Brazilian Journal of Medical and Biological Research (2003) 36: 369-375
ISSN 0100-879X

The X-X-/E+E+ genotype of the *Xba*I/*Eco*RI polymorphisms of the apolipoprotein B gene as a marker of coronary artery disease in a Brazilian sample

M. Scartezini[1], M.A. Zago[4], E.A. Chautard-Freire-Maia[2], A. Pazin-Filho[4], J.A. Marin-Neto[4], J.K.S. Hotta[4], A.J. Nascimento[3] and J.E. Dos-Santos[4]

Departamentos de [1]Patologia Médica, [2]Genética, and [3]Bioquímica, Universidade Federal do Paraná, Curitiba, PR, Brasil
[4]Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

Studies that consider polymorphisms within the apolipoprotein B (apo B) gene as risk factors for coronary artery disease (CAD) have reported conflicting results. The aim of the present study was to search for associations between two DNA RFLPs (*Xba*I and *Eco*RI) of the apo B gene and CAD diagnosed by angiography. In the present study we compared 116 Brazilian patients (92 men) with CAD (CAD+) to 78 control patients (26 men) without ischemia or arterial damage (CAD-). The allele frequencies at the *Xba*I (X) and *Eco*RI (E) sites did not differ between groups. The genotype distributions of CAD+ and CAD- patients were different ($\chi^2_{(1)} = 6.27$, P = 0.012) when assigned to two classes (X-X-/E+E+ and the remaining *Xba*I/*Eco*RI genotypes). Multivariate logistic regression analysis showed that individuals with the X-X-/E+E+ genotype presented a 6.1 higher chance of developing CAD than individuals with the other *Xba*I/*Eco*RI genotypes, independently of the other risk factors considered (sex, tobacco consumption, total cholesterol, hypertension, and triglycerides). We conclude that the X-X-/E+E genotype may be in linkage disequilibrium with an unknown variation in the apo B gene or with a variation in another gene that affects the risk of CAD.

Correspondence
M. Scartezini
Setor de Ciências da Saúde
Sede Botânico, UFPR
Rua Pref. Lothario Meissner, 3400
80210-170 Curitiba, PR
Brasil
Fax: +55-41-360-4127
E-mail: marileia@ufpr.br

Research supported by FAPESP, CAPES and CNPq.

Received April 12, 2002
Accepted December 16, 2002

Key words
- Apo B *Xba*I/*Eco*RI polymorphisms
- Coronary artery disease
- CAD risk

Introduction

Two restriction fragment length polymorphisms detected with the restriction enzymes *Xba*I and *Eco*RI represent single base alterations in the coding region of the apolipoprotein B (apo B) gene (1).

The *Xba*I RFLP in exon 26 of the apo B gene involves the 2,488th nucleotide (ACC→ACT). The presence of thymine creates a restriction site for the *Xba*I enzyme characterizing the X+ allele, whereas its absence determines the X- allele. These are synonymous variations and so they do not affect the amino acid sequence of apo B (2-4). The *Eco*RI polymorphism of the apo B gene ap-

pears in exon 29 (GAA→AAA; 4,154th nucleotide) (1,2), resulting in an amino acid change (Glu→Lys) (5). A restriction site for the *Eco*RI enzyme is present with the guanine (E+ allele); otherwise it is lost (E- allele).

The allele lacking the *Xba*I site (X-) and/or its homozygous genotype (X-X-) have been reported as more common in survivors of myocardial infarction and in patients with coronary artery disease (CAD) than in controls (6-10).

In the present prospective, cross-sectional study, the apo B alleles detected with the *Xba*I and *Eco*RI restriction enzymes were examined for association with CAD by evaluating their frequency distributions in patients with CAD (CAD+) as compared to patients with the confirmed absence of this disease (CAD-).

