Gene polymorphisms affecting HDL-cholesterol levels in the normolipidemic population

George Miltiadous [a], Marilena Hatzivassiliou [b],
Evagelos Liberopoulos [a], Eleni Bairaktari [c],
Alexandros Tselepis [d], Marios Cariolou [b], Moses Elisaf [a,*]

[a] *Department of Internal medicine, Medical School, University of Ioannina,
GR 451 10 Ioannina, Greece*
[b] *Molecular Genetics Department, B-DNA Identification Laboratory,
The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*
[c] *Department of Biological Chemistry, Medical School, University of Ioannina, Ioannina, Greece*
[d] *Department of Biochemistry, University of Ioannina, Ioannina, Greece*

Summary   *Background and aim:* HDL-cholesterol (HDL-C) is inversely related to the risk of ischemic heart disease. Many genes are reported to affect HDL-C serum levels in both hyperlipidemic and normolipidemic populations, though the data are controversial. We examined the effect of common gene polymorphisms known to interfere with HDL-C metabolism (apolipoprotein E, cholesterol ester transfer protein and apolipoprotein A-IV gene polymorphisms) on HDL-C plasma levels in normolipidemic subjects.
Methods and results: The study population consisted of 200 normolipidemic individuals visiting our clinic for a routine check-up. None of the above gene polymorphisms affected HDL-C levels in our population. However, participants carrying the allele E4 of the apolipoprotein (apo) E gene, the allele B1 of the TaqIB polymorphisms in the cholesterol ester transfer protein (CETP) gene and the allele T of the apoA-IV gene (A to T polymorphism at site 347) ($n=28$) had statistically significantly lower HDL-C levels compared to those not carrying the above allele combination (0.99 ± 0.33 vs 1.28 ± 0.35 mmol/L, $p=0.04$).
Conclusion: In this study, we describe a subgroup of normolipidemic individuals with low HDL-C levels due to genetic variability, and we discuss the underlying possible mechanisms involved.
© 2005 Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +30 2651097509; fax: +30 2651097016.
  E-mail address: me00521@cc.uoi.gr (M. Elisaf).

Introduction

HDL-cholesterol (HDL-C) is inversely related to the risk of ischemic heart disease. The atheroprotective mechanism of the high density lipoproteins (HDL) is mainly focused on the reverse cholesterol transport [1]. Apolipoproteins (apo) A-I and A-IV are the primary acceptors of cellular cholesterol [2,3]. The effluxed cholesterol is then transferred to bigger HDL particles in a process in which enzymes play an important role [4]. The esterification of the free cholesterol by lecithin cholesterol acyl transferase (LCAT) allows the package of the cholesterol inside HDL molecules. Cholesterol ester transfer protein (CETP) plays a notable role in cholesteryl ester (CE) transport in plasma by transferring CE from HDL to triglyceride-rich lipoproteins. Finally, the delivery of HDL-C to the liver proceeds through receptors possibly mediated by apoE.

ApoA-IV polymorphisms have been associated with variations in plasma concentrations of HDL-C, LDL-cholesterol (LDL-C), triglycerides (TRG), lipoprotein (a) [Lp(a)] as well as apoA-1 and apoB [5–12]. However, other studies failed to detect any association between apoA-IV polymorphisms and plasma lipid parameters [13–15]. TaqIB polymorphism of the CETP gene influences the CETP serum levels, and therefore affects HDL-C levels [16–18]. Numerous studies have shown an effect of apoE polymorphisms on plasma lipid parameters. Indeed, this effect was consistent for total cholesterol (TCHOL) and LDL-C but controversial for TRG, HDL-C and Lp(a) [19–21]. Thus, it is suggested that HDL-C may be influenced by a number of gene polymorphisms.

We examined the effect of the above common gene polymorphisms known to participate in HDL-C metabolism on HDL-C levels in normolipidemic individuals visiting our outpatient internal medicine clinic for a routine check-up.

