Association of the 12669G>A Apolipoprotein B Gene Polymorphism with Apo-B Serum Level and Lipid Profile in Patients with Coronary Artery Disease Comparing with Individuals Without Coronary Artery Disease in Zanjan Population of Iran

Fariborz Ahmadi¹ · Yousef Mortazavi² · Koorosh Fouladsaz¹ · Saeede Mazloomzadeh³

Received: 4 July 2015 / Accepted: 21 September 2015 / Published online: 6 October 2015
© Association of Clinical Biochemists of India 2015

Abstract Atherosclerosis is a pathologic disorder which has an important role in the occurrence of coronary heart disease. It is determined as a focal, inflammatory proliferative response to several types of endothelial damage. Apolipoprotein B which is a requirement in the sustenance of cholesterol homeostasis, and is the major protein component of low density lipoprotein, characterized by multitude polymorphic sites, one of which (12669G>A) is related to the levels of serum lipid profiles, coronary artery disease and/or myocardial infarction. One Common polymorphism which is more important in this process is 12669G>A that is appraised in this research. We recruited 80 patients from the Mousavi hospital, Zanjan, Iran, diagnosed with coronary artery disease by the clinician on the basis of clinical symptoms, echocardiogram result, and angiography. Seventy-seven healthy individuals without any evident symptoms of Coronary stenosis and any past history of the disease were taken as controls from the

general population. We carried out PCR using specific primers. Then, we digested PCR product by RFLP. Lipid parameters by biochemical methods and Apolipoprotein B serum level by immunoturbidometry method were done. Genotype frequencies for 12669G>A polymorphism were determined: 55 % R+R+, 45 % R+R– in case group, and 55.8 R+R+, 44.2 % R+R– in controls. The R–R– genotype was not seen. There was no significant relationship between this polymorphism and the risk of Coronary stenosis ($P = 0.6$). In the present study, higher plasma levels of cholesterol and low density lipoproteins in the subjects with R+R– genotype were found while there was no association between this polymorphism and coronary stenosis with ≥ 50 % in the Zanjan population.

Keywords Atherosclerosis · Apolipoprotein B · Coronary artery disease

Introduction

Atherosclerosis, as a chronic inflammatory disease originating from a state of disequilibrium in lipid metabolism and showing faulty adaptation in immune reaction compelled forcibly by the accretion of cholesterol-filled macrophages in the artery wall [1]. Atherosclerosis-related cardiovascular and cerebrovascular (CV) cases are the causal agent resulting in death in rather 50 % of cases in developed countries [2]. The existence of atherosclerotic disease in more than one arterial system can be linked to a higher risk of repeated symptoms and complications. The rates of myocardial infarction, stroke, vascular death or rehospitalization are almost 25.5 % for patients with symptomatic disease in one vascular system and 40.5 % for multiple vascular systems [3]. The constituent lipids of the

✉ Yousef Mortazavi
youmort@yahoo.com

Fariborz Ahmadi
fariborz.ahmadi@hotmail.com

Koorosh Fouladsaz
Koorosh.fouladsaz@hotmail.com

Saeede Mazloomzadeh
smazloomzadeh@zums.ac.ir

¹ Department of Clinical Biochemistry, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

² Department of Pathology and Metabolic Diseases Research Center, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

³ Zanjan Social Determinants of Health Research Center, Zanjan University of Medical Sciences, Zanjan, Iran