Material and Methods

Study population

A total of 116 (92 men and 24 women) CAD+ patients and 78 control patients (26 men and 52 women) without ischemia or arterial damage (CAD-) were diagnosed by angiography at the University Hospital of Ribeirão Preto, SP, Brazil. Mean age was 44 ± 7 years (ascertainment below 56 years) for both groups. The CAD+ group comprised consecutive patients with acute ischemic syndromes and angiographically proven CAD (with at least one coronary stenosis with ≥50% of narrowing of the luminal diameter). The CAD- group comprised consecutive patients being catheterized for clinical reasons and presenting non-cyanogenic congenital cardiopathy or valvulopathies. All patients in the CAD- group had angiographically normal coronaries. All coronary angiograms were analyzed visually by two experienced interventional cardiologists according to conventional American Heart Association methods. No exclusion criteria, other than not signing the informed consent form, were applied to the consecutively enrolled patients in either group. This study was approved by the Ethics Committee of the Hospital das Clínicas, USP, Ribeirão Preto, SP, Brazil.

Blood (10 ml) was collected without anticoagulant after a 12-h fast and the serum was used for the determination of lipids, lipoproteins and apolipoproteins. The levels of total cholesterol (TC) and triglycerides (TG) were obtained by an enzymatic procedure (Roche Diagnostic GmbH, Mannheim, Germany) using a Cobas Mira S autoanalyzer (Roche). HDL-cholesterol (HDL-C) was measured by an enzymatic method (Roche) in the supernatant after precipitation with phosphotungstate-$MgCl_2$ (Roche). LDL-C levels were estimated by the method of Friedewald et al. (11). Apo A-I, A-II, B, E and Lp(a) were measured by immunonephelometry (Behring, Marburg, Germany).

Determination of DNA polymorphism

Leukocyte genomic DNA was extracted from 10 ml of whole blood collected with EDTA by the Super Quick Gene procedure (Analytical Genetic Testing Center, Inc., Denver, CO, USA). The desired segments were amplified by PCR (12) using the apo B *Xba*I and apo B *Eco*RI protocols (13) with the respective primers (Gibco BRL, Rockville, MD, USA): 5'(5'GGAGACTATTCAG AAGCTAA3') and 3'(5'GAAGAGCCTG AAGACTGACT3'); 5'(5'CTGAGAGAAGT GTCTTCGAAG3') and 3'(5'CTCGAAAGG AAGTGTAATCAC3'). The final amplification products were submitted to digestion with the respective restriction enzymes (*Xba*I and *Eco*RI) and the variations were visualized after electrophoresis on 1.5% agarose gel and 6% polyacrylamide gel, respectively, with ethidium bromide under UV light, followed by photographic documentation.

Statistical analysis

Means were compared by the Student *t*-

test and logarithmic transformation was used when the data did not fit a normal distribution. Allelic frequencies were calculated by gene counting. Comparison of observed and expected genotypes under Hardy-Weinberg equilibrium, as well as comparison of the CAD+ and CAD- groups were made by the χ^2 test. Haplotype frequencies and linkage disequilibrium were estimated with the Arlequin program (14). A multivariate logistic regression (Statistica for Windows, version 4.2, Statsoft Inc., 1993) was performed considering CAD as the dependent variable and the following independent variables: *Xba*I/*Eco*RI genotypes, sex, hypertension, tobacco consumption, TC, and TG. For this analysis numbers were assigned to the variables (CAD- = 0, CAD+ = 1; female = 0, male = 1; absence of hypertension = 0, presence = 1; absence of tobacco consumption = 0, presence = 1). The *Xba*I/*Eco*RI genotypes were classified as X-X-/E+E+ (= 1) and other genotypes as 0. TC and TG were classified as 0 when below the median (195 mg/dl and 122 mg/dl, respectively) and as 1 when equal or above the median. The odds ratios were calculated as explained in Hosmer Jr. and Lemeshow (15).

Results

Comparisons concerning the frequency distributions of ethnic origin and risk factors in the CAD+ and CAD- groups are shown in Table 1.

