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ORIGINAL ARTICLE

Haplotype analysis of Apo AI-CIII-AIV gene cluster and lipids level: Tehran lipid and glucose study

Maryam S. Daneshpour · Bita Faam · Mohamad Ali Mansournia · Mehdi Hedayati · Sohrab Halalkhor · Seyed Alireza Mesbah-Namin · Shahla Shojaei · Maryam Zarkesh · Fereidoun Azizi

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Abstract Iranian populations show an increased tendency for abnormal lipid levels and high risk of Coronary artery disease. Considering the important role played by the ApoAI-CIII-AIV gene cluster in the regulation of the level and metabolism of lipids, this study aimed at elucidating the association between five single nucleotide polymorphisms on the Apo11q cluster gene and lipid levels. A cross-sectional study of 823 subjects (340 males and 483 females) from the Tehran lipid and glucose study (TLGS) was conducted. Levels of TG, Chol, HDL-C, Apo AI, Apo AIV, Apo B, and Apo CIII were measured, and the selected segments of the APOAI-CIII-AIV gene cluster were amplified by PCR and the polymorphisms were revealed by RFLP using restriction enzymes. The allele frequencies for each SNP between males and females were not significantly different. The distribution of Genotypes and alleles was in Hardy–Weinberg equilibrium except for Apo AI (+83C>T). The results showed a significant association between TG, HDL-C, HDL_2, Apo AI, and Apo B levels and the presence of some alleles in the polymorphisms studied. After haplotype analysis not only did the association between these variables and SNPs remain but also levels of Chol and LDL-C were added. This study demonstrates that the level of lipids such as TG, HDL-C, HDL_2, Apo AI, and Apo B, maybe regulated partly by genetic factors and their haplotype within the Apo11q gene cluster.

Keywords AI-CIII-AIV gene cluster · Coronary artery disease · Haplotype · Lipid and TLGS

M. S. Daneshpour · B. Faam · M. Hedayati · S. Shojaei · M. Zarkesh
Obesity Research Center, Research Institute for Endocrine Sciences, Shaheed Beheshti University of Medical Sciences, P.O. Box 19195-4763, Tehran, I.R. Iran

M. A. Mansournia
Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, I.R. Iran

S. Halalkhor · S. A. Mesbah-Namin
Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, I.R. Iran

F. Azizi (✉)
Endocrine Research Center, Research Institute for Endocrine Sciences, Shaheed Beheshti University of Medical Sciences, Tehran, I.R. Iran
e-mail: azizi@endocrine.ac.ir

Introduction

One of the most important multi factorial diseases is coronary artery disease (CAD). Genetic and environmental factors play an important role in complex disorders. Lipids and lipoproteins increase the risk of CAD. The Apo AI-CIII-AIV gene cluster, located on the 11q23, is the most characterized region in the human genome. This gene cluster is associated with the levels of lipids and lipoproteins [1]. According to a molecular study, the interaction between this region and other genetic variations has important effects on lipids [2]. Approximately 182 single nucleotide polymorphisms (SNPs) and four ins/del variants in this genetical region are reported [3]. A complex pattern of gene expression has been demonstrated in vivo studies, wherein the Apo CIII enhancer acts as a common regulatory element for the ApoAI-CIII-AIV gene cluster [4]. Based on population-based studies, there is an association between the level of TG and the ApoAI-CIII-AIV gene cluster in individuals with hyper triglyceridemia [5]. Increasing the level of TG is a major independent risk

factor for CAD across populations from America [6, 7], Europe [8, 9], and Asia [10, 11]. In addition, there are other studies assessing the association between ApoE and ApoB gene polymorphisms with lipid levels in Iranians [12], and they show that this population has an inherent tendency for dyslipidemia associated with high risk of CAD [12, 13]. The aim of this study was to investigate the association between the −75G>A and +83C>T SNPs in Apo AI gene, 360G>T SNP in exon 3 of Apo AIV gene and the −482C>T, and 3238C>G SNPs of Apo CIII gene, with lipids levels.

Materials and methods

Subjects and biochemical variables: Subjects were selected from the Tehran Lipid and Glucose Study (TLGS), designed to determine the risk factors for major non-communicable disorders in a Tehranian population [14]. Written informed consent was obtained from each subject and the research council of the Research Institute of Endocrine Sciences of the Shahid Beheshti University of Medical Sciences, approved this study. At study entry, all subjects answered a questionnaire covering data on demographic factors and smoking habits [15]. In this cross-sectional study, subjects were 823 randomly selected Tehranian adult aged 19–70 years. Biochemical variables were measured as described previously [16, 17]. LDL-C concentrations were calculated using Friedewald's equation [18]. Coefficients of variation (CV) for total cholesterol, HDL-C and triglyceride measurements were below 5%.