Methods

Our study population consisted of normolipidemic individuals (fasting TCHOL and TRG plasma levels below 6.2 and 2.1 mmol/L, respectively) visiting our outpatient internal medicine clinic for their yearly check-up. None of the participants was receiving drugs affecting plasma lipid levels such as hypolipidemic drugs (statins, fibrates), β-blockers, diuretics, estrogens, etc. Individuals with diabetes mellitus (fasting glucose >6.9 mmol/L), hypertension (blood pressure $>140/90$ mmHg), abnormal thyroid function (TSH >20 mU/L or <0.03 mU/L), renal failure (creatinine >150 μmol/L), proteinuria (urinary protein >0.150 g/24 h), hepatic disease (aspartate or alanine aminotransferase >2 times the upper normal values), obesity (body mass index (BMI) >30 kg/m^2) and alcoholism were also not included. Finally, 200 individuals participated in our study.

After informed consent of all participants, blood samples were obtained after a 14-h overnight fast for gene genotype detection as well as the determination of lipid parameters. Blood samples were centrifuged for 30 min ($3600 \times g$) and then the plasma was separated and stored at 4 °C for analysis of lipid parameters. Plasma for the assay of Lp(a) was frozen and stored at -70 °C. DNA was extracted from the whole blood specimens and the genetic analysis was performed only in patients who fulfilled the inclusion criteria.

Concentrations of TCHOL and TRG were determined enzymatically on the Olympus AU600 Clinical Chemistry analyzer (Olympus Diagnostica, Hamburg, Germany). HDL-C was determined in the supernatant, after precipitation of the apoB-containing lipoproteins with dextran sulphate-Mg^{2+} (Sigma Diagnostics, St. Louis, MO, USA). LDL-C was calculated using the Friedewald formula. ApoA1, -B and -E were measured with

Table 1 The characteristics of the study population (the values are expressed as mean $\pm$ SD)

Characteristics	Study population ($n=200$)	Reference range
Male/female	101/99	
Age, years	39±10	
BMI, kg/m^2	23±6	
Cigarette habit, no/yes	143/57	
TCHOL, mmol/L	5.2±0.95	[a]
TRG, mmol/L	1.44±0.86	[a]
LDL-C, mmol/L	3.49±1.17	[a]
HDL-C, mmol/L	1.2±0.4	[a]
apoA1, g/L	1.45±0.22	1.15–1.90
apoB, g/L	0.98±0.26	0.70–1.60
apoE, g/L	0.039±0.014	0.023–0.063
Lp(a), g/L	0.08 (0.008–0.62)	0.01–0.30

TCHOL, total cholesterol; TRG, triglycerides; LDL-C, LDL cholesterol; HDL-C, HDL-cholesterol; apo, apolipoprotein; Lp(a), lipoprotein (a).
[a] According to the recently published NCEP guidelines, levels of TCHOL, LDL-C and TRG greater than 6.21, 4.14 and 2.26 mmol/L, respectively are regarded as elevated, while a low HDL-C should be defined as a level of <1.04 mmol/L in both men and women.

Table 2 Lipid profile and gene polymorphisms of the study population (the values are expressed as mean±SD)