human body include the most parts of phospholipids, cholesterol, TG and cholesteryl esters, which are carried through blood forming lipoprotein complexes of lipids and one or more proteins, called apolipoproteins, and are under an uninterrupted synthesis/degradation turnover. Low density lipoprotein (LDL) is the product of metabolism of very low density lipoprotein (VLDL), and LDL is built by 75 % lipid (cholesteryl esters and cholesterol) and remaining 25 % protein. Increased LDL levels are connected to cardiovascular disease [4]. Dyslipidemia are known to come from the interactions between genetics and environmental (particularity diet, exercise and tobacco smoking) factors in different populations. The biological, genetics and diet risk factors are likely to account for the diversity in the CAD risk in a number of populations. Several factors appear in the diet that can interpolate the lipids density including the high concentration of lipids as well as a low intake of vegetables and fruits [5]. Apolipoprotein B (Apo B) is significant for the sustainment of cholesterol homeostasis, and is the main protein element of LDL. It acts as the ligand for the identification and catabolism of plasma LDL by LDL-receptor [6]. The increasing levels of serum apo B are associated with an increased risk of premature atherosclerosis [7]. Apo B circulates in two separate forms (Apo B100 and Apo B48), encoded by a single gene confined or restricted to chromosome 2 p23-25. Apo B100, the larger form, is synthesized in the liver as a translational product of the entire apo B mRNA [8]. Various polymorphisms in the Apo B gene have been distinguished, and the rest have been linked to TC, LDL cholesterol, high density cholesterol (HDL) and VLDL cholesterol levels [9–13]. The Apo B gene is known to have several sequences which are the identification places for various restriction enzymes. Polymorphism in the Apo B gene in regard with the EcoRI restriction site exists because of a change in guanine to adenine at exon 29, resulting in the exchange for glutamine for lysine at position 4154 in the Apo B polypeptide product [14]. This nucleotide modification resulting in the loss of the EcoRI restriction site. The wild type and the mutated alleles are assigned as R+ and R–, respectively. The frequency of the R– allele has been shown to be more dominant in CAD patients as in comparison with normal subjects in some, but not all populations; hence, indicating that subjects having the R– allele are more likely to be amenable to CAD development [15–20]. Several researchers reported the positive connection between the R– allele with elevated serum lipid levels [19], compared to others suggesting no such link [18]. The available reports show significant fluctuation in linking Apo B gene polymorphism (12669G>A) with CAD risk in various populations. The current research tries to examine the role of this Apo B gene polymorphism with CAD in the Iranian population.

Iranian are believed to have a fat-rich diet and genetically have more tendency to CAD development. The present study would help describe a subset population with a higher or lower risk of CAD. To the knowledge of the researcher, no such study has been carried out on the Iranian population so far.

Material and Methods

Study Population

We recruited 80 CAD+ Subjects (31 men and 49 women) with a median age of 60.75 years old and 77 control Subjects (51 men and 26 women) without ischemia or arterial injury with a median age of 55.6 years old at the Mousavi hospital in Zanjan-Iran. The CAD+ group included patients with having at least one coronary vessel narrowing 50 % or greater in diameter. The CAD– group included individuals with angiographically normal coronaries and presenting non-signs of CHD upon clinical or laboratory Measurements. The consent form was signed by all participants in the study. This study was approved by the Ethics Committee of the Zanjan University of Medical Sciences.

Measurement of Biochemical Tests

After 12-h overnight fasting, 5 ml whole blood was collected from all subjects without anticoagulant and the serum was used for biochemical analysis. High-density lipoprotein (HDL) was isolated from serum by sedimentation LDL and very low density lipoprotein (VLDL) with magnesium chloride and sodium phosphotungstate chemical agent [21]. TC, TG and HDL cholesterol serum levels were calculated by using an accessible kit from Pars Azmoon Co., Iran (via Hitachi system 717). The LDL cholesterol levels were estimated from the formula given by Friedwald et al. [22]. Serum Apo B levels were measured by an immunoturbidimetric kit.

Genotyping

Two millilitre venous blood were taken for DNA extraction in EDTA. DNA was isolated from leukocytes using spin column (Bioron Co. Germany). DNA sequence containing Apo B gene was amplified by pcr using set of primers (Takapozist, Iran), pcr products electrophoresed on 2 % agarose gel and a fragments of 480 bp containing EcoRI restriction site was observed for each individual. PCR condition was as follow: 0.2 mM of each dNTP, 0.2 μM of each forward (5'-CTG AGA GAA GTG TCT TCG AAG-3') and reverse (5'-CTC GAA AGG AAG TGT AAT CAC-

3') primers, 1.5 mM MgCl₂, 0.1 units/μl Ampliqon Taq DNA polymerase and 2 μl of DNA (1 μg/μl). The total volume was Adjusted to 50 μl with dH₂O. The amplified sequence digested at 37 °C with 20 U of EcoR1. Digests were run on 2 % agarose gel at 50 V in 1X Tris–borate-EDTA (TBE) buffer. Subsequent to 3-h digestion with EcoR1 that resulted in fragments of 253 and 227 bp in the presence of the restriction site (R+ allele). In the absence of the EcoR1 cutting site, the only produced band on the agarose gel was the 480 bp sequence.

