

Polymorphisms of the low-density lipoprotein receptor gene in Brazilian individuals with heterozygous familial hypercholesterolemia

L.A. Salazar¹, S.A. Cavalli¹,
M.H. Hirata¹, J. Diament²,
N. Forti², S.D. Giannini²,
E.R. Nakandakare³,
M.C. Bertolami⁴
and R.D.C. Hirata¹

¹Departamento de Análises Clínicas e Toxicológicas,
Faculdade de Ciências Farmacêuticas, ²Instituto do Coração, and
³Divisão de Endocrinologia, Hospital das Clínicas, Faculdade de Medicina,
Universidade de São Paulo, São Paulo, SP, Brasil
⁴Instituto Dante Pazzanese de Cardiologia, São Paulo, SP, Brasil

Abstract

Correspondence

L.A. Salazar
Departamento de Análises Clínicas
e Toxicológicas, FCF, USP
Av. Prof. Lineu Prestes, 580, B17
05508-900 São Paulo, SP
Brasil
Fax: +55-11-813-2197
E-mail: luasn@usp.br

Research supported by FAPESP
and CAPES. L.A. Salazar is the
recipient of a FAPESP fellowship
(No. 98/09759-8).

Received March 15, 2000
Accepted July 25, 2000

Familial hypercholesterolemia (FH) is a metabolic disorder inherited as an autosomal dominant trait characterized by an increased plasma low-density lipoprotein (LDL) level. The disease is caused by several different mutations in the LDL receptor gene. Although early identification of individuals carrying the defective gene could be useful in reducing the risk of atherosclerosis and myocardial infarction, the techniques available for determining the number of the functional LDL receptor molecules are difficult to carry out and expensive. Polymorphisms associated with this gene may be used for unequivocal diagnosis of FH in several populations. The aim of our study was to evaluate the genotype distribution and relative allele frequencies of three polymorphisms of the LDL receptor gene, *HincII*₁₇₇₃ (exon 12), *AvaII* (exon 13) and *PvuII* (intron 15), in 50 unrelated Brazilian individuals with a diagnosis of heterozygous FH and in 130 normolipidemic controls. Genomic DNA was extracted from blood leukocytes by a modified salting-out method. The polymorphisms were detected by PCR-RFLP. The FH subjects showed a higher frequency of A+A+ (*AvaII*), H+H+ (*HincII*₁₇₇₃) and P1P1 (*PvuII*) homozygous genotypes when compared to the control group ($P < 0.05$). In addition, FH probands presented a high frequency of A+ (0.58), H+ (0.61) and P1 (0.78) alleles when compared to normolipidemic individuals (0.45, 0.45 and 0.64, respectively). The strong association observed between these alleles and FH suggests that *AvaII*, *HincII*₁₇₇₃ and *PvuII* polymorphisms could be useful to monitor the inheritance of FH in Brazilian families.

Key words

- Familial hypercholesterolemia
- DNA polymorphism
- Atherosclerosis
- Genetics
- LDL receptor

Familial hypercholesterolemia (FH) is a common autosomal dominant disease with an estimated world prevalence of 0.2% (1). It is caused by defects in the gene coding for the low-density lipoprotein receptor (LDLR) preventing normal clearance of plasma LDL. The disease is clinically characterized by elevated LDL-cholesterol (LDL-C) levels in blood, tendon xanthomas, and premature coronary heart disease (1). However, these symptoms are not always present, especially in children, and hence do not allow an unequivocal diagnosis. As an appropriate diet and drug treatment of FH patients can reduce their risk of future coronary heart disease, it would be advantageous to identify the affected subjects before the onset of clinical symptoms. Thus, molecular characterization of the LDLR gene appears to be a useful tool for diagnosis. It has been demonstrated that the disease is caused by several different mutations in the LDLR gene (2-5). Although the early identification of individuals carrying the defective gene could be useful in reducing the risk of atherosclerosis and myocardial infarction, the available techniques for determining the number of the functional LDLR molecules are difficult and expensive. However, several authors have shown that polymorphisms associated with this gene may be used to monitor the inheritance of the defective allele in FH families (6-8).

In the present study, we report the genotype distribution and the relative allele frequencies for three polymorphisms of the LDLR gene, *HincII*₁₇₇₃ (exon 12), *AvaII* (exon 13) and *PvuII* (intron 15), in 50 unrelated Brazilian individuals clinically diagnosed as FH heterozygotes and in 130 normolipidemic controls.