Gene	Genotypes	n	Sex (m/f)	Age (years)	BMI (kg/m²)	Smoke habit (no/yes)	TCHOL (mmol/L)	TRG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	apoA-I (g/L)	apoB (g/L)	apoE (g/L)	Lp(a) (g/L)
apoA-IV 347	AA	126	63/63	37±12	23±3	90/36	5.19±1	1.44±0.9	3.46±1.2	1.22±0.5	1.46±0.3	0.98±63	0.04±0.01	0.09 (0.02−0.58)
	AT+TT	74	38/36	35±10	24±6	51/23	5.20±0.9	1.44±0.9	3.51±1.1	1.16±0.3	1.43±0.4	0.99±0.2	0.04±0.01	0.08 (0.008−0.52)
p-value			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
apoE	nonE4	148	72/76	36±10	24±7	105/43	5.20±0.9	1.54±1.3	3.52±1.3	1.23±0.5	1.45±0.2	0.97±0.3	0.05±0.02	0.07 (0.008−0.52)
	E4	52	29/23	36±15	22±5	37/15	5.10±1	1.36±1.7	3.48±1	1.15±0.4	1.44±0.2	1.00±0.3	0.04±0.01	0.09 (0.02−0.62)
p-value			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CETP	B2B2	70	40/30	37±9	23±4	50/20	5.19±1.1	1.35±0.9	3.44±1.2	1.22±0.3	1.46±0.2	0.98±0.3	0.03±0.02	0.08 (0.008−0.62)
		130	61/69	35±13	23±3	93/37	5.21±0.9	1.60±1.7	3.55±1.1	1.13±0.5	1.44±0.3	0.98±0.3	0.05±0.03	0.08 (0.02−0.60)
p-value	B1B1+B1B2		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, not statistically significant; CETP, cholesterol ester transfer protein TaqIB polymorphism; apoA-IV 347; apolipoprotein A-IV polymorphism at position 347; apoE, apolipoprotein E.

Table 3 Lipid profile of individuals carrying allele E4 of the apoE gene, allele B1 of the TaqIB polymorphism in the CETP gene and allele T of the apoA-IV gene compared to the other individuals (the values are expressed as mean±SD)

Variable	Group 1 (n=172)	Group 2 (n=28)	p-value
Sex, m/f	84/86	17/13	NS
Age, years	38±16	39±10	NS
BMI, kg/m²	24±4	23±4	NS
Smoking habit, no/yes	118/52	25/5	NS
TC, mmol/L	5.2±0.9	5.17±0.95	NS
TRG, mmol/L	1.38±0.98	1.55±1.01	NS
LDL-C, mmol/L	3.48±1.01	3.50±1.16	NS
HDL-C, mmol/L	1.28±0.35	0.99±0.33	0.04
apoA-I, g/L	1.48±0.33	1.39±0.44	NS
apoB, g/L	0.94±0.18	1.03±0.25	NS
apoE, g/L	0.038±0.035	0.039±0.010	NS
Lp(a), g/L	0.08 (0.008−0.62)	0.06 (0.02−0.56)	NS

Group 2: individuals carrying the allele E4 of the apoE gene, allele B1 of the TaqIB polymorphisms in the CETP gene and allele T of the apoA-IV gene (A to T polymorphism at site 347). Group 1: other individuals. NS, not statistically significant.

a Behring Nephelometer BN100 using reagents (antibodies and calibrators) from Date Behring Holding Gmbh (Liederbach, Germany). The apoA1 and -B assays were calibrated according to the International Federation of Clinical Chemistry (IFCC) standards. Lp(a) levels were determined by the enzyme immunoassay Macra Lp(a) (Trinity Biotech, Jamestown, NY, USA). The lower limit of detectability was 0.8 mg/dL.

DNA was extracted from the whole blood specimens [22]. The TaqIB polymorphism of the CETP gene, the apoE genotyping and the apoA-IV 347 gene polymorphism site were performed as previously described [23−25].

Statistical analysis was performed with SAS statistical software. Student's t-test for independent samples was used to estimate the effects of the gene polymorphisms on lipid parameters. Since serum triglycerides were not normally distributed in all groups, triglyceride data were transformed into natural logarithms. The effect of the gene polymorphisms on Lp(a) levels was tested using Mann−Whitney U-test. A general linear model was finally used to analyze the effect of the combination of genetic and environmental factors on HDL-C.

Results

The clinical characteristics and the lipid profile of the study population are shown in Table 1. All genotype frequencies were in Hardy Weinberg equilibrium. None of the alleles studied influenced plasma lipid parameters (Table 2). However, as shown in Table 3, individuals carrying allele E4 of the apoE gene, allele B1 of the TaqIB polymorphisms in the CETP gene and allele T of the apoA-IV gene (A to T polymorphism at site 347) had statistically significantly lower HDL-C levels compared to those not carrying the above allele combination ($p=0.04$). On the contrary, there were no significant differences in the values of other lipid parameters or other variables, such as age, sex, BMI and smoking habit between the two groups (Table 3). Finally, the above genetic combination still affected HDL-C levels significantly when the general linear model was applied taking into account age, sex, BMI, smoking habit and TRG levels ($p=0.05$) (Table 4).