The genotype and allele frequency distributions for the CAD+ and CAD- groups with respect to *Xba*I and *Eco*RI polymorphisms were compared by χ^2 tests (Table 2). None of the differences in allele or genotype frequencies between the CAD+ and CAD- groups were statistically significant. In both groups, these genotype frequencies for *Xba*I and *Eco*RI were distributed according to Hardy-Weinberg equilibrium.

Table 3 shows haplotype frequencies and estimates of linkage disequilibrium for *Xba*I/

Table 1. Frequency distribution of ethnic origin and risk factors for coronary artery disease found in the CAD+ (N = 116) and CAD– (N = 78) groups.

Variable	CAD+ (%)	CAD- (%)	P
Ethnic origin			
Euro-Brazilians	78	81	>0.05
Risk factors			
Male sex	79	33	<0.0001
Hypertension	62	38	<0.01
Diabetes mellitus	16	8	>0.05
Tobacco consumption	70	31	<0.01
Familial hypercholesterolemia	31	24	>0.05
Obesity (BMI >30)	25	21	>0.05

Data were compared statistically by the χ^2 test. The complementary ethnic group consisted of Afro-Brazilians. BMI = body mass index.

Table 2. Genotype and allele frequencies of *Xba*I and *Eco*RI (apo B gene) in the CAD+ (N = 114) and CAD– (N = 78) groups.

Apo B	Genotype			χ^2 (P)	Allele		χ^2 (P)
	X+X+	X+X–	X–X–		X+	X–	
*Xba*I							
CAD+	15.8	45.6	38.6		38.6	61.4	
CAD–	18.0	56.4	25.6	3.54 (0.17)	46.2	53.8	2.18 (0.14)
	E+E+	E+E–	E–E–		E+	E–	
*Eco*RI							
CAD+	62.0	34.5	3.50		79.3	20.7	
CAD–	60.0	36.0	4.00	0.07 (0.96)	78.2	21.8	0.07 (0.79)

Data are reported as percent and the two groups were compared by the χ^2 test.

Table 3. Absolute and relative frequencies of the apo B (*Xba*I/*Eco*RI) haplotypes and results of linkage disequilibrium analyses in the CAD+ and CAD– groups.

	CAD+		CAD–		χ^2 (P)
	N	%	N	%	
*Xba*I/*Eco*RI					
X+E+	78	34.2	67	43.0	3.00 (0.08)
X+E–	10	4.4	5	3.2	0.34 (0.56)
X–E+	103	45.2	55	35.2	3.76 (0.052)
X–E–	37	16.2	29	18.6	0.36 (0.55)
Total	228	100.0	156	100.0	
D	0.036		0.071		
χ^2 (P)	7.50 (<0.01)		17.29 (<0.001)		
D_{max}	0.080		0.101		
D/D_{max}	0.45		0.70		

Haplotypes were estimated by the Arlequin program (14). X+E+ and X–E– were considered to be the cis conformations. The χ^2 test was used to compare the two groups (upper part of the table) and to test independent segregation in each group (lower part of the table).

*Eco*RI and the results of comparisons between the CAD+ and CAD- groups.

When the CAD+ and CAD- haplotype frequencies were compared (Table 3), the difference closest to the significance limit was shown by the X-E+ haplotype. This result led to a comparison between the CAD+ and CAD- groups, as classified on the basis of only two genotype classes, i.e., X-X-/E+E+ and all the other genotypes (Table 4). The distributions of these genotypes differed in the CAD+ and CAD- groups ($\chi^2_{(1)} = 6.27$, P = 0.012).