Genetic analysis

DNA was extracted by Proteinase K, salting out method [5, 19]. For genotyping the fragments of selected genes, PCRs were done by using the following primers; AI (−75G>A and +83C>T) (F: 5′-AGG GAC AGA GCT GAT CCT TGA ACT CTT AAG-3′; R: 5′-TTA GGG GCA CCT AGC CCT CAG GAA GAG AGC A-3′); AIV(360G>T) (F: 5′-CCT GAG GGA CAA GGT CAA CTC-3′; R: 5′-CAC CTG CTC CTG CTA CTG CTC C-3′); CIII(3238C>G) (F: 5′-GGT GAC CGA TGG CTT CAG TT-3′; R:5′-CAG AAG GTG GAT AGA GCG CT-3′); CIII(-482C>T) (F: 5′-GGT GAC CGA TGG CTT CAG TT-3′; R:5′-TCA CAC TGG AAT TTC AGG CC-3′). Hybridization was carried out in a DNA Thermal cycler (Corbett co. Australia) in which the DNA templates annealing temperatures were: 60°C (AI, AIV), 55°C (CIII3228), and 58°C (CIII 482). For Apo AI polymorphisms after restriction enzyme digestion, $M_1$and M_2 allele were detected [20], and the products obtained by SstI digestion, were submitted [21]. For AIV polymorphism, restriction fragment length polymorphism products were detected [22]. The details of restriction enzyme and digestion of CIII3228 and CIII482 polymorphisms were determined [23].

Definition of terms

Cigarette smokers included daily smokers defined as those who smoke cigarettes at least once a day and never smokers (never smoked before or smoked too little in the past) categorized in the non smoker groups. Participants were classified as diabetic if they met at least one of these criteria: FPG $\geq$ 7 mmol/l, or 2 h-PCPG $\geq$ 11.1 mmol/l or taking anti-diabetic medication.

Statistical analysis

Continuous data were presented as mean ± SD, whereas, categorical variables were summarized as frequencies and percentages. One-way analysis of variance (ANOVA) was used for comparisons of genotype with lipid variables by normal distribution. If necessary, a logarithmic transformation was performed to normalize the error distribution and stabilize the error variance. Kruskale-Wallis Analysis was used for other variables. Five genetic models were considered: A co-dominant model (three genotype groups separated), a dominant model (heterozygotes grouped with the homozygotes for the minor allele); a recessive model (heterozygotes grouped with the homozygotes for the major allele); a log-additive model (scores were assigned counting the number of minor alleles: score 0 for the homozygotes of the major allele, score 1 for the heterozygotes, and score 2 for the homozygotes of the minor allele) and an over dominant model (homozygotes for the major allele grouped with the homozygotes for the minor allele). The Akaike information criterion was used to choose the genetic model that best fits the data. Multiple linear regressions was used to control the effects of potential confounders. For each SNP, deviation from Hardy–Weinberg equilibrium was tested using Fisher's exact test, as implemented by the HWE exact function in the R package "genetics". Pair-wise linkage disequilibrium between SNPs was estimated by pairwise Lewontin's D' and product-moment correlation coefficient (r) using LD function in the R package "genetics". Haplotype frequencies were estimated using the Haplo.em, an implementation of the EM algorithm included in the R package "haplo.stats". The association of haplotypes with lipid variables was examined by means of the haplo.glm function of the R package "haplo.stats". Statistical analyses were performed using SPSS version 16 (SPSS, Inc., Chicago, IL), STATA version 9.1 (Stata Corp LP, College Station, TX) and R version 2.10.1 (http://www.r-project.org). For all

tests, a 2-sided p value below 0.05 was considered significant.

Results

Associations of Apo AI-CIII-AIV genotypes with lipids profiles levels: The demographics, biochemical characteristics, and allele frequency of the selected subjects are shown in Table 1. In females, the means of BMI, Apo AI, CIII, and HDL-C and its sub fractions, were significantly increased, whereas in males only systolic and diastolic blood pressure were increased. The results show that more males are smokers, while more females are diabetic. In all cases, genotypes and alleles distributions were in Hardy–Weinberg equilibrium, except for Apo AI (+83C>T). Allele frequencies for each SNP in males and females were not significantly different. The means of anthropometric and biochemical variables in the three groups of genotype and different genetic models for confounders analysis are presented in Tables 2 and 3, respectively. In the presence of the T allele in the CIII (−482C>T) polymorphism, HDL-C and HDL_2 concentrations significantly increased (TT: 49.1 ± 1.3 vs. CC: 45.7 ± 1.2 mg/dl *P* 0.036; HDL-C); (TT^{-}: 18.3 ± 1.6 vs. CC: 14.8 ± 1.6 mg/dl *P* 0.015; HDL_2); these associations were retained following covariate adjustment for sex and BMI, based on recessive model (*P* 0.0130, 0.003, respectively). In the CIII (3238C>G) polymorphism, presence of the G allele significantly increased the triglyceride concentration (GG:

Table 1 Summery of demography and biochemistry characters

Variables	Total (n = 823)	Males (n = 340)	Females (n = 483)	P
Age (years)	44.4 ± 15.8	46.1 ± 16.5	43.3 ± 15.1	0.011*
Body mass index (kg/m^2)	27.4 ± 4.79	26.2 ± 3.87	28.2 ± 5.20	<0.001
Cigarette smokers (%)	9.5	19.5	2.5	<0.001
Lipid drug (%)	5.7	3.5	7.2	0.024
Diabetes mellitus drug (%)	6.0	4.7	6.8	NS
Triglyceride (mg/dl)	149 ± 86.7	153 ± 75.4	146 ± 93.8	NS
Total cholesterol (mg/dl)	191 ± 40.9	189 ± 36.4	192 ± 43.8	NS
HDL cholesterol (mg/dl)	46.1 ± 11.4	42.3 ± 9.50	48.8 ± 11.9	<0.001
HDL_2 (mg/dl)	16.5 ± 8.10	13.2 ± 6.21	18.8 ± 8.49	<0.001
HDL_3 (mg/dl)	29.9 ± 6.97	29.1 ± 6.43	30.5 ± 7.29	0.008
LDL cholesterol (mg/dl)	115 ± 36.8	115 ± 33.7	115 ± 38.8	NS
Diastolic blood pressure (mm Hg)	73.9±10.1	75.5 ± 9.72	72.7 ± 10.1	<0.001
Systolic blood pressure (mm Hg)	115 ± 18.7	117 ± 17.5	114 ± 19.3	0.004
Apo lipoprotein A_I (mg/dl)	144 ± 33.1	133 ± 27.9	152 ± 34.1	<0.001
Apo lipoprotein B (mg/dl)	113 ± 35.9	115 ± 34.5	112 ± 36.8	NS
Apo lipoprotein AIV (mg/dl)	19.6 ± 8.43	20.7 ± 9.76	18.9 ± 7.42	NS
Apo lipoprotein CIII (mg/dl)	145 ± 68.6	125 ± 54.9	158 ± 73.5	0.006
Apo lipoprotein CIII (3238C>G) allele frequency (%)				
C	83.27	84.78	83.98	NS
G	16.73	15.22	16.02	
Apo lipoprotein CIII (−482C>T) allele frequency (%)				
C	63.68	65.26	64.21	NS
T	36.32	34.74	35.79	
Apo lipoprotein AI (M1) (−75G>A) allele frequency (%)				
X+	86.22	87.08	85.71	NS
X−	13.78	12.92	14.29	
Apo lipoprotein A1 (M2) (+83C>T) allele frequency (%)				
X+	94.62	94.28	94.57	NS
X−	5.38	5.72	5.43	
Apo lipoprotein AIV (360G>T) allele frequency (%)				
G	92.36	91.88	92.21	NS
T	7.64	8.13	7.79	

* *P* values show the differences between genders

Table 2 Association of genotypes with lipid profile variables

	CIII-482C>T			CIII3238C>G			AI-75G>A		
	CC ($n = 199$)	CT ($n = 207$)	TT ($n = 62$)	CC ($n = 561$)	CG ($n = 205$)	GG ($n = 21$)	X^+X^+ ($n = 436$)	X^+X^- ($n = 139$)	X^-X ($n = 11$)
Age (years)	45.8 ± 16.3	44.1 ± 16.3	42.8 ± 15.8	44.1 ± 15.9	44.7 ± 15.6	43.2 ± 17.7	43.6 ± 15.3	44.8 ± 16.8	46.1 ± 15.1
BMI (kg/m^2)	27.4 ± 1.2	26.6 ± 1.2	26.9 ± 1.2	26.9 ± 1.2	26.9 ± 1.2	28.7 ± 1.2	26.9 ± 1.2	27.2 ± 1.2	27.1 ± 1.2
Smokers (%)	11.5	8.2	6.4	10	7.3	0	10.8	5	9
DI (%)	6.1	4.3	6.1	4.7	6	5.6	4.6	7.2	0
DM (%)	2.7	8	4.1	3.7	7.8	11.1	5.1	5	9.1
TG (mg/dl)	125 ± 1.6	135 ± 1.8	121 ± 1.6	125 ± 1.6	142 ± 1.6	167 ± 1.6*	132 ± 1.6	132 ± 1.6	140 ± 1.5
Total Chol (mg/dl)	190 ± 1.2	189 ± 1.2	186 ± 1.2	186 ± 1.2	192 ± 1.2	188 ± 1.2	188 ± 1.2	189 ± 1.2	183 ± 1.8
HDL-C (mg/dl)	45.7 ± 1.2	44.9 ± 1.3	49.1 ± 1.3*	44.8 ± 1.3	45.2 ± 1.3	43.9 ± 1.3	44.9 ± 1.3	44.9 ± 1.3	40.5 ± 1.2
HDL_2 (mg/dl)	14.8 ± 1.6	15.3 ± 1.6	18.3 ± 1.6*	14.6 ± 1.6	15.4 ± 1.6	15.1 ± 1.9	14.5 ± 1.6	15.1 ± 1.6	12.4 ± 1.6
HDL_3 (mg/dl)	29.8 ± 1.2	28.8 ± 1.2	30.1 ± 1.2	29.2 ± 1.2	29.8 ± 1.1	26.6 ± 1.3	29.7 ± 1.3	29.2 ± 1.21	27.2 ± 1.8
LDL-C (mg/dl)	113 ± 1.4	108 ± 1.4	106 ± 1.3	109 ± 1.4	112 ± 1.4	105 ± 1.5	109 ± 1.4	110 ± 1.4	109 ± 1.2
SBP (mm Hg)	115 ± 1.2	113 ± 1.2	113 ± 1.2	114 ± 1.7	115 ± 1.6	122 ± 1.2	114 ± 1.2	113 ± 1.2	117 ± 1.1
DBP (mm Hg)	74.2 ± 1.1	73.2 ± 1.2	74.1 ± 1.1	73.1 ± 1.1	73.7 ± 1.2	75.2 ± 1.2	73.3 ± 1.2	71.7 ± 1.1	74.2 ± 1.0
Apo AI (mg/dl)	143 ± 1.2	145 ± 1.3	148 ± 1.3	139 ± 1.2	146 ± 1.3	141 ± 1.3*	143 ± 1.2	143 ± 1.3	144 ± 1.1
Apo B (mg/dl)	113 ± 1.4	111 ± 1.4	106 ± 1.3	107 ± 1.4	113 ± 1.4	103 ± 1.5*	110 ± 1.3	110 ± 1.4	126 ± 1.2
Apo AIV (mg/dl)	16.9 ± 1.6	16.5 ± 1.6	16.5 ± 1.4	17.4 ± 1.6	18.7 ± 1.5	17.8 ± 1.7	18.1 ± 1.5	19.6 ± 1.5	13.4
Apo CIII (mg/dl)	130 ± 1.7	130 ± 1.6	114 ± 2.9	129 ± 1.9	130 ± 1.6	154 ± 1.0	123 ± 1.6	151 ± 1.6	114

