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ORIGINAL ARTICLE

The G360t Polymorphism in the APO AIV Gene and its Association with Combined HDL/LDL-Cholesterol Phenotype: Tehran Lipid and Glucose Study

Daneshpour MS[a], Zarkesh M[a], Hedayati M[a], Mesbah Namin SA[b], Halalkhor S[b], Faam B[a], Azizi F[c]

[a]Obesity Research Center, Research Institute for Endocrine Sciences, Shaheed Beheshti University of Medical Sciences, [b]Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, [c]Endocrine Research Center, Research Institute for Endocrine Sciences, Shaheed Beheshti University of Medical Sciences, Tehran, I.R. Iran

Identifying genetic polymorphisms as risk factors for complex diseases can facilitate their prevention, diagnosis, and prognosis. The purpose of this study is to assess the association between Apo AIV polymorphism and lipid factors based on high density lipoprotein cholesterol (HDL-C) levels in a population of the Tehran Lipid and Glucose Study (TLGS). Materials and Methods: A total of 181 elderly TLGS subjects with Combined HDLC/low density lipoprotein-Cholesterol (LDL-C) phenotype were investigated. The distributions of a polymorphic site in the apolipoprotein gene APO AIV and its relationship with total cholesterol, LDL-C, HDL-C, and triglycerides were investigated in subjects with LDL-C> 121 mg/dL and HDL-C< 40 mg/dL (case group) and those with LDL-C< 90 mg/dL and HDL-C> 50 (controls). Results: All the variables studied in the case and control groups were statistically different. At the APO AIV locus the G360T polymorphism at codon 360 showed a significant impact on total cholesterol (G: 211±1.16 vs, T: 228±1.20 mg/dL p 0.038) concentration in the case group and on Apo CIII (G: 157±66.9 vs, T: 83.18±17.1 mg/dL p <0001) level in the controls. These associations remained after adjustment for age, sex and smoking (P values: P Chol: 0.028 and P Apo CIII: 0.021). Conclusion: Difference in the apolipoprotein A-IV (G360T) polymorphism in the two groups with the combined HDL/LDL-C phenotype indicates that this phenotype can be a selective phenotype for genetic analysis in this field.

Key Words: Apolipoprotein AIV, polymorphism, cholesterol
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Correspondence: Fereidoun Azizi, Professor of Internal Medicine & Endocrinology, Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University (M.C.), Tehran, I.R. Iran
E-mail: azizi@endocrine.ac.ir

Introduction

Preventing atherosclerosis holds the key to reducing the burden of cardiovascular disease (CVD). A detailed understanding of the pathophysiology of atherosclerotic disease would hence facilitate the designing of innovative therapeutic strategies for the management of dyslipidaemia and the prevention of morbid cardiovascular events[1]. Related contributions of individual lipoproteins to overall cardiovascular risk have been intensively studied over several decades[1]. Recent studies have shown that low high density lipoprotein cholesterol (HDL-C) is common in the insulin-resistant states,

such as the metabolic syndrome and type 2 diabetes, which may account for a substantial increase in cardiovascular disease observed in patients with these conditions[2]. CVD risk is the result of complex interactions between genetic and environmental factors. During the past few decades, much attention has focused on plasma lipoproteins as CVD risk factors. Current data provide further evidence for the concept that gene-environment interactions modulate plasma lipid concentrations and potential CVD risk[3]. Genetic polymorphism studies play an important role in identifying the difference between alleles and in ascertaining their association with longevity and the diseases most commonly affecting elderly people, such as CVD, diabetes, hypertension and low cognitive function. Selection of favorable genotypes and low frequency of risk alleles can lead to successful aging. The genetic factors that participate in lipid metabolism could make the difference between susceptibility and resistance to atherogenesis. It has been proposed that isoproteins of the apolipoprotein AIV gene (APO AIV) may play different roles in lipids modulation[4]. The Apolipoprotein AIV locus provides confirmation for the potential application of genetics in the context of personalized nutritional recommendations for CVD prevention[3]. The Human apolipoprotein (APO) AIV, involved in triglyceride-rich lipoprotein metabolism[5-7], and in reverse cholesterol transport[8-10], is mainly synthesized by the intestine[11] and is secreted with chylomicrons, from the surface of which it rapidly dissociates during catabolism[12, 13] and about 70% associates with HDL-C[14,15]. Based on data available, the structure, nucleotide sequence, and chromosomal location of the apo AIV gene[11,16],the human apo AIV gene is mapped to chromosome 11q23 and contains 3 exons separated by two introns[11, 17,18]. One polymorphism in the apo AIV gene, the apo AIV G360T polymorphism, is caused by a G-to-T substitution in exon 3 of the gene, which causes the glutamine-to-histidine substitution at position 360 in the apo AIV protein[19]. Genetic studies have been conducted to find the gene responsible for low HDL-C, a most common metabolic abnormality in Iranians [20-23]. The purpose of this study was to assess the association between the Apo AIV polymorphism and lipid factors, based on HDL-C levels in a population of Tehran Lipid and Glucose Study (TLGS).

