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Lipid-Lowering Response of the HMG-CoA Reductase Inhibitor Fluvastatin Is Influenced by Polymorphisms in the Low-Density Lipoprotein Receptor Gene in Brazilian Patients With Primary Hypercholesterolemia

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Although the efficacy of fluvastatin (HMG-CoA reductase inhibitor) in the treatment of primary hypercholesterolemia is well documented, a wide interindividual variation treatment response has been observed. We have studied the possible role of the *Ava*II (exon 13), *Hinc*II (exon 12), and *Pvu*II (intron 15) polymorphisms at the low-density lipoprotein receptor (LDLR) gene on lipid-lowering response in 55 patients (36 to 70 years old) with primary hypercholesterolemia treated with fluvastatin for 16 weeks. LDLR genotypes were determined by PCR-RFLP. The results indicate that the *Ava*II and *Pvu*II polymorphisms influence the cholesterol-lowering response of the HMG-CoA reductase inhibitor Fluvastatin. Patients carrying A+A+ (*Ava*II) or P1P1 (*Pvu*II) homozygous genotypes presented lower reduction in total cholesterol, LDL-C and apolipoprotein B levels after 16 weeks of treatment with fluvastatin, when compared to other genotypes (P <0.05). Our data also support the previous assumption that the *Ava*II, *Hinc*II, and *Pvu*II polymorphisms of the LDLR gene are associated with variation of serum cholesterol levels. Therefore, the identification of the LDLR genetic profile may provide better prediction of a patient's clinical response to fluvastatin. J. Clin. Lab. Anal. 14:125–131, 2000.

Key words: low-density lipoprotein receptor gene; genetic polymorphisms; primary hypercholesterolemia; fluvastatin; treatment response

INTRODUCTION

High blood cholesterol levels, particularly low-density lipoprotein cholesterol (LDL-C), increase the risk of coronary artery disease (CAD), and the lowering of both total cholesterol and LDL-C has been shown to reduce the incidence of CAD (1–3). Treatment has been recommended for those patients with high LDL-C levels (≥4.1 mmol/L) and for those with borderline high values (3.4 to 4.1 mmol/L) in the presence of definitive CAD or two (or more) risk factors for CAD (4).

The U.S. National Cholesterol Education Program (NCEP) recommends treatment goals of LDL-C <3.4 mmol/L and triglyceride (TG) levels < 2.3 mmol/L (4). High-density lipoprotein cholesterol (HDL-C) also appears to be an independent risk factor for CAD, with higher levels being protective (5). Although there does not appear to be a comparable causality between elevated plasma TG levels and CAD, some TG-rich lipoproteins can be atherogenic, and high TG concentrations can produce increases in concentration of several clotting factors and decreases in fibrinolytic activity (6).

Therefore, treatments that reduce serum LDL-C and TG and, at the same time, enhance HDL-C levels are ideally suited to treat hypercholesterolemic patients that do not respond to dietary intervention.

Recently, several 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors have been developed (7–9). These agents reduce endogenous cholesterol biosynthesis by competitive inhibition of the main rate-limiting enzyme HMG-CoA reductase. The resulting low intracellular levels of cholesterol lead to increased production of high-affinity LDL receptors on hepatocytes and increased hepatic uptake of circulating LDL (10). In addition, HDL-C levels are increased and TG levels are reduced.

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Fluvastatin was the first, totally synthetic member of this class of agents. Its absorption is virtually complete (> 98%) and unaffected by the presence of food. Systemic exposure is limited due to a characteristically short half-life (< 30 minutes) (11).

The pharmacokinetic and pharmacodynamic properties of fluvastatin are not modified by age or sex (12). In controlled clinical trials in young and middle-aged patients with primary hypercholesterolemia, fluvastatin, at a dose of 20 to 80 mg once daily, reduced the LDL-C, TG, and apolipoprotein B (apo B) by 22 to 36%, 12 to 18%, and 19 to 23%, respectively (13,14). Other effects of fluvastatin on the plasma lipid profile that improve CAD risk include increasing HDL-C levels (3.3 to 5.6%) and decreasing the LDL-C:HDL-C ratio. In addition, fluvastatin has antiatherogenic, antithrombotic, and antioxidant effects, can improve vascular function, and may have immunomodulatory effects (13,15).