The subjects were selected according to the following criteria: normolipidemic controls had total cholesterol levels ≤ 5.7 mmol/l with LDL-C < 4.0 mmol/l, triglycerides ≤ 1.8 mmol/l, and a negative family history of hyperlipidemias, coronary heart disease, or other clinical signs of atherosclerosis. FH

patients had total cholesterol levels ≥ 7.5 mmol/l with LDL-C ≥ 5.2 mmol/l, a triglyceride level not exceeding 2 mmol/l, plus two of the following: tendon xanthomas in the proband or in one or more first degree relatives, hypercholesterolemic children in the family, cholesterol levels ≥ 7.5 mmol/l in two or more family members, and a family history of coronary artery disease in one or more first or second degree relatives younger than 50 years, and absence of the 3500 mutation, characteristic of familial defective apolipoprotein B-100 (9). All subjects gave informed consent to participate in the study.

Serum lipid and lipoprotein concentrations were determined after an overnight (> 12 h) fast by standard procedures. Genomic DNA was extracted from peripheral blood leukocytes by a salting-out procedure (10). The 3500 mutation in the apolipoprotein B-100 gene was detected according to the procedure described by Cavalli et al. (11). The *AvaII*, *HincII*₁₇₇₃, and *PvuII* polymorphic sites of the LDLR gene were detected by PCR-RFLP, according to previously described procedures (12,13). Allele frequencies and genotype distribution for each polymorphic site were estimated by gene counting. Chi-square analysis was used to test Hardy-Weinberg equilibrium and for comparison of allele frequencies and genotype distribution between the studied groups, with the level of significance set at 5%.

Clinical details, lipid and lipoprotein concentrations (mean \pm SD) in serum from 50 unrelated Caucasian FH subjects and 130 normolipidemic controls are shown in Table 1. As expected, serum total cholesterol, triglyceride and LDL-C concentrations were higher in FH subjects ($P < 0.0001$). HDL-C concentrations were similar for the two groups.

All subjects, FH and controls, showed absence of the 3500 mutation in the apolipoprotein B-100 gene.

Table 2 gives the genotype distribution and the relative allele frequencies for FH

patients and controls for the polymorphisms studied. None of the genotype distributions differed significantly from the one expected for a sample in Hardy-Weinberg equilibrium. The FH patients showed a greater frequency of A+A+ (*AvaII*), H+H+ (*HincII*₁₇₇₃) and P1P1 (*PvuII*) homozygous genotypes when compared to the normolipidemic controls ($\chi^2 = 7.07$, $P = 0.0292$; $\chi^2 = 8.92$, $P = 0.0116$ and $\chi^2 = 10.1$, $P = 0.0064$, respectively). In addition, FH probands presented a high frequency of A+, H+ and P1 alleles when compared to normolipidemic individuals (Table 2). However, the relative allele frequencies found in the Brazilian FH patients were similar to those for other FH individuals from Caucasian populations (6-8).

In conclusion, the strong association observed between A+, H+ and P1 alleles and FH suggests that *AvaII*, *HincII*₁₇₇₃ and *PvuII* polymorphisms of the LDLR gene could be useful as genetic markers to monitor the inheritance of familial hypercholesterolemia in Brazilian families. However, family studies are required to support this assumption.

Acknowledgments

We are grateful to Dr. Nga Y. Nguyen (CBER, FDA, Bethesda, USA) for providing the primers used in the amplification of the LDLR gene.

References

1. Brown MS & Goldstein JL (1986). A receptor-mediated pathway for cholesterol homeostasis. *Science*, 232: 34-47.
2. Hobbs H, Brown M & Goldstein J (1992). Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Human Mutation*, 1: 445-466.
3. Varret M, Rabes JP, Villegier L, Junien C, Beroud C & Boileau C (1999). Analysis of the 525 point mutation in the human LDL receptor gene database. *Atherosclerosis*, 144: 182-183 (Abstract).
4. Alberto FL, Figueiredo MS, Zago MA, Araújo AG & Dos-Santos JE (1999). The Lebanese mutation as an important cause of familial hypercholesterolemia in Brazil. *Brazilian Journal of Medical and Biological Research*, 32: 739-745.
5. Soutar AK (1998). Update on low density lipoprotein receptor mutations. *Current Opinion in Lipidology*, 9: 141-147.
6. Chaves FJ, Puig O, Garcia-Sogo M, Real J, Gil JV, Ascaso J, Carmena R & Armegod ME (1996). Seven DNA polymorphisms in the LDL receptor gene: application to the study of familial hypercholesterolemia in Spain. *Clinical Genetics*, 50: 28-35.
7. Humphries SE, King-Underwood L, Gudnason V, Seed M, Delattre S, Clavey V & Fruchart J-CH (1993). Six DNA polymorphisms in the low density lipoprotein receptor gene: their genetic relationship and an example of their use for identifying affected relatives of patients with familial hypercholesterolemia. *Journal of Medical Genetics*, 30: 273-279.