With respect to the mean values of serum lipids, lipoproteins, apoproteins and of the apo B/apo A-I ratio (Table 5), considering only the X-X-/E+E+ genotype and comparing the CAD+ (N = 22) and CAD- (N = 6) groups, only HDL-C showed a statistically significant difference. When only the other *Xba*I/*Eco*RI genotypes were considered together and the means of these same variables were compared in the CAD+ (N = 87) and CAD- (N = 72) groups, all means were higher in the CAD+ than in the CAD- group, except for HDL-C and apo A-I that did not differ statistically between these two groups. Comparing the X-X-/E+E+ genotype with the class formed by all the other *Xba*I/*Eco*RI genotypes, no statistically significant difference was detected for the means of these variables in the CAD+ group (22 and 87 patients, respectively) or in the CAD- group (6 and 72 individuals, respectively). Calcu-

Table 4. Frequency distributions of two apo B genotype classes (*Xba*I/*Eco*RI) compared by the χ^2 test in the CAD+ and CAD– groups.

Genotypes	CAD+		CAD–		χ^2 (P)
	N	%	N	%	
X–X–/E+E+	24	21.1	6	7.7	
Other genotypes	90	78.9	72	92.3	6.27 (0.012)
Total	114	100.0	78	100.0	

Table 5. Serum levels of lipids, lipoproteins, apolipoproteins (apo) and of the apo B/apo A-I ratio of the X–X–/E+E+ genotype and of the other *Xba*I/*Eco*RI genotypes in the CAD+ and CAD– groups, as compared by the Student *t*-test.

Variable	X–X–/E+E+		P	Other *Xba*I/*Eco*RI genotypes		P
	CAD+ (N = 22)	CAD– (N = 6)		CAD+ (N = 87)	CAD– (N = 72)	
Total Cholesterol	215.0 ± 15.2	182.8 ± 10.8	>0.20	205.2 ± 4.9	176.2 ± 5.1	$<10^{-4}$
Triglycerides	170.8 ± 27.9	88.8 ± 14.0	>0.10	161.3 ± 10.5	119.4 ± 8.3	<0.01
HDL-Cholesterol	37.4 ± 3.0	53.3 ± 7.3	=0.027	38.9 ± 1.4	42.9 ± 1.7[a]	>0.05
LDL-Cholesterol	138.9 ± 11.3[b]	111.8 ± 12.3	>0.20	134.9 ± 4.1[c]	107.9 ± 4.1[a]	$<10^{-5}$
Lipoprotein (a)	47.4 ± 9.5	47.2 ± 11.8	>0.90	52.6 ± 5.2	32.9 ± 3.6	<0.01
Apoprotein B	154.5 ± 12.2	109.0 ± 10.0	>0.05	143.5 ± 3.8	109.0 ± 3.9	$<10^{-6}$
Apoprotein A-I	135.0 ± 7.6	146.8 ± 10.2	>0.40	137.4 ± 3.7	138.3 ± 4.3	>0.80
Apoprotein A-II	31.1 ± 2.0	30.5 ± 1.2	>0.80	31.7 ± 1.0	27.1 ± 0.9	<0.001
Apoprotein E	5.0 ± 0.5	3.6 ± 0.5	>0.10	4.1 ± 0.2	3.5 ± 0.2	<0.01
apo B/apo A-I	1.2 ± 0.1	0.8 ± 0.1	>0.10	1.1 ± 0.04	0.83 ± 0.04	$<10^{-4}$

Data are reported as means ± SEM in mg/dl.
[a]N = 71, [b]N = 21, [c]N = 86.

Table 6. Results of the Student *t*-test and the odds ratios obtained by multivariate logistic regression analysis, considering CAD as the dependent variable and *Xba*I/*Eco*RI genotypes, sex, hypertension, tobacco consumption, total cholesterol (TC) and triglycerides (TG) as independent variables in CAD+ (N = 108) and CAD– (N = 78) individuals.