Although the efficacy of fluvastatin in the treatment of primary hypercholesterolemia is well documented (13–18), a wide interindividual variation treatment response has been observed in a number of studies (13–21). This variation can be partially explained by various environmental and genetic factors that affect the disposition of fluvastatin (i.e., absorption, distribution, biotransformation, excretion, or a combination of these) in each individual. However, environmental and genetic also may exert their effects by modulating substrates and/or structures mandatory for the action of the drug. Thus, bearing in mind the mechanism of action of fluvastatin, all factors that alter the function of the LDL receptor (LDLR) or the ligands for the receptor (i.e., apo B or apo E) might be expected to contribute to the variation in treatment response to fluvastatin. This hypothesis is supported by the observation that approximately 50% of the interindividual variability in lipid levels may be attributable to genetic influence (22).

Serum apolipoproteins serve as structural components of lipoproteins, cofactors for lipid-metabolizing enzymes, and ligands for receptor-mediated uptake of lipoprotein particles. These genes are polymorphic and some of the reported polymorphisms have been found to be associated with alterations in serum lipid levels (23).

Recently, our group have associated the *Ava*II (exon 13), *Hinc*II (exon 12), and *Pvu*II (intron 15) polymorphisms at the LDLR gene with differences on the serum lipid profiles in Brazilian individuals with high risk for coronary artery disease (24,25). In this study, we have investigated the possible influence of these polymorphisms of the LDLR gene on treatment response to fluvastatin in 55 Brazilian patients with primary hypercholesterolemia.

MATERIALS AND METHODS

Subjects

After completing an 8-week placebo period, a total of 55 patients (15 men and 40 women; mean age, 59 ± 3 years) with type IIA primary hypercholesterolemia, according to Fredrickson's classification (LDL-C ≤4.1 mmol/L; TG ≤3.0 mmol/L), were enrolled in a multicenter, randomized study. After enrollment, 24 patients were treated with a dose of 40 mg and 31 patients were treated with 80 mg of fluvastatin daily for 16 weeks.

Patients with secondary forms of dyslipidemia and those with diabetes mellitus, hypothyroidism, or those controlled with drug therapy were excluded, as were those who were obese (Body mass index ≥ 30 kg/m^2) or had abnormal liver or renal function. Patients with neoplasm and who had suffered acute myocardial infarction or had undergone coronary bypass surgery also were excluded. The ethical committee of our hospital accepted the study protocol. Each patient provided informed consent before participating in the study.

Lipid Measurements

At end of the placebo period (baseline) and after 16 weeks of fluvastatin treatment, serum lipid levels were determined from blood samples collected after overnight (>12 hours) fast. Triglycerides (TG) were determined by enzymatic assay (26), and total cholesterol (TC) was assayed by the esterase-oxidase method (27). High-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic assay after phosphotungstic acid and magnesium precipitation (28). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (29). The serum levels of apolipoprotein A-I and B were determined by RIA (30).

DNA Analysis

Genomic DNA was extracted from blood leukocytes by a salting-out procedure modified in our laboratory (31). The *Ava*II, *Hinc*II, and *Pvu*II polymorphic regions at the LDLR gene were amplified by polymerase chain reaction (PCR) as previously described (24,25). Amplified products were digested with *Ava*II, *Hinc*II, or *Pvu*II and the resulting fragments were separated on 2 or 4%-agarose gels stained with ethidium bromide, and visualized on UV light.

The correct assessment of genotype for *Ava*II, *Hinc*II, and *Pvu*II polymorphisms at the LDLR gene was evaluated using a homozygous sample for restriction site (A+A+, H+H+, or P2P2, respectively) as a positive control. In addition, all gels were reread blindly by two persons without any change, and 10% of the analysis were repeated randomly.