	AI+83C>T			AIV360G>T		
	X^+X^+ ($n = 528$)	X^+X^- ($n = 51$)	X^-X^- ($n = 7$)	TT ($n = 2$)	TG ($n = 117$)	GG ($n = 644$)
Age (years)	44.2 ± 15.7	39.8 ± 13.7	52.7 ± 14.7	31.1 ± 4.2	44.2 ± 15.7	44.2 ± 15.9
BMI (kg/m^2)	27.1 ± 1.2	25.9 ± 1.2	25.3 ± 1.1	24.4 ± 1.2	27.1 ± 1.2	27.1 ± 1.2
Smokers (%)	9.3	12	0	0	8.5	10
DI (%)	5.5	2	0	0	3.2	5.3
DM (%)	5.3	2	14.3	0	3.2	5.3
TG (mg/dl)	133 ± 1.6	120 ± 1.7	128 ± 1.6	90.6 ± 2.2	138 ± 1.7	130 ± 1.6
Total Chol (mg/dl)	188 ± 1.2	180 ± 1.2	206 ± 1.2	169 ± 1.3	192 ± 1.2	186 ± 1.2
HDL-C (mg/dl)	44.8 ± 1.3	45.2 ± 1.3	47.7 ± 1.2	49.4 ± 1.3	43.3 ± 1.2	44.9 ± 1.3
HDL_2 (mg/dl)	14.6 ± 1.6	15.3 ± 1.7	15.2 ± 1.9	19.7 ± 1.9	14.1 ± 1.2	14.7 ± 1.6
HDL_3 (mg/dl)	29.6 ± 1.2	29.4 ± 1.3	30.7 ± 1.2	28.7 ± 1.0	28.2 ± 1.2	29.3 ± 1.2
LDL-C (mg/dl)	110 ± 1.4	105 ± 1.4	121 ± 1.4	95.1 ± 1.6	111 ± 1.5	109 ± 1.4
SBP (mm Hg)	114 ± 1.2	110 ± 1.2	118 ± 1.2	113 ± 1.0	114 ± 1.2	114 ± 1.2
DBP (mm Hg)	73.1 ± 1.1	71.7 ± 1.1	68.5 ± 1.1	75.5 ± 1.3	74.2 ± 1.2	73.2 ± 1.1

Table 2 continued

	AI+83C>T			AIV360G>T		
	X^+X^+ ($n = 528$)	X^+X^- ($n = 51$)	X^-X^- ($n = 7$)	TT ($n = 2$)	TG ($n = 117$)	GG ($n = 644$)
Apo AI (mg/dl)	143 ± 1.2	144 ± 1.3	145 ± 1.3	105 ± 1.0	135 ± 1.2	142 ± 1.2*
Apo B (mg/dl)	111 ± 1.4	107 ± 1.3	124 ± 1.4	62.9 ± 1.6	107 ± 1.4	108 ± 1.4*
Apo AIV (mg/dl)	18.3 ± 1.5	18.9 ± 1.3	17.8 ± 1.6	10.2	17.5 ± 1.6	17.9 ± 1.5
Apo CIII (mg/dl)	127 ± 1.6	125 ± 1.5	210 ± 1.4	95.4	111 ± 1.5	131 ± 1.6