Materials and Methods

Population

The TLGS, designed to determine the risk factors for major non-communicable disorders (including atherosclerosis), occurring in an urban population of Tehran, the capital city of Iran, is an ongoing study involving about 15,005 participants of all ages. The study aims at developing population-based measures to alter life-styles of the Tehranian population and prevent the rising trend of diabetes mellitus, dietary disorders and dyslipidemia[24,25]. Subjects selected from the third TLGS phase(2001-2005), at enrollment for this study, completed a questionnaire on demographics, biochemistry factors and smoking habits. Written informed consent was obtained from each subject and the study was approved by the research council of the Endocrine Research Center of the Shahid Beheshti University of Medical Science.

Variables and lipids analysis

Demographic data and blood pressure of all subjects was obtained and biochemical factors were measured[26]. Total cholesterol, HDL-C and triglyceride levels were measured as described previously[27]. ApoAI, apoB were measured by immunot-urbidometery methods (Pars Azmoun Co, Tehran, Iran) and apoAIV and apoCIII measured by the ELISA method. Serum HDL-C levels was measured after precipi-tation of apo AIV containing lipoproteins with dextran-magnesium sulfate[28]. HDL-C

subtractions were separated by differential poly anion precip-itation[29]. LDL-C concentrations in samples with serum triglyceride levels <400mg/dl were calculated using Friedewald's equation[30]. Coefficients of variation (CV) for total cholesterol, HDL-C and triglyceride measurements were below 5%.

Sample selection

In each sample separately, phenotypically diverse subjects (atherogenic 'cases' and non-atherogenic 'controls') were defined as those individuals comprising the relevant reverse-highest and lowest tertiles (T1, T3 =33.3rd and 66.7th percentiles respectively) of the gender-specific joint LDL-C and HDL-C distri-butions. Originally we selected subjects from the upper and lower range of the joint LDL-C and HDL-C phenotype distribution for a case-control study, excluding subjects in the intermediate region. This approach provided a set of subjects who were either 'cases' (highest LDL-C; more than 121 mg/dl and lowest HDL-C; less than 40 mg/dl) or 'controls' (lowest LDL-C; less than 90 mg/dl and highest HDL-C; more than 50), but who still belonged to the normolipidemic population.

Amplification of DNA and material detection

For analysis of the apo AIV polymorphism, buffy coats were separated from the non coagulated blood samples and stored at -70°C until processing when genomic DNA was extracted by the Proteinase K, salting out method[31]. Amplification of DNA (100ng) was performed in a final volume of 25 µL containing: 1 µl each of the primers forward SatI (5'- CCT GAG GGA CAA GGT CAA CTC -3') and reverse SatI (5'-CAC CTG CTC CTG CTA CTG CTC C-3'), 40 pmol of each oligonucleotide, 0.2 mmol L-1 of each dNTP, 1.5 mmol L-1 MgCl2, 10 mmol L-1 Tris (pH 8.4) and 0.25 units of Taq polymerase (Fermentase Co. Canada). Hybridization was carried out in a DNA thermal cycler (Corbett co. Australia). The reaction mixture was heated at 95 °C for 10 min for denaturation, the cycle of which (50s at 95◦C), annealing (45 s at 60 °C) and extension (55s at 72 °C) was repeated 35 times. Amplified DNA (10 µl) was digested with 0.2 µl of SatI restriction enzyme (Roche Co. City, Germany) for 3 h at 37 °C. Digested PCR products were electrophoresed on a 2% agarose gel, identified by ethidium bromide staining and visualized under UV light and by gel documentation (Optigo Co. City, Holland). The GG sample contained 127 bp fragments, The GT sample also contained 127, 101, 26 bp fragments. The TT sample also contained 101 bp and 26 bp fragments.