Statistical Analysis

The mean difference between CAD+ and CAD− groups were analyzed by Student *t* test and rendered as means ± standard deviation (SD); Chi square test was applied to compare distinct variables as well as the comparison of observed and expected genotypes under Hardy–Weinberg equilibrium. The Mann–Whitney test was used to examine the means and standard deviations of plasma level concentrations between CHD and control groups. The

odds ratio (OR) and 95 % confidence intervals (CI) was estimated. A multivariate logistic regression was implemented considering CAD as the dependent variable and the following independent variables: 12669G>A genotypes, age, sex, TG, TC and FBS. *P* values of 0.05 or less were believed to be statistically important. All the statistical analyses were carried out using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The baseline characteristics of study subjects are shown in Table 1. The two groups were well equalized and compared for age and sex. The patients in the case group were older than the control and male to female ratio was higher in the CAD+ group significantly. The difference in positive exercise test, history of STEMI, history of blood pressure, and history of stable anginas was significant. Left ventricular dysfunction was revealed by left ventricular

Table 1 Baseline characteristics in CAD+ and CAD− groups

Characteristics	Case group (<i>n</i> = 80)	Control group (<i>n</i> = 77)	<i>P</i> value
Male gender	52 (65)	31 (40.3)	0.002
Age (year)	60.75 ± 9.4	55.6 ± 10.9	<0.001
Body mass index (kg/m ²)	26.7 ± 4.1	24.7 ±	0.357
Current smoking	18 (22.5)	9 (11.7)	0.073
History of hyperlipidemia	13 (16.2)	6 (7.8)	0.104
History of hypertension	40 (50)	35 (45.5)	0.004
History of diabetes	13 (16.2)	6 (7.8)	0.104
History of unstable angina	52 (65)	44 (57.1)	0.313
History of stable angina	7 (8.8)	20 (26)	0.004
History of STEMI	52 (65)	44 (57.1)	<0.001
History of NSTEMI	3 (3.8)	1 (1.3)	0.330
Positive exercise test	5 (6.2)	13 (16.9)	0.037
Pace maker implantation	3 (3.8)	2 (2.6)	0.681
History of CABG	4 (5.0)	2 (2.6)	0.432
Abnormal cardiac MRI	2 (2.5)	10 (13)	0.013
Left dominance	5 (6.2)	3 (3.9)	0.503
LV ejection fraction <30 %	7 (8.8)	1 (1.3)	0.007
Systolic blood pressure	157.5 ± 30.0	156.2 ± 28.9	0.777
Diastolic blood pressure	86.1 ± 9.2	87.9 ± 10.98	0.251
Fasting blood sugar	16.6 ± 53.1	98.6 ± 25.7	0.008
Triglycerides	178.12 ± 86.0	152.3 ± 84.3	0.060
Total cholesterol	184.1 ± 54.5	172.6 ± 54.9	0.192
High density lipoprotein	46.79 ± 8.0	48.51 ± 11.3	0.274
Low density lipoprotein	102.4 ± 44.24	95.97 ± 49.10	0.389
Very low density lipoprotein	35.64	30.46 ± 16.86	0.059
Apolipoprotein-B	114.4 ± 31.2	106.94 ± 28.7	0.120

P < 0.05 is significant

Table 2 The genotype distribution for Apo-B polymorphism in CAD+ and CAD− groups

Characteristics	Case group (<i>n</i> = 80)	Control group (<i>n</i> = 77)	<i>P</i> value
G12669A polymorphism			
GG	44 (55.0)	43 (55.8)	0.6
GA	36 (45.0)	34 (44.2)	