* P value < 0.05

BMI Body mass index, *DL* Lipid drug, *DM* Diabetes mellitus drug, *Chol* Cholesterol, *TG* triglyceride, *HDL* High density lipoprotein, *LDL* Low density lipoprotein, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *Apo* Apolipoprotein

167 ± 1.6 vs. CC: 125 ± 1.6 mg/dl P 0.001). Age, sex, BMI, and Diabetes Mellitus Drug usage (dm) were common confounding factors in the additive model that showed the same significant interaction ($P < 0.001$); for this polymorphism, the level of Apo B and Apo AI in the presence of CG is the highest (GG: 103 ± 1.5 vs. CG: 113 ± 1.4 vs. CC: 107 ± 1.4 mg/dl P 0.056); (GG: 141 ± 1.3 vs. CG: 146 ± 1.3 vs. CC: 139 ± 1.2 mg/dl P 0.027), respectively; therefore, the best model for assessing the effects of covariates was the over dominant model which was selected according to the AIC factor. These associations were significant after adjustment for suitable contributory factors based on the over dominant model (P 0.017, 0.004, respectively). No significant association between Apo AI polymorphisms and studied variables was seen. Results show that the G allele significantly increased Apo B and AI levels across the AIV360G>T genotypes (TT: 62.9 ± 1.6 vs. GG: 108 ± 1.4 mg/dl P 0.047; Apo B); (TT: 105 ± 1.0 vs. GG: 142 ± 1.2 mg/dl P 0.014; Apo AI). The association between AIV360G>T SNP and Apo AI was retained following adjustment of covariates for age, sex and dl by additive model (P 0.008).

Linkage disequilibrium across the Apo AI-CIII-AIV gene cluster: The assessment for the genotypic associations could be explained by the linkage disequilibrium (LD). The two Apo AI variants showed strong positive allelic association with each other (D' 0.991), and there was a strong association between ApoA1 (−75G>A) and Apo AIV (360G>T) (D' 0.994) (Table 4).

Identification the lipid factors with Apo AI-CIII-AIV haplotype: The allele frequency of Apo AI (+83C>T) SNP was not in the Hardy–Weinberg equilibrium; therefore, it was omitted from the haplotype groups. From the 16 theoretically possible haplotypes derived from all four polymorphic sites, only 14 were represented that their frequencies were higher than 1% in the selected population. The most common haplotype (45.7%) was defined by the common alleles of the four variant sites named the haplotype base which had not significant association with lipid factors. Haplotypes with frequencies above 0.10 were X^+GTC (0.191) and X^+GTG (0.111). Association of the X^+GTC with HDL-C (P 0.028) and HDL_2 (P 0.001) were significant. After adjustment for sex, age, and BMI for HDL-C, only a trend can be seen (P 0.083). The association with HDL_2 remained significant after adjustment for sex, BMI, dm, and smoking (P 0.005) (Fig. 1a). The haplotypes that were associated with triglyceride, HDL_2 and Apo AI concentration, carried the −482C>T and 3238C>G rare alleles, compared with the common background (X^+GTG) (P values: TG, 0.001; HDL_2, 0.007; apo AI, 0.019). These associations were significant after adjustment for BMI, age, lipid drug, and dm for triglyceride (P 0.003); sex, BMI, lipid drug, and smoking for

Table 3 The relation between ApoAI/CIII/AIV gene cluster and biochemical variables after adjustment

Variables	Genetics' models	Estimate	*P* value	(CI 95%)
Apo AIV/[APOAI]	Additive	0.021	0.008	(−0.096, −0.014)
ApoCIII3238/[ApoAI]	Over dominant	0.051	0.004	(0.016, 0.086)
ApoCIII3238/[TG]	Additive	0.123	0	(0.062, 0.185)
ApoCIII3238/[ApoB]	Over dominant	0.059	0.017	(0.010, 0.108)
ApoCIII482/[HDL2]	Recessive	0.185	0.003	(0.062, 0.307)
ApoCIII482/[HDL]	Recessive	0.075	0.013	(0.016, 0.134)

Model AIV/[Apo AI]: Age + sex + lipid drug
Model ApoCIII/[ApoAI]: Age + Sex + dm
Model ApoCIII/[ApoB]: Age + Smoking + dm
Model ApoCIII482/[HDL2]: Sex + BMI
Model ApoCIII482/[HDL]: Sex + BMI
Model ApoCIII/[TG]: Age + Sex + BMI + dm

Table 4 Linkage disequilibrium between all the variants under study in the ApoAI/CIII/AIV gene cluster

	ApoAI −75G>A	ApoAI+83C>T	ApoAIV 360G>T	ApoCIII −482C>T	ApoCIII3238C>G
ApoAI −75G>A		0.991($P = 0.001$)	0.994 ($P < 0.005$)	0.270 ($P = 0.032$)	0.522 ($P = 0.002$)
ApoAI+83C>T			0.535 ($P = 0.199$)	0.313 ($P = 0.005$)	0.448 ($P = 0.114$)
ApoAIV+360G>T				0.156 ($P = 0.314$)	0.126 ($P = 0.538$)
ApoCIII −482C>T					0.626 ($P < 0.005$)
ApoCIII3238C>G					

HDL_2 (*P* 0.007) and age, lipid drug, sex, and dm for Apo AI (*P* 0.028), respectively (Fig. 1b). The levels of Apo lipoprotein B and LDL-C were affected by X-GTC haplotype (*P* value: Apo B; 0.025, LDL-C 0.016). These associations were retained following covariate adjustment for age, dm, smoking, and BMI for ApoB and for BMI, age, and smoking for LDL-C, respectively (*P* value: Apo B 0.008, LDL-C 0.038) (Fig. 1c).