Discussion

We examined the effect of common gene polymorphisms involved in HDL-C metabolism on HDL-C levels in normolipidemic individuals. HDL-C levels are inversely related to ischemic heart disease and gene polymorphisms associated with low HDL-C levels may identify individuals at high risk for ischemic heart disease.

None of the individual gene alleles studied (apoA-IV and -E and CETP) influenced HDL-C levels. However, subjects carrying allele E4 of the apoE gene, allele B1 of the TaqIB polymorphism in the CETP gene and allele T of the apoA-IV gene tended to have lower HDL-C levels compared to those not

Table 4 General linear model for HDL-C significance taking age, sex, BMI, smoking habit, TRG and the allelic combination of apoE, apoA-IV 347 and CETP as covariates

General linear model[a]	F-value	p-value
Gene combination[b]	3.888	0.05
Sex	0.583	0.44
Age	0.290	0.59
BMI	3.998	0.05
Smoking habit	0.44	0.49
TRG	11.067	0.001

[a] $R^2=0.23$, $p=0.01$.
[b] apoE, apoA-IV 347 and CETP gene polymorphism combination.
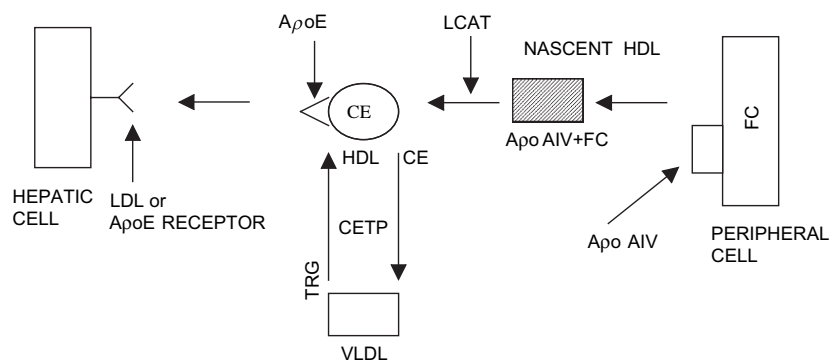
Figure 1 Schematic representation of the reverse cholesterol transport: Cellular free cholesterol (FC) is transported from peripheral cells to plasma through receptors. This process is possibly mediated at least in part by apolipoprotein (apo) A-IV. The esterification of FC to cholesteryl ester (CE) by LCAT allows the package of cholesterol inside bigger particles forming mature HDL molecules. CETP mediates the lipid exchange between HDL and VLDL, a process that is a part of the reverse cholesterol transport. HDL particles finally enter hepatic cells through apoE binding to cellular receptors. Genes encoding for proteins involved in the formation of HDL particles (apoA-IV or LCAT) or in the metabolism of HDL (apoE or CETP) may affect HDL-cholesterol levels.