Statistical Methods

The drug efficacy within and between groups was assessed by analyzing the percentage changes from baseline after 16 weeks of treatment with fluvastatin. Differences among lipid and lipoprotein concentrations in different groups of individuals were compared using the Student's *t*-test (32). Allele frequencies and genotype distribution for each polymorphic site

were estimated by gene counting. Chi-square analysis was used to test Hardy-Weinberg equilibrium. The sampling distributions of all the quantitative variables were tested for normality, and were $\log_e$ transformed to obtain normal distribution. To evaluate the effect of each polymorphism on the variation of quantitative variables of lipid, one-way ANOVA was performed (32). Significance was considered to be at the 5% level.

RESULTS

Drug Efficacy

Table 1 presents lipid parameters at baseline and percentage changes after 16 weeks of treatment with different doses of fluvastatin. Both doses (40 or 80 mg daily) produced significant ($P < 0.001$) reductions in TC (mean, –20%), LDL-C (mean, –26%) and apo B levels (mean, –21%), and increasing the HDL-C and apo A-I levels. The percentage changes, observed in Brazilian hypercholesterolemic individuals after 16 weeks of treatment, are similar to that previously described for other hypercholesterolemic patients treated with fluvastatin from several populations (13–21). However, the percentage changes were not significantly different between the two treatment groups.

Considering that the percentage changes after 16 weeks in lipid parameters were not significantly different between the two treatment groups, we have grouped the individuals to study the influence of genetic polymorphisms at the LDLR gene on serum lipid levels and treatment response to fluvastatin.

LDLR Polymorphisms and Baseline Parameters

The distribution pattern of the *Ava*II, *Hinc*II, and *Pvu*II polymorphisms of the Brazilian individuals with primary hypercholesterolemia is shown in Table 2. The allele frequencies are similar to that previously reported for hypercholesterolemic subjects from the Brazilian population (24,25). Moreover, when the Hardy-Weinberg equilibrium (HWE) was evaluated we observed that *Ava*II, *Hinc*II, and *Pvu*II genotype distributions did not differ from what was projected.

TABLE 2. Genotype distribution and relative allele frequency of polymorphisms at the LDLR gene in 55 Brazilian patients with primary hypercholesterolemia[a]

Polymorphisms	Genotype distribution			Allele frequency	
*Ava*II[b]	A+A+	A+A–	A–A–	A+	A–
	31%	55%	14%	0.582	0.418
*Hinc*II[b]	H+H+	H+H–	H–H–	H+	H–
	31%	51%	18%	0.564	0.436
*Pvu*II[c]	P1P1	P1P2	P2P2	P1	P2
	64%	29%	7%	0.782	0.218

[a]Hardy-Weinberg Equilibrium, *Ava*II Genotypes: $\chi^2 = 0.80$ (1 df, P = NS); *Hinc*II Genotypes: $\chi^2 = 0.07$ (1df, P = NS); *Pvu*II Genotypes: $\chi^2 = 1.19$ (1df, P = NS); NS, not significant.
[b]+/– indicates the presence/absence of restriction site.
[c]P1/P2 indicates the absence/presence of restriction site.

As shown in Figure 1 (Panels A–C), significant variability among *Ava*II, *Hinc*II, and *Pvu*II genotypes was observed for lipid traits in the hypercholesterolemic patients. Individuals carrying the A+A+ (*Ava*II), H+H+ (*Hinc*II), and P1P1 (*Pvu*II) homozygous genotype presented greater TC, LDL-C, and apo B levels when compared to other genotypes ($P < 0.001$). These data are similar to those previously described in Brazilian individuals with high risk for CAD (24,25).

LDLR Polymorphisms and Treatment Response to Fluvastatin

The effects of the LDLR genotypes on treatment response to fluvastatin are shown in Tables 3–5. To study the effect of LDLR polymorphisms on treatment response to fluvastatin, we grouped the individuals carrying the A+A– and A–A–, H+H– and H–H– and P1P2 and P2P2 genotypes for *Ava*II, *Hinc*II, and *Pvu*II polymorphisms, respectively, due to the small sample sizes for the A–A–, H–H–, and P2P2 genotypes (Table 2).