Discussion

This study demonstrates the association between selected genetic variations in the Apo AI-CIII-AIV gene cluster and the levels of lipid related factors. Concentrations of TG, HDL-C, HDL_2, ApoAI, and ApoB maybe changed in the presence of some alleles of these SNPs. After haplotype analysis, not only did the associations of these variables with polymorphisms remain, but the cholesterol and LDL-C were added. The results of this study represent an approach to the association between genetic with lipid profiles in Tehranians. CAD is a complex and multi factorial disorder with strong interaction between the genetics and environmental factors [24], which could differ from one population to another [10, 25]. Apolipoprotein genes which play an important role in lipoprotein synthesis and their metabolism, are excellent candidates for research. There are many reports about the association between apolipoprotein gene polymorphisms and lipid factors [26].

In this study, levels of TG and ApoA1 were increased in the presence of G allele in the CIII (3238C>G) polymorphism; also the level of these variables were affected by the X^+GTG haplotype that carry G allele for this SNP. In the same polymorphism, the presence of C allele was associated with increased level of ApoB. It was shown that the concentration of ApoB was related to the X^-GTC haplotype. The level of HDL-C increased in the presence of T allele of CIII (482C>T) and X^+GTC haplotype. Presence of the T allele in ApoAIV SNP and X^-GTC and X^+GTG haplotypes had increased associations with levels of ApoB and ApoAI. There was no association between two ApoAI SNPs with lipids profiles. A study in 2007 showed that The SstI polymorphism of ApoAI-CIII-AIV cluster differentiated the plasma levels of TG and Apo AI [27]. Another 2002 study showed that the ApoCIII-AIV-AV gene cluster is associated with TG level [28]. Data showed that genetic variations in the APOAI-CIII-AIV gene cluster are likely to be significant marker for clinical studies.[29] In a study of Asian-Indians, it was found that Sac-1 and −75G>A SNPs had significant associations with total cholesterol, TG, and ApoB levels. The results of this study also show that the APOAI-CIII-AIV gene cluster is related to 1% of the

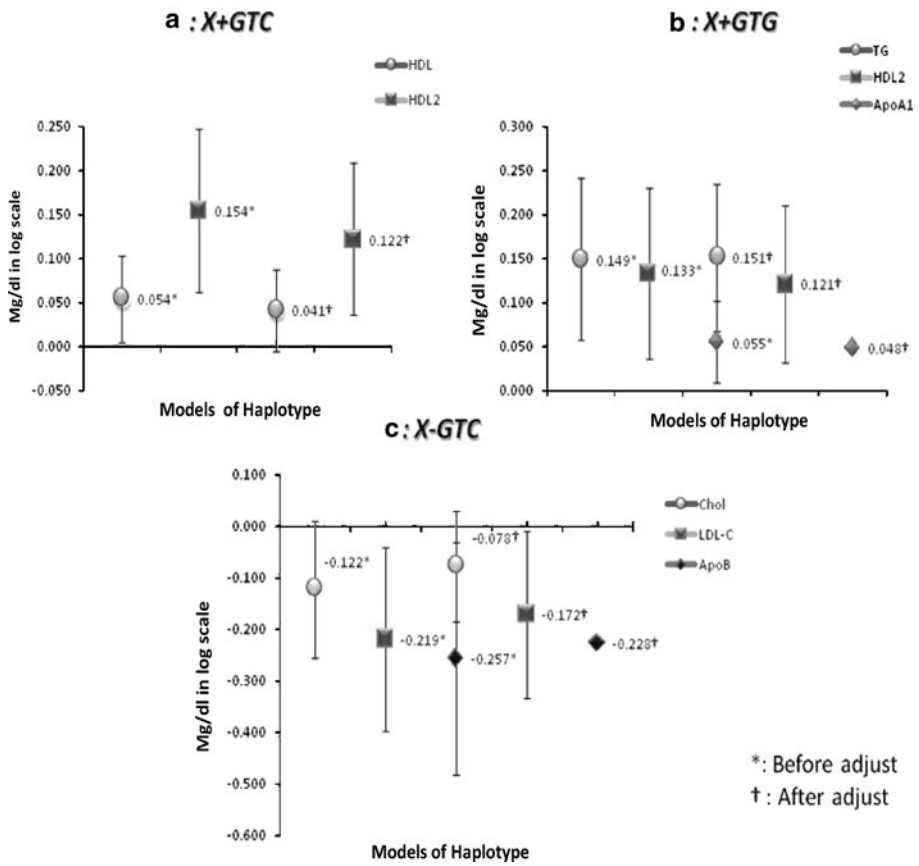

Fig. 1 Coefficient changes of haplotypes before and after adjustment: ApoAI −75G>A, ApoAIV 360G>T, ApoCIII −482C>T and ApoCIII3238C>G

variation in the lipid levels [29]. According to another study, the ApoCIII SNP is associated with CAD through its interaction with lipids levels [30].The present study has some shortcomings. First, the size of our study population was small, which might not have had the power to detect direct evidence. Second, only four SNPs were evaluated in this region, in spite of the region spanning a large genomic distance. The whole sequencing of these genes in case–control or family based studies is suggested.