	Constant $ß_o$	*Xba*I/*Eco*RI	Sex	Hypertension	Tobacco	TC	TG
Estimate of ß	-3.748	1.803	1.970	1.414	1.740	1.432	0.950
SE of ß	0.626	0.683	0.437	0.435	0.445	0.456	0.467
$t_{(179)}$	-5.982	2.640	4.502	3.250	3.908	3.138	2.035
P	0.0000	0.009	0.0000	0.0014	0.0001	0.0019	0.0433
		X–X–/E+E+	Male	Hypertension	Tobacco	TC[b]	TG[b]
Odds ratio		6.1[a]	7.2	4.1	5.7	4.2	2.6

[a]1.6 to 23.1 with 95% confidence interval; [b]equal to or above the median.

lations using the data from Table 5 showed that the CAD+ group (N = 109) presented significantly higher means than the CAD- group (N = 78) for TC, TG, LDL-C, Lp(a), apo A-II, apo B, apo E, apo B/apo A-I, whereas mean HDL-C concentration was significantly higher in the CAD- than in the CAD+ group. Only mean apo A-I concentrations did not differ significantly between these groups.

Table 6 shows the results of multivariate logistic regression analysis which considered CAD as the dependent variable and the following independent variables: *Xba*I/*Eco*RI genotypes, sex, hypertension, tobacco consumption, TC, and TG levels. The table also shows the odds ratios obtained for each of these statistically significant risk factors for CAD. The other variables that differed between the CAD+ and CAD- groups (HDL-C, LDL-C, Lp(a), apo B, apo A-II, apo E, and apo B/apo A-I ratio) were not included in the analysis presented in Table 6, since they did not present statistically significant values of ß when considered in the regression analysis.

Discussion

The allele frequencies found in the present study referring to the *Xba*I and *Eco*RI sites do not differ from those reported for Brazilian CAD patients and controls (16). Bohn and Berg (17) cited significant positive associations between CAD and the X- allele and/or the X-X- genotype in five studies (6-10), and reported that this association was not observed in seven other studies (18-24). The X-X- genotype was more frequent in Brazilian women with CAD than in control women (25). Studies on Caucasians from Europe (26) and on Chinese subjects (27) did not detect significant associations between *Xba*I variability and CAD.

Bohn et al. (10), applying multivariate logistic regression analysis, obtained an odds ratio of 2.16 ($P<0.01$) for the X-X- homozygotes having myocardial infarction compared to the combined group of X+X+ and X+X- genotypes. This increased risk for the X-X- genotype was not apparently conferred by higher levels of TC, LDL-C or apo B, but by some mechanism not closely related to the traditional risk factors. However, the X-X- genotype was associated with higher serum concentrations of TC and LDL-C in Brazilian women classified as presenting a high risk lipid profile for CAD (28).

In the present study the difference in the frequencies of the X-E+ haplotype between CAD+ and CAD- individuals (Table 3) was close to the significance limit and the frequency of the X-X-/E+E+ genotype was significantly higher in the CAD+ group when

compared to the CAD- group (Table 4), suggesting that this genotype may be a risk factor for CAD. Data in Table 5 for the X-X-/E+E+ genotype show a significantly lower mean HDL-C in the CAD+ group than in the CAD- group. At first we may assume the existence of an association between this genotype and lower mean levels of HDL-C. However, analysis of these data showed 73 and 17% of men with the X-X-/E+E+ genotype in the CAD+ and CAD- groups, respectively. So, the reason for this difference in mean HDL-C may reside in the different sex proportions of these two groups. Possibly, a case-control study with matching for gender would have been more appropriate. However, we set out to form a control group of patients being referred for cardiac catheterization due to clinical conditions other than ischemic heart disease.

The importance of the X-X-/E+E+ genotype as an independent risk factor for CAD can be seen in the results of the regression analysis shown in Table 6. These results seem to exclude the possibility that the higher risk found for this genotype (Table 4) could be due to sample stratification caused by the differences between the two CAD groups (Table 1) in terms of the proportions of sex, hypertension and tobacco consumption. It is important to note that these three variables also appear to be independent risk factors in this analysis (Table 6). Among the six independent risk factors found in this study, the male sex and the X-X-/E+E+ genotype presented the highest odds ratios (7.2 and 6.1, respectively).

