133.48	5.67	
Apo A4(+347)	0	11	125.27	6.72	
	1	12	128.90	6.58	
	2	22	140.81	8.61	
Logarithm Triglyceride					
ApoA1 (-75)	0	11	4.658	0.031	
	1	12	4.621	0.037	

	2	22	4.553	0.058	
Apo A1 (+83)	0	11	4.638	0.026	
	1	12	4.502	0.096	
	2	22			
Apo C3 (+3175)	0	11	4.601	0.034	
	1	12	4.649	0.035	
	2	22	4.743	0.067	
Apo C3 (+3206)	0	11	4.484	0.124	
	1	12	4.610	0.037	
	2	22	4.653	0.030	
Apo A4(+347)	0	11	4.594	0.036	
	1	12	4.633	0.034	
	2	22	4.769	0.061	