P < 0.05 is significant **Fig. 1** Agarose gel electrophoresis for 12669G>A polymorphism digestion. SM; 100 bp DNA size marker, column 1; undigested (without enzyme), column 2, 7, 9, 10; R+R− genotype, column 3, 4, 5, 6, 8, 11; R+R+ genotype**Table 3** The frequency of genotypes of the 12669G>A polymorphism according to Baseline characteristics in CAD+ and CAD− participants

Characteristics	GA genotype	GG genotype	<i>P</i> value
Systolic blood pressure	155/0 ± 27/8	158/3 ± 30/7	0.483
Diastolic blood pressure	85/7 ± 11/4	88/1 ± 8/8	0.142
Fasting blood sugar	110/4 ± 45/4	105/6 ± 40/7	0.482
Triglycerides	179/8 ± 77/4	153/9 ± 90/9	0.060
Total cholesterol	208/3 ± 55/0	154/4 ± 41/4	<0.001
High density lipoprotein	46/5 ± 9/4	48/51 ± 10/0	0.214
Low density lipoprotein	127/0 ± 46/2	76/7 ± 32/8	<0.001
Very low density lipoprotein	35/9 ± 15/4	30/8 ± 18/2	0.062
Apolipoprotein-B	123/2 ± 29/8	100/7 ± 26/6	<0.001

P < 0.05 is significant

ejection fraction and abnormal cardiac MRI was also more severe in the CAD+ group than the control. Also, high blood sugar level was more common in those with significant CAD+ than normal subjects. Concerning laboratory parameters, the CAD+ group had higher mean value of lipid profile including TC, TG, LDL, VLDL and Apo B serum level while mean HDL was higher in the control group. The findings of the G12889A polymorphism as well as the genotype distribution for Apo B polymorphism are shown in Table 2. There were two genotypes of the polymorphism in CAD+ and CAD− groups with no remarkable difference in the frequency of these genotypes across the two groups. The subjects under study had either the R+R+ genotype or R+R− genotype. No individual with

homozygous mutant alleles, i.e., R−R− was identified (Fig. 1).

When the R+/R− genotype was compared between the study groups, statistical significance was observed in Apo B, LDL and cholesterol level (*P* < 0.05) among CAD+ and CAD− participants. Highlighted differences also seen in mean BMI (kg/m²) (*P* < 0.05) between CAD+ and CAD− participants with the R+/R− genotype (Table 3).

Multivariable logistic regression analysis (Table 4) showed that among all baseline variables, male gender, progressed age and higher level of fasting blood sugar could foretell the appearance of coronary atherosclerosis. However, the types of the genotypes of 12669G>A polymorphism could not predict CAD.

Table 4 Main determinants of CAD in a multivariable regression model

Characteristics	OR (95 % CI)	P value
12669G>A polymorphism (GA vs. GG)	1/14 (0/50–2/60)	0.75
Age	1/05 (1/02–1/09)	<0.001
Female versus male	0/27 (0/130–0/57)	<0.001
Triglyceride	1/00 (0/99–1/00)	0.23
Total cholesterol	1/00 (0/99–1/01)	0.41
Fasting blood sugar	1/01 (1/00–1/02)	0.008

P < 0.05 is significant

Discussion

The role of the Apo B gene polymorphism (12669G>A) in the present study, was investigated in Iranian subjects suffering from CAD and only two genotypes R+R+ and R+R– were observed in both control subjects and CAD patients. The R–R– genotype was not seen. The occurrence of this genotype in other populations has been reported to differ from sporadic to nil [8]. In our study, no important connection was observed between the appearance of CAD and gene polymorphism. Also, in a study in the Italian population which compared 55 Italian patients to 117 control patients, the allele frequencies at EcoRI (E) sites did not differ between groups [23]. Similar results have been reported in the Brazilian population [18, 24], Marshal et al. [25] and also in South Asian subjects living in Britain [17].