In conclusion, the results of this and other studies confirmed that, the levels of the lipids and lipoproteins are regulated partly by the genetic factors and their haplotype within the Apo11q gene cluster.

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References

1. L.A. Pennacchio, M. Olivier, J.A. Hubacek, J.C. Cohen, D.R. Cox, J.C. Fruchart, R.M. Krauss, E.M. Rubin, An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science **294**, 169–173 (2001)
2. Q.F. Wang, X. Liu, J. O'Connell, Z. Peng, R.M. Krauss, D.L. Rainwater, J.L. VandeBerg, E.M. Rubin, J.F. Cheng, L.A. Pennacchio, Haplotypes in the APOA1-C3-A4-A5 gene cluster affect plasma lipids in both humans and baboons. Hum. Mol. Genet. **13**, 1049–1056 (2004)
3. M. Olivier, X. Wang, R. Cole, B. Gau, J. Kim, E.M. Rubin, L.A. Pennacchio, Haplotype analysis of the apolipoprotein gene cluster on human chromosome 11. Genomics **83**, 912–923 (2004)
4. J. Gao, Y. Wei, Y. Huang, D. Liu, G. Liu, M. Wu, L. Wu, Q. Zhang, Z. Zhang, R. Zhang, C. Liang, The expression of intact and mutant human apoAI/CIII/AIV/AV gene cluster in transgenic mice. J. Biol. Chem. **280**, 12559–12566 (2005)
5. M. Daneshpour, M. Hedayati, P. Eshraghi, F. Azizi, Association of Apo E gene polymorphism with HDL level in a Thehranian population. Eur. J. Lipid Sci. Technol. (2010 in press)
6. S. Eichenbaum-Voline, M. Olivier, E.L. Jones, R.P. Naoumova, B. Jones, B. Gau, H.N. Patel, M. Seed, D.J. Betteridge, D.J. Galton, E.M. Rubin, J. Scott, C.C. Shoulders, L.A. Pennacchio, Linkage and association between distinct variants of the APOA1/C3/A4/A5 gene cluster and familial combined hyperlipidemia. Arterioscler. Thromb. Vasc. Biol. **24**, 167–174 (2004)
7. R. Mar, P. Pajukanta, H. Allayee, M. Groenendijk, G. Dallinga-Thie, R.M. Krauss, J.S. Sinsheimer, R.M. Cantor, T.W. de Bruin, A.J. Lusis, Association of the APOLIPOPROTEIN A1/C3/A4/A5 gene cluster with triglyceride levels and LDL particle size in familial combined hyperlipidemia. Circ. Res. **94**, 993–999 (2004)
8. M. Groenendijk, R.M. Cantor, T.W. de Bruin, G.M. Dallinga-Thie, The apoAI-CIII-AIV gene cluster. Atherosclerosis **157**, 1–11 (2001)
9. D.M. Hallman, S.R. Srinivasan, W. Chen, E. Boerwinkle, G.S. Berenson, Longitudinal analysis of haplotypes and polymorphisms of the APOA5 and APOC3 genes associated with