Statistical analysis

Statistical analysis was performed using the SPSS V version. 16 (SPSS, Inc. Chicago, IL) statistical package.

Allele frequencies of apo A-IV polymorphism were estimated by the Power Marker software[32]. Chi-square test was applied to verify the Hardy–Weinberg equilibrium. A descriptive test was applied for categorical variables. Continuous data were presented as mean and standard deviation, whereas categorical variables were summarized as frequencies and percentages. For comparisons of biochemical variables with normal distribution in different genotypes, T-test analysis of variance was used. If necessary, a logarithmic transformation was performed to normalize the error distribution and stabilize the error variance. To test the effect of the carriers in each group of risk, we applied ANCOVA analysis using sex, age and smoking as co- variables (confidence interval of 95% and significant when $P<0.05$).

Results

Demographic, biochemical characteristics and genotypes frequencies of the 181 studied individuals are presented in Table 1.

Table 1. Demographic, biochemical parameters and genotypic frequencies of 181 participants: Tehran Lipid and Glucose Study

Variables	Cases (n=103)	Controls (n=78)	P Value
Age (years)	48.4±13.9	34.3±13.0	<0.001
Cigarette smokers (%)	87.5	12.5	0.009
Systolic Blood Pressure (mmHg)	116±17.7	107±14.5	<0.001
Diastolic Blood Pressure (mmHg)	74.4±9.46	70.2±9.08	0.003
Total cholesterol (mg/dl)	217±37.9	162±32.1	<0.001
Triglycerides (mg/dl)	164±62.1	109±59.9	<0.001
High density lipoprotein cholesterol (mg/dl)	34.8±4.02	62.5±12.1	<0.001
High density lipoprotein cholesterol 2 (mg/dl)	10.6±3.33	26.4±9.09	<0.001
High density lipoprotein cholesterol 3 (mg/dl)	24.4±3.40	36.6±9.37	<0.001
Low density lipoprotein cholesterol (mg/dl)	152±31.3	71.3±16.3	<0.001
Apolipoprotein A1 (mg/dl)	135±30.1	152±33.1	0.001
Apolipoprotein B (mg/dl)	137±35.6	87.2±30.8	<0.001
Apolipoprotein C3 (mg/dl)	136±60.9	150±67.5	0.246
Apolipoprotein A IV (mg/dl)	19.8±8.81	19.1±7.80	0.612
Apolipoprotein A IV carriers Frequency (%):			
G	78.6	83.3	<0.001
T	21.4	16.7	

Continues variables are presented with mean ± SD

All lipids related variables were significantly different in case and control groups, except apolipoprotein AVI and CIII level. The allele frequencies of G and T alleles were 0.0764 and 0.9236, respectively and the relative frequencies of the apo AIV G and apo AIV T carriers in the case and control groups were 78.6%, 83.3% and 21.4%, 16.7%, respectively, T carriers being more frequent in the case group. Allele frequencies were in conformity with the Hardy-Weinberg equilibrium. The lipid related biochemical changes, in relation to the different carriers were examined and the association between the two groups, with regard to age, smoking and total cholesterol was significant (P <0.001, 0.008 and 0.015), respectively. Table 2 shows the lipid related biochemical changes for the different carriers; presence of T carrier was significantly associated with higher total cholesterol (G: 211±1.16 vs, T: 228±1.20 mg/dl p 0.038) in the case group and with lower Apolipoprotein CIII (G: 157±66.9 vs, T: 83.18±17.1 mg/dL p <0001) levels in the controls. These associations were retained following covariate adjustment for age, sex and smoking (P values: P Chol: 0.028 and P apo CIII: 0.021).