On the other hand, remarkable positive connections in the frequency of the R– allele and CAD has been reported in other population [15, 16, 26, 27]. Being incompatible with other studies, in our population Apo B serum levels was remarkably higher in individuals with the R+R– genotype. A number of Apo B polymorphisms have been found to be linked to serum lipoprotein levels in many children and adult populations [28, 29]. In fact, genetic polymorphisms at the Apo B have been associated with elevated plasma concentrations of LDL cholesterol, and hence, it has been conjectured to be also linked to atherosclerosis and increased risk for CAD [30]. In the present study, we observed higher levels of LDL and cholesterol in individuals with the R+R– genotype in comparison with those having the R+R+ genotype. In a study on the Austrian population, the level of serum lipids was higher in the individuals carrying the R+R– Apo B genotype [26] while insignificant difference in lipid profile with different Apo B genotypes has been reported in the other populations [15–17, 23, 24].

Acknowledgments We are grateful to all the subjects for their cooperation.

Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

- Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol*. 2013;13(10):709–21.
- Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, et al. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation*. 2012;125(1):e2.
- Alberts MJ, Bhatt DL, Mas JL, Ohman EM, Hirsch AT, Rother J, et al. Three-year follow-up and event rates in the international REDuction of Atherothrombosis for Continued Health Registry. *Eur Heart J*. 2009;30(19):2318–26.
- Vuorela T, Caste A, Niemelä PS, Hall A, Hyvönen MT, Marrink S-J, et al. Role of lipids in spheroidal high density lipoproteins. *PLoS Comput Biol*. 2010;6(10):e1000964.
- Bøggild H, Knutsson A. Shift work, risk factors and cardiovascular disease. *Scand J Work Environ Health*. 1999;25:85–99.
- Rebhi L, Omezzine A, Kchok K, Belkahla R, Ben HadjMbarek I, Rejeb J, et al. 5' ins/del and 3' VNTR polymorphisms in the apolipoprotein B gene in relation to lipids and coronary artery disease. *Clin Chem Lab Med*. 2008;46(3):329–34.
- Sorell L, Simón R. Triglyceride and Lp (a) concentrations in hyperapobetalipoproteinemia. *Clin Chim Acta*. 2000;294(1):199–203.
- Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell*. 1987;50(6):831–40.
- Benn M, Nordestgaard BG, Jensen JS, Grande P, Sillesen H, Tybjaerg-Hansen A. Polymorphism in APOB associated with increased low-density lipoprotein levels in both genders in the general population. *J Clin Endocrinol Metab*. 2005;90(10):5797–803.