carrying the above alleles. Interestingly, the combination of these alleles significantly affected HDL-C levels. As shown in Table 3, individuals with E4, TaqIBI and T allele combination had lower HDL-C levels compared to those not carrying the above allele combination. ApoA-IV favors the uptake of cell cholesterol [2,3]. The esterification of the free cholesterol by LCAT allows the package of the cholesterol inside HDL molecules [4]. CETP plays an important role in cholesteryl ester (CE) transport in plasma by transferring CE from HDL to triglyceride-rich lipoproteins [4]. ApoA-IV has been proved to be an activator of the LCAT, as well as a modulator of the CETP [26,27]. The effect of the apoA-IV gene polymorphisms at site 347 on the uptake of cell cholesterol and the activation of the LCAT and CETP has not yet been established. Speculation is that the T allele is associated with a lower rate of cell cholesterol efflux or lower LCAT activity, resulting in low HDL-C levels [2,3,26]. The B1 allele of the TaqIB polymorphism of the CETP gene is associated with higher CETP plasma levels and lower HDL-C levels [16—18]. The co-existence of allele BI of the CETP gene and allele T of the apoA-IV gene may be associated with both higher plasma levels and enzyme activity, resulting in even lower HDL-C levels [27]. Finally, the delivery of HDL-C to the liver proceeds through receptors possibly mediated by apoE. Since the allele E4 shows greater affinity with cellular receptors, HDL carrying apoE might exhibit higher catabolic rates when present [28] (Fig. 1). These protein interventions in HDL metabolism may be the target of newly designed drugs aiming to elevate HDL-C plasma levels in individuals at high risk for cardiovascular disease. The fact

that none of the above gene polymorphisms affect HDL-C levels separately suggests that the impact of the above alleles on HDL-C levels is low.

In conclusion, we describe a subgroup of normolipidemic individuals with low HDL-C levels due to genetic variability and we put forward a relevant speculation.

References

[1] Stein O, Stein Y. Atheroprotective mechanisms of HDL. Atherosclerosis 1999;144:285—301.

[2] Steinmetz A, Barbaras R, Ghalim N, Clavey V, Fruchart JC, Ailhaud G. Human apolipoprotein A-IV binds to apolipoprotein A-1/A-II receptor sites and promotes cholesterol efflux from adipose cells. J Biol Chem 1990;265(14): 7859—63.

[3] Dvorin E, Gorder NL, Benson DM, Gotto AM. Apolipoprotein A-IV, a determinant for binding and uptake of high density lipoproteins by rat hepatocytes. J Biol Chem 1986;261(33): 15714—8.

[4] von Eckardstein A, Jauhiainen M, Huang Y, Metso J, Langer CL, Pussinen WS, et al. Phospholipid transfer protein mediated conversion of high density lipoproteins generates pre b_1 HDL. Biophys Acta 1996;1301:255—62.

[5] Menzel HJ, Boerwinkle E, Schrangl-Will S, Uterman G. Frequency and effects on lipid and lipoprotein levels. Hum Genet 1988;79:368—72.

[6] Menzel HJ, Sigurdsson G, Boerwinkle E, Schrangl-Will S, Uterman G. Frequency and effect of human apolipoprotein A-IV polymorphism on lipid and lipoprotein levels in an Icelandic population. Hum Genet 1990;84:563—7.

[7] Eichner JE, Kuller LH, Ferrel RE, Kamboh KI. Phenotypic effects of apolipoprotein structural variation on lipid profiles, apolipoprotein A-IV and quantitative lipid measures in the healthy woman study. Genet Epidemiol 1989;6:493—9.

[8] de Knijff P, Rosseneu MY, Biesiegel U, De Keersgieter W, Frants RR, Havekes LM. Apolipoprotein A-IV polymorphism

and its effect on serum lipid apolipoprotein concentrations. J Lipid Res 1988;29:1621–7.

[9] Hanis CL, Douglas TC, Hewett-Emmett D. Apolipoprotein A-IV protein polymorphism: frequency and effects on lipids, lipoproteins and apolipoproteins among Mexican-Americans in Starr County, Texas. Hum Genet 1991;86:323–5.

[10] von Eckardstein A, Funke H, Schulte M, Erren M, Schulte H, Assman G. Nonsynonymous polymorphic sites in the apolipoprotein (apo) A-IV gene are associated with changes in the concentration of apoB and apoA-I containing lipoproteins in a normal population. Am J Hum Genet 1992;50:1115–28.

[11] Pepe G, Di Perna V, Resta F, Lovecchio M, Chimienti G, Colacicco AM, et al. In search of a biological pattern for human longevity: Impact of apoA-IV genetic polymorphisms on lipoproteins and the hyper-Lp(a) in centenarians. Atherosclerosis 1998;137:407–17.