Table 2. Lipid profile variables in various apo AIV carriers

Variables	Cases		Controls	
	G Carrier (n= 81)	T Carrier (n= 22)	G Carrier (n= 65)	T Carrier (n= 13)
Systolic Blood Pressure (mm Hg)	115±1.15	118±1.18	108±14.6	103±13.9
Diastolic Blood Pressure (mm Hg)	73.7±9.14	76.9±10.4	70.5±8.85	68.6±10.4
Total cholesterol (mg/dL)	211±1.16	228±1.20*	165±32.92	150±25.1
Triglycerides (mg/dL)	162±60.3	172±68.9	97.1±1.67	95.1±1.53
High density lipoprotein cholesterol (mg/dL)	34.4±4.03	36.3±3.69	61.8±1.19	59.9±1.20
High density lipoprotein cholesterol 2 (mg/dL)	10.4±3.30	11.1±3.46	26.8±9.59	24.1±5.47
High density lipoprotein cholesterol 3 (mg/dL)	24.2±3.51	24.9±3.00	35.4±1.25	36.3±1.31
Low density lipoprotein cholesterol (mg/dL)	149±1.17	155±1.27	72.1±15.8	67.7±19.1
Apolipoprotein A1 (mg/dL)	137±31.7	128±27.3	154±34.2	140±24.4
Apolipoprotein B (mg/dL)	137±35.3	138±37.6	88.5±31.8	80.3±25.5
Apolipoprotein C3 (mg/dL)	138±62.1	130±58.1	157±66.9	83.2±17.1*
Apolipoprotein A IV (mg/dL)	18.1±1.55	17.6±1.65	19.4±7.99	16.2±5.75

*$p<0.05$

Discussion

The present study investigated the apo AIV G360T polymorphism in the risk and non risk groups, and found that the T allele frequency is significantly more frequent in case group, indicating that the presence of this allele may increase the risk of cardiovascular disease; also in this group, cholesterol concentration was significantly increased in the T carriers.

As long ago as 1976, the Framingham study showed that depressed levels of HDL-C were significantly and independently associated with an increased risk of coronary death[1], a finding confirmed by further analyses based on longer follow-ups[33,34]. Further, cohort studies have strengthened the association among low HDL-C and adverse coronary[35] and cerebrovascular[36] outcomes. Responses to restriction in dietary cholesterol and saturated fat have been reported to differ between carriers and non-carriers of the mutation[37,38]. A significant gene-environment interaction between this locus and LDL-C particle size has also been observed in a Costa Rican population[39]. In our research, a significant effect of the apo AIV T carrier on high total cholesterol in the cases and low apo CIII in the controls was noted. Our study indicates that the while apo AIV polymorphism at position 360 has no significant effect on plasma HDL-C and triglyceride levels, it does affect plasma total cholesterol and Apo CIII levels. A study of American Samoans showed that apo AIV genotypes were significantly associated with total cholesterol, LDL-C, and apo-B levels[40]. Ejchel and et al indicated that the presence of the rare allele in elderly people can play a significant role in the occurrence of multifactorial diseases[41]. A previous 2006 study indicated that the T allele was associated with a significantly high risk of CAC progression among patients with type 1 diabetes, but not in the controls. Logistic regression analysis confirmed that the presence of the apo AIV T allele predicts an increased risk of progression of coronary atherosclerosis in adults with type 1 diabetes of long duration after adjustment for covariates associated with CAD risk[42]; a Brazilian study investigated the association of these polymorphisms in children and reported that the APOC3/-455 and APOA4 T347S variants had significant effects on HDL-C in girls and higher total cholesterol

and LDL-C levels in boys, who were carriers of the 3238G allele at the APOC3/3238 C>G site. These results disclosed an overall absence of associations between these polymorphisms and lipids in children, a finding not unexpected because expression of the effect of these polymorphisms might depend on the interaction with environmental variables both internal and external to the individual[43].

The strength of the study is that it investigated the apo AIV G360T polymorphism for the first time in a large sample of Iranians. To mention a limitation, it would have been better to examine more polymorphisms in this gene and other genes related to lipid metabolism.

In conclusion, results of this study suggest that the combined HDL/LDL-Cholesterol Phenotype can be a selective phenotype for genetic analysis in this field.

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