variation in serum triglyceride levels: the Bogalusa heart study. Metabolism **55**, 1574–1581 (2006)
10. M.C. Huang, T.N. Wang, Y.L. Liu, T.H. Pa, H.P. Tu, Y.C. Huang, W.T. Chang, Y.C. Ko, Effect of SstI polymorphism of the apolipoprotein CIII gene and environmental factors on risks of hypertriglyceridemia in Taiwan aborigines. Circ. J. **70**, 1030–1036 (2006)
11. J. Song, J.W. Park, H. Park, J.Q. Kim, Linkage disequilibrium of the Apo AI-CIII-AIV gene cluster and their relationship to plasma triglyceride, apolipoprotein AI and CIII levels in Koreans. Mol. Cells **8**, 12–18 (1998)
12. M.S. Daneshpour, M. Hedayati, P. Eshraghi, M. Hooshmand, F. Azizi, Asociation of apolipoprotein E gene polymorphism and lipid level in an Iranian population: Tehran Lipid and Glucose study. Iran. J. Diabet. Lipid **7**, 399–405 (2008)
13. M.S. Daneshpour, M. Hedayati, F. Azizi, Association of the TaqI and −629C>A polymorphisms in CETP gene with low level of HDL-C in Tehran. Pajouhesh Q. Res. J. **29**, 13–17 (2005)
14. F. Azizi, M. Rahmani, H. Emami, P. Mirmiran, R. Hajipour, M. Madjid, J. Ghanbili, A. Ghanbarian, Y. Mehrabi, N. Saadat, P. Salehi, N. Mortazavi, P. Heydarian, N. Sarbazi, S. Allahverdian, N. Saadati, E. Ainy, S. Moeini, Cardiovascular risk factors in an Iranian urban population: Tehran lipid and glucose study (phase 1). Soz. Praventivmed. **47**, 408–426 (2002)
15. F. Azizi, M. Rahmani, H. Emami, M. Madjid, Tehran lipid and glucose study: rationale and design. CVD Prev. **3**, 242–247 (2000)
16. F. Azizi, H. Emami, P. Salehi, A. Ghanbarian, P. Mirmiran, M. Mirbolooki, T. Azizi, Cardiovascular risk factors in the elderly: the Tehran lipid and glucose study. J. Cardiovasc. Risk **10**, 65–73 (2003)
17. M. Daneshpour, M. Hedayati, P. Eshraghi, and F. Azizi, Association of Apo E gene polymorphism with HDL level in a Tehranian population. Eur J. Lipid Sci. Technol. (2010)
18. W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. **18**, 499–502 (1972)
19. G.E. Truett, P. Heeger, R.L. Mynatt, A.A. Truett, J.A. Walker, M.L. Warman, Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques **29**, 52–54 (2000)
20. Y. Zou, D. Hu, X. Yang, X. Jia, L. Wang, L. Cui, X. Liu, M. Gao, Y. Wei, Z. Xu, Relationships among apolipoprotein A1 gene polymorphisms, lipid levels and coronary atherosclerosis disease. Chin. Med. J. (Engl) **116**, 665–668 (2003)
21. J. Salas, S. Jansen, J. Lopez-Miranda, J.M. Ordovas, P. Castro, C. Marin, M.A. Ostos, M.D. Bravo, J. Jimenez-Pereperez, A. Blanco, F. Lopez-Segura, F. Perez-Jimenez, The SstI polymorphism of the apolipoprotein C-III gene determines the insulin response to an oral-glucose-tolerance test after consumption of a diet rich in saturated fats. Am. J. Clin. Nutr. **68**, 396–401 (1998)
22. T.F. Ejchel, L.M. Araujo, L.R. Ramos, M.S. Cendoroglo, M. de Arruda Cardoso Smith, Association of the apolipoprotein A-IV: 360 Gln/His polymorphism with cerebrovascular disease, obesity, and depression in a Brazilian elderly population. Am. J. Med. Genet. B **135B**, 65–68 (2005)
23. S. Tas, Strong association of a single nucleotide substitution in the 3′-untranslated region of the apolipoprotein-CIII gene with common hypertriglyceridemia in Arabs. Clin. Chem. **35**, 256–259 (1989)
24. E.J. Topol, J. Smith, E.F. Plow, Q.K. Wang, Genetic susceptibility to myocardial infarction and coronary artery disease. Hum. Mol. Genet. **15**(Spec No 2), 117–123 (2006)
25. R. Bahri, E. Esteban, P. Moral, M. Hassine, K. Ben Hamda, H. Chaabani, Apolipoprotein gene polymorphisms and plasma levels in healthy Tunisians and patients with coronary artery disease. Lipids Health Dis. **7**, 46 (2008)
26. S.Y. Hung, H.H. Liou, L.P. Ger, L.K. Chen, M.C. Liu, H.M. Chung, K.J. Chou, T.H. Chen, C.K. Lin, Y. Yang, Clustering of unconventional cardiovascular risk factors among Taiwanese hemodialysis patients. Am. J. Nephrol. **30**, 222–231 (2009)
27. T. Nieminen, M. Kahonen, S. Islam, O.T. Raitakari, N. Hutri-Kahonen, J. Marniemi, M. Juonala, R. Rontu, J. Viikari, T. Lehtimaki, Apolipoprotein A-I/C-III/A-IV SstI and apolipoprotein B XbaI polymorphisms do not affect early functional and structural changes in atherosclerosis: the cardiovascular risk in young Finns study. Circ. J. **71**, 741–745 (2007)
28. P.J. Talmud, E. Hawe, S. Martin, M. Olivier, G.J. Miller, E.M. Rubin, L.A. Pennacchio, S.E. Humphries, Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. Hum. Mol. Genet. **11**, 3039–3046 (2002)
29. S.H. Hong, W.H. Park, C.C. Lee, J.H. Song, J.Q. Kim, Association between genetic variations of apo AI-CIII-AIV cluster gene and hypertriglyceridemic subjects. Clin. Chem. **43**, 13–17 (1997)
30. J. Shanker, G. Perumal, V.S. Rao, N.B. Khadrinarasimhiah, S. John, S. Hebbagodi, M. Mukherjee, V.V. Kakkar, Genetic studies on the APOA1-C3-A5 gene cluster in Asian Indians with premature coronary artery disease. Lipids Health Dis. **7**, 33 (2008)