Two limitations could be pointed out in relation to the present study. One is the fact that the CAD+ group was composed of patients with a diagnosis of acute coronary syndrome reflecting their tendency to coronary thrombotic complications. However, all patients were studied after the acute phase, and the angiography clearly showed significant atherosclerotic disease in all of them. The second limitation refers to the relatively small number of patients that may lead to type II errors. However, the patient number in the present study is comparable to those reported in several similar investigations. In spite of these limitations, the present study is the first in which the X-X-/E+E+ genotype is compared to all the other *Xba*I/*Eco*RI genotypes, suggesting this sort of comparison for further studies.

Since the X- and X+ variations are synonymous, it is assumed that the X-X-/E+E+ genotype may be in linkage disequilibrium with an unknown variation in the apo B gene or with a variation in another gene that influences the risk for CAD. The chance of detecting the effect of this putative variation would be two times higher if the homozygous genotype rather than the haplotype were considered in the analyses.

Acknowledgments

The authors gratefully acknowledge Marly Tavela and Amélia G. Araújo for excellent technical collaboration.

References

1. Barni N, Talmud PJ, Carlsson P et al. (1986). The isolation of genomic recombinants for the human apolipoprotein B gene and the mapping of three common DNA polymorphisms of the gene - a useful marker for human chromosome 2. *Human Genetics*, 73: 313-319.
2. Priestley L, Knott T, Wallis S, Powell L, Pease R, Brunt H & Scott J (1985). RFLP for the human apolipoprotein B gene: I, *Bam*HI; II, *Eco*RI; III, *Eco*RV; IV, *Msp*I; V, *Xba*I. *Nucleic Acids Research*, 13: 6789-6793.
3. Law A, Powell LM, Brunt H et al. (1986). Common DNA polymorphism within coding sequence of apolipoprotein B gene associated with altered lipid levels. *Lancet*, 1: 1301-1303.
4. Blackhart BD, Ludwig EM, Pierotti VR et al. (1986). Structure of the human apolipoprotein B gene. *Journal of Biological Chemistry*, 261: 15364-15367.
5. Shoulders CC, Myant NB, Sidoli A, Rodriguez JC, Cortese C, Baralle FE & Cortese R (1985). Molecular cloning of human LDL-apolipoprotein B cDNA. Evidence for more than one gene per haploid genome. *Atherosclerosis*, 58: 277-292.
6. Hegele RA, Huang L, Herbert PN, Blum CB, Buring JE, Hennekens CH & Breslow JL (1986). Apolipoprotein B-gene DNA polymor-