Table 1 - Clinical details of Brazilian heterozygous familial hypercholesterolemia (FH) patients and normolipidemic controls.

Values are reported as mean \pm SD. ns, Nonsignificant (Student t-test).

	FH	Controls	P
N (female/male)	50 (40/10)	130 (85/45)	
Age (years)	50.0 \pm 8.3	54.1 \pm 8.8	ns
Body mass index (kg/m ²)	28.4 \pm 4.7	26.6 \pm 6.0	ns
Total cholesterol (mmol/l)	11.4 \pm 1.7	4.4 \pm 0.6	<0.0001
Triglycerides (mmol/l)	1.3 \pm 0.4	1.0 \pm 0.4	<0.0001
HDL-cholesterol (mmol/l)	1.2 \pm 0.2	1.3 \pm 0.3	ns
LDL-cholesterol (mmol/l)	9.7 \pm 1.8	2.7 \pm 0.6	<0.0001
Presence of xanthomas (%)	60	0	
Positive family history of premature atherosclerosis (%)	90	0	

Table 2 - Comparison of genotype distribution and relative allele frequencies of three polymorphisms of the LDLR gene in Brazilian individuals with heterozygous familial hypercholesterolemia (FH) and normolipidemic controls (NC).

^a+/- Indicates presence/absence of a restriction site; ^bP1/P2 indicates absence/presence of a restriction site.

Polymorphism	Genotype distribution (%)			Allele frequency	
	A+A+	A+A-	A-A-	A+	A-
<i>AvaII</i> ^a					
FH (N = 50)	34	48	18	0.58	0.42
NC (N = 130)	16	58	26	0.45	0.55
	$\chi^2 = 7.07$ (2 d.f., $P = 0.0292$)			$\chi^2 = 4.38$ (1 d.f., $P = 0.0364$)	
<i>HincII</i> ₁₇₇₃ ^a					
FH (N = 50)	35	52	13	0.61	0.39
NC (N = 130)	18	55	27	0.45	0.55
	$\chi^2 = 8.92$ (2 d.f., $P = 0.0116$)			$\chi^2 = 6.77$ (1 d.f., $P = 0.0093$)	
<i>PvuII</i> ^b					
FH (N = 50)	P1P1	P1P2	P2P2	P1	P2
	64	29	7	0.78	0.22
NC (N = 130)	38	52	10	0.64	0.36
	$\chi^2 = 10.1$ (2 d.f., $P = 0.0064$)			$\chi^2 = 5.99$ (1 d.f., $P = 0.0144$)	

8. Puig O, Chaves FJ, Garcia-Sogo M, Real J, Gil JV, Ascaso J & Armegod ME (1996). A three-allelic polymorphic system in exon 12 of the LDL receptor gene is highly informative for segregation analysis of familial hypercholesterolemia in the Spanish population. *Clinical Genetics*, 50: 50-53.
9. Myant NB (1993). Familial defective apolipoprotein B-100: a review, including some comparisons with familial hypercholesterolemia. *Atherosclerosis*, 104: 1-18.
10. Salazar LA, Hirata MH, Cavalli SA, Machado MO & Hirata RDC (1998). Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. *Clinical Chemistry*, 44: 1748-1750.
11. Cavalli SA, Hirata MH & Hirata RDC (1999). Optimized procedure for detection of 3500 and 3531 mutations and MspI polymorphism in exon 26 of the apolipoprotein B-100 gene. *Brazilian Journal of Pharmaceutical Sciences*, 35: 15 (Abstract).
12. Salazar LA, Hirata MH, Giannini SD, Forti N, Diament J, Issa J & Hirata RDC (1999). Effects of Avall and HincII polymorphisms at the LDL receptor gene on serum lipid levels of the Brazilian individuals with high risk for coronary heart disease. *Journal of Clinical Laboratory Analysis*, 13: 251-258.
13. Salazar LA, Hirata MH, Forti N, Diament J, Giannini SD, Issa J & Hirata RDC (2000). Pvull intron 15 polymorphism at the LDL receptor gene is associated with differences in serum lipid concentrations in individuals with low and high risk for coronary artery disease from Brazil. *Clinica Chimica Acta*, 293: 75-88.