The baseline levels and percentage changes in serum TC, LDL-C, and apo B in the *Ava*II genotypes after 16 weeks of treatment are shown in Table 3. Here individuals carrying A+A+ homozygous genotype presented lower reduction in TC, LDL-C, and apo B levels after treatment, when com-

TABLE 1. Baseline levels and percentage changes in lipid parameters after 16 weeks of treatment with fluvastatin in 55 Brazilian patients with primary hypercholesterolemia[a]

	40 mg (n = 24)		80 mg (n = 31)		
Parameters[b]	Baseline	Change (%)	Baseline	Change (%)	P[d]
TC, mmol/L[c]	7.86 ± 1.64	–20 ± 12	7.88 ± 1.61	–21 ± 11	NS
HDL-C, mmol/L	1.25 ± 0.24	5 ± 7	1.24 ± 0.26	6 ± 7	NS
LDL, mmol/L[c]	5.83 ± 1.68	–26 ± 12	5.83 ± 1.57	–27 ± 12	NS
Apo B, g/L[c]	1.75 ± 0.32	–21 ± 8	1.71 ± 0.40	–21 ± 9	NS
Apo A-I, g/L	1.30 ± 0.34	3 ± 5	1.31 ± 0.29	3 ± 5	NS

[a]TC, total cholesterol; HDL-C, HDL cholesterol, LDL-C, LDL cholesterol; Apo, apolipoprotein; NS, not significant.
[b]Values are mean ± SD.
[c]Differences between baseline and treatment values were significant ($P < 0.001$).
[d]P values from Student's t-test (% change, 40 mg vs. 80 mg).