[12] Zhongyan W, Mingpeng S, Man X, Zongli W, Renyi X. A study of apolipoprotein A-IV genetic polymorphisms, serum lipids and lipoproteins in Beijing habitants. Chin Med J 1997; 110(4):264–8.

[13] Zaiou M, Visvikis S, Gueguen R, Parra H-J, Fruchart JC, Siest G. DNA polymorphisms of human apolipoprotein A-IV gene: frequency and effects on lipid, lipoprotein and apolipoprotein levels in a French population. Clin Genet 1994;46:248–54.

[14] Ehnholm C, Tenkanen P, Knijff P, Havekes L, Rosseneu M, Menzel HJ, et al. Genetic polymorphism of apolipoprotein A-IV in five different regions of Europe. Relations to serum lipoproteins and to history of myocardial infarction: the EARS study. Atherosclerosis 1994;107:229–38.

[15] Menzel HJ, Dieplinger H, Sandholzer C, Karadi I, Uterman G, Csaszar A. Apolipoprotein A-IV polymorphism in the Hungarian population: gene frequencies, effect on lipid levels and sequence of two new variants. Hum Mutat 1995;5:58–65.

[16] Kondo I, Berg K, Drayna DT, Lawn RM. DNA polymorphism at the locus for human cholesterol ester transfer protein (CETP) is associated with high density lipoprotein cholesterol and apolipoprotein levels. Clin Genet 1989;35:49–56.

[17] Freeman DJ, Griffin BA, Holmes AP, Lindsay GM, Gaffney D, Packard CJ, et al. Regulation of serum HDL cholesterol and subfraction distribution by genetic and environmental factors: associations between Taq1 B RFLP in the CETP gene and smoking and obesity. Arterioscler Thromb 1994; 14:166–344.

[18] Hannuksela ML, Liinamaa MJ, Kesaniemi YA, Savolainen MJ. Relation of polymorphisms in the cholesterol ester transfer protein gene to transfer protein activity and serum lipoprotein levels in alcohol drinkers. Atherosclerosis 1994;110:35–45.

[19] Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Atherosclerosis 1988; 8:1–21.

[20] Smit M, de Knijff P, Rosseneu M, Bury J, Klasen E, Frants R, et al. Apolipoprotein E polymorphism in the Netherlands and its effect on serum lipid and apolipoprotein levels. Hum Genet 1988;80:287–92.

[21] de Knijff P, van den Maagdenberg A, Frants R, Havekes LM. Genetic heterogeneity of apolipoprotein E and its influence on serum lipid and lipoprotein levels. Hum Mutat 1994;4:178–94.

[22] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.

[23] Kuivenhoven JA, Knijff P, Boer J, Smalheer H, Botma G, Seidell J, et al. Heterogeneity at the CETP gene locus-Influence on serum CETP concentration and HDL cholesterol levels. Arterioscler Thromb Vasc Biol 1997;17: 560–8.

[24] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 1990;31:545–8.

[25] Miltiadous G, Hatzivassiliou M, Bashiardes E, Bairaktari E, Cariolou MA, Elisaf M. Genetic polymorphisms of the apolipoprotein A-IV in a Greek population and their relation to serum lipid and lipoprotein levels. Clin Genet. 2002;62(3):208–13.

[26] Steinmetz A, Utermann G. Activation of lecithin: cholesterol acyltransferase by human lipoprotein A-IV. J Biol Chem 1985;260:2258–64.

[27] Guyard-Dangremont V, Lagrost L, Gambert P. Comparative effects of purified apolipoproteins A-I, A-II, and A-IV on cholesterol ester transfer protein activity. J Lipid Res 1994;35:982–92.

[28] Miltiadous G, Cariolou MA, Elisaf M. HDL cholesterol levels in patients with molecularly defined familial hypercholesterolemia. Ann Clin Lab Sci 2002;32(1):50–4.