phisms associated with myocardial infarction. *New England Journal of Medicine*, 315: 1509-1515.
7. Monsalve MV, Young R, Jobsis J, Wiseman SA, Dhamu S, Powell JT, Greenhalgh RM & Humphries SE (1988). DNA polymorphisms of the gene for apolipoprotein B in patients with peripheral arterial disease. *Atherosclerosis*, 70: 123-129.
8. Myant NB, Gallagher J, Barbir M, Thompson GR, Wile D & Humphries SE (1989). Restriction fragment length polymorphism in the apo-B-gene in relation to coronary-artery disease. *Atherosclerosis*, 77: 193-201.
9. Tybjaerg-Hansen A, Nordestgaard BG, Gerdes LU & Humphries SE (1991). Variation of apolipoprotein B gene is associated with myocardial infarction and lipoprotein levels in Danes. *Atherosclerosis*, 89: 69-81.
10. Bohn M, Bakken A, Erikssen J & Berg K (1993). *Xba*I polymorphism in DNA at the apolipoprotein B locus is associated with myocardial infarction (MI). *Clinical Genetics*, 44: 241-248.
11. Friedewald WT, Levy RI & Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18: 499-502.
12. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB & Erlich HA (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239: 487-491.
13. Renges HH, Peacock R, Dunning AM, Talmud P & Humphries SE (1992). Genetic relationship between the 3'VNTR and diallelic apolipoprotein B gene polymorphisms: haplotype analysis in individuals of European and South Asian origin. *Annals of Human Genetics*, 56: 11-33.
14. Excoffier L, Schneider S, Kueffer JM & Roessli D (1997). Arlequin: version 1.1. Geneva. Download in 1998. http://anthropologie.unige.ch/arlequin.
15. Hosmer Jr DW & Lemeshow S (1989). *Applied Logistic Regression*. John Wiley & Sons, Inc., New York, NY, USA, 44.
16. Machado MO, Hirata MH, Bertolami MC & Hirata RDC (2001). Apo B gene haplotype is associated with lipid profile of higher risk for coronary heart disease in Caucasian Brazilian men. *Journal of Clinical Laboratory Analysis*, 15: 19-24.
17. Bohn M & Berg K (1994). The *Xba*I polymorphism at the apolipoprotein B locus and risk of atherosclerotic disease. *Clinical Genetics*, 46: 77-79.
18. Deeb S, Failor A, Brown BG, Brunzell JD, Albers JJ & Motulsky AG (1986). Molecular genetics of apolipoprotein and coronary heart disease. *Cold Spring Harbor Symposia on Quantitative Biology*, 56: 403-409.
19. Ferns GAA, Robinson D & Galton DJ (1988). DNA haplotypes of the human apolipoprotein B gene in coronary atherosclerosis. *Human Genetics*, 81: 76-80.
20. Wiklund O, Darnfors C, Bjursell G, Nilsson J, Linden T, Olofsson SO, Wilhelmsen L & Bondjers G (1989). *Xba*I restriction fragment length polymorphism of apolipoprotein B in Swedish myocardial infarction patients. *European Journal of Clinical Investigation*, 19: 255-258.
21. Genest JJ, Ordovas JM, McNamara JR et al. (1990). DNA polymorphisms of the apolipoprotein B gene in patients with premature coronary artery disease. *Atherosclerosis*, 82: 7-17.
22. Paulweber B, Frield W, Krempler F, Humphries SE & Sandhofer F (1990). Association of DNA polymorphism at the apolipoprotein B gene locus with coronary heart disease and serum very low density lipoprotein levels. *Arteriosclerosis*, 10: 17-24.
23. Peacock R, Dunning A, Hamsten A, Tornvall P, Humphries SE & Talmud P (1992). Apolipoprotein B gene polymorphism, lipoproteins and coronary atherosclerosis: a study of young myocardial infarction survivors and healthy population-based individuals. *Atherosclerosis*, 92: 151-164.
24. Nieminen MS, Mattila KJ, Aalto-Setälä K, Kuusi T, Kontula K, Kauppinen-Mäkelin R, Ehnholm C, Jauhiainen M, Valle M & Taskinen M-R (1992). Lipoproteins and their genetic variation in subjects with and without angiographically verified coronary artery disease. *Arteriosclerosis and Thrombosis*, 12: 58-69.
25. Salazar LA, Hirata MH, Giannini SD, Forti N, Diament J, Lima TM & Hirata RD (2000). Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. *Clinica Chimica Acta*, 300: 139-149.
26. Turner PR, Talmud PJ, Visvikis S, Ehnholm C & Tiret L (1995). DNA polymorphisms of the apoprotein B gene are associated with altered plasma lipoprotein concentrations but not with perceived risk of cardiovascular disease: European Atherosclerosis Research Study. *Atherosclerosis*, 116: 221-234.
27. Pan J, Chiang A, Tai JJ, Wang S & Chang M (1995). Restriction fragment length polymorphisms of apolipoprotein B gene in Chinese population with coronary heart disease. *Clinical Chemistry*, 41: 424-429.
28. Guzman EC, Hirata MH, Quintão EC & Hirata RD (2000). Association of the apolipoprotein B gene polymorphisms with cholesterol levels and response to fluvastatin in Brazilian individuals with high risk for coronary heart disease. *Clinical Chemistry and Laboratory Medicine*, 38: 731-736.