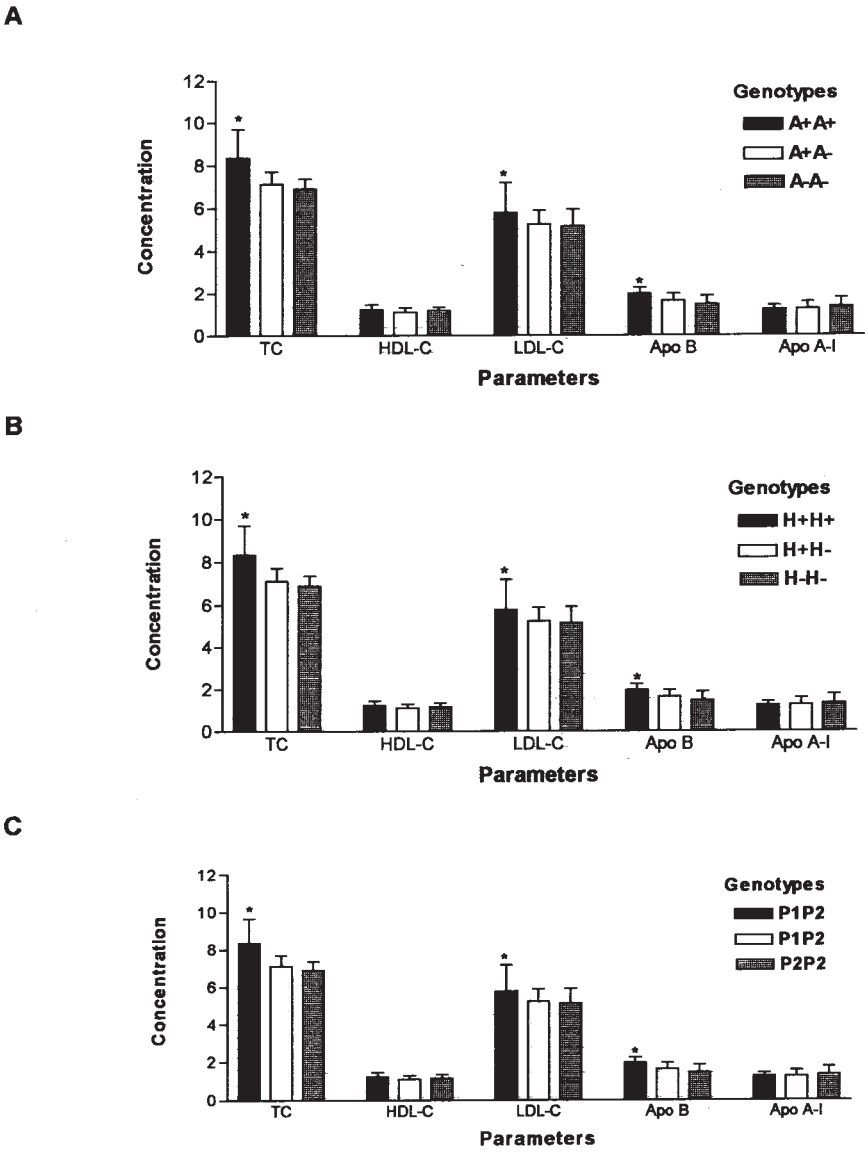

Fig. 1. Lipid parameters at baseline (mean ± SD) in 55 Brazilian individuals with primary hypercholesterolemia grouped in *Ava*II (**A**), *Hinc*II (**B**), and *Pvu*II (**C**) genotypes. TC, HDL-C, and LDL-C values are expressed in mmol/L and apolipoprotein values in g/L. TC, indicates total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; Apo, apolipoprotein; +/–, presence/absence of restriction site and P1/P2, absence/presence of restriction site; *$P < 0.05$ (ANOVA).

TABLE 3. Lipid parameters at baseline and percent change after 16 weeks of treatment with fluvastatin, according to the *Ava*II genotypes[a]

	Baseline levels			Change at week 16 (%)		
Parameters[b]	A+A+ (n = 17)	A+A–/A–A– (n = 38)	P^c	A+A+	A+A–/A–A–	P^c
TC, mmol/L[d]	8.42 ± 1.31	7.02 ± 0.55	<0.05	–20 ± 9	–28 ± 12	0.043
LDL-C, mmol/L	5.81 ± 1.43	5.25 ± 0.63	<0.05	–23 ± 9	–27 ± 7	0.023
Apo B, g/L	2.03 ± 0.33	1.62 ± 0.32	<0.05	–20 ± 9	–28 ± 12	0.043

[a]+/– indicates the presence/absence of restriction site.
[b]Values are mean ± S.D.
[c]*P* values from one-way ANOVA.
[d]TC, total cholesterol; LDL-C, LDL cholesterol; Apo, apolipoprotein.

TABLE 4. Lipid parameters at baseline and percent change after 16 weeks of treatment with fluvastatin, according to the *Hinc*II genotypes[a]

	Baseline levels			Change at week 16 (%)		
Parameters[b]	H+H+ (n = 17)	H+H–/H–H– (n = 38)	P^c	H+H+	H+H–/H–H–	P^c
TC, mmol/L[d]	8.38 ± 1.32	7.13 ± 0.64	<0.05	–21 ± 11	–20 ± 12	NS
LDL-C, mmol/L	5.82 ± 1.35	5.18 ± 0.83	<0.05	–27 ± 12	–25 ± 12	NS
Apo B, g/L	2.02 ± 0.34	1.53 ± 0.42	<0.05	–21 ± 3	–20 ± 3	NS

[a]+/– indicates the presence/absence of restriction site.
[b]Values are mean ± S.D.
[c]*P* values from one-way ANOVA.
[d]TC, total cholesterol; LDL-C, LDL cholesterol; Apo, apolipoprotein; NS, not significant.

pared to other genotypes (P <0.05). On the other hand, no differences in percentage changes in TC, LDL-C, and apo B after 16 weeks of treatment were observed between *Hinc*II genotypes (Table 4).

Table 5 shows the lipid parameters at baseline and percentage changes after 16 weeks of treatment with fluvastatin, according to the *Pvu*II genotypes