10. Moreno-Luna R, Perez-Jimenez F, Marin C, Perez-Martinez P, Gomez P, Jimenez-Gomez Y, et al. Two independent apolipoprotein A5 haplotypes modulate postprandial lipoprotein metabolism in a healthy Caucasian population. *J Clin Endocrinol Metab.* 2007;92(6):2280–5.
11. Glišić S, Savić I, Alavantić D. Apolipoprotein B gene DNA polymorphisms (EcoRI and MspI) and serum lipid levels in the Serbian healthy population: interaction of rare alleles and smoking and cholesterol levels. *Genet Epidemiol.* 1995;12(5):499–508.
12. Peacock R, Dunning A, Hamsten A, Tornvall P, Humphries S, Talmud P. Apolipoprotein B gene polymorphisms, lipoproteins and coronary atherosclerosis: a study of young myocardial infarction survivors and healthy population-based individuals. *Atherosclerosis.* 1992;92(2):151–64.
13. Delghandi M, Thangarajah R, Nilsen M, Grimsgaard S, Bønaa K, Tonstad S, et al. DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls. *Acta Cardiol.* 1999;54(4):215–25.
14. Shoulders C, Myant N, Sidoli A, Rodriguez J, Cortese C, Baralle F, et al. Molecular cloning of human LDL apolipoprotein B cDNA: evidence for more than one gene per haploid genome. *Atherosclerosis.* 1985;58(1):277–89.
15. Hegele RA, Huang L-S, Herbert PN, Blum CB, Buring JE, Hennekens CH, et al. Apolipoprotein B—gene DNA polymorphisms associated with myocardial infarction. *N Engl J Med.* 1986;315(24):1509–15.
16. Genest JJ Jr, Ordovas JM, McNamara JR, Robbins AM, Meade T, Cohn SD, et al. DNA polymorphisms of the apolipoprotein B gene in patients with premature coronary artery disease. *Atherosclerosis.* 1990;82(1):7–17.
17. Renges H, Wile D, McKeigue P, Marmot M, Humphries S. Apolipoprotein B gene polymorphisms are associated with lipid levels in men of South Asian descent. *Atherosclerosis.* 1991;91(3):267–75.
18. Scartezini M, Zago M, Chautard-Freire-Maia E, Pazin-Filho A, Marin-Neto J, Hotta J, et al. The XX-/E+ E+ genotype of the XbaI/EcoRI polymorphisms of the apolipoprotein B gene as a marker of coronary artery disease in a Brazilian sample. *Braz J Med Biol Res.* 2003;36(3):369–75.
19. Mustafina O, Novikova L, Nasibullin T, Kolchina E, Tuktarova I. An analysis of association between the apolipoprotein B gene EcoRI polymorphism and ischemic stroke. *Zh Nevrol Psikhiatr Im S S Korsakova.* 2005;17(2):66–70.
20. Padmaja N, Kumar MR, Adithan C. Association of polymorphisms in apolipoprotein A1 and apolipoprotein B genes with lipid profile in Tamilian population. *Indian Heart J.* 2008;61(1):51–4.
21. Burstein M, Scholnick H, Morfin R. Estimation of high density lipoprotein cholesterol. *J Lipid Res.* 1970;19(6):583–93.
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499–502.
23. Corbo R, Vilaro T, Mantuano E, Ruggeri M, Gemma A, Scacchi R. Apolipoproteins B and E, and angiotensin I-converting enzyme (ACE) genetic polymorphisms in Italian women with coronary artery disease (CAD) and their relationships with plasma lipid and apolipoprotein levels. *Clin Genet.* 1997;52(2):77–82.
24. Machado MO, Hirata MH, Bertolami MC, Hirata RD. Apo B gene haplotype is associated with lipid profile of higher risk for coronary heart disease in Caucasian Brazilian men. *J Clin Lab Anal.* 2001;15(1):19–24.
25. Marshall HW, Morrison LC, Wu LL, Anderson JL, Corneli PS, Stauffer DM, et al. Apolipoprotein polymorphisms fail to define risk of coronary artery disease. Results of a prospective, angiographically controlled study. *Circulation.* 1994;89(2):567–77.
26. Paulweber B, Friedl W, Krempler F, Humphries SE, Sandhofer F. Association of DNA polymorphism at the apolipoprotein B gene locus with coronary heart disease and serum very low density lipoprotein levels. *Arterioscler Thromb Vasc Biol.* 1990;10(1):17–24.
27. Sharma R, Mahajan M, Singh B, Singh G, Singh P. Role of the APOB gene polymorphism (c. 12669G> A, p. Gln4154Lys) in coronary artery disease in the Indian Punjabi population. *Balkan J Med Genet.* 2011;14(2):35–40.
28. Gallegos-Arreola MP, Valdez Y, Zúñiga-Corona M, Figuera LE, Arnaud-Lopez L, Robles-Cervantes JA, et al. Association between the Xba I polymorphism of APOB gene and plasma lipid level in Mexican patients with coronary artery disease. *Asia Pac J Clin Nutr.* 2012;21(2):312.
29. Aalto-Setälä K, Viikari J, Akerblom H, Kuusela V, Kontula K. DNA polymorphisms of the apolipoprotein B and AI/C-III genes are associated with variations of serum low density lipoprotein cholesterol level in childhood. *J Lipid Res.* 1991;32(9):1477–87.
30. Cavalli SA, Hirata MH, Salazar LA, Diament J, Forti N, Giannini SD, et al. Apolipoprotein B gene polymorphisms: prevalence and impact on serum lipid concentrations in hypercholesterolemic individuals from Brazil. *Clin Chim Acta.* 2000;302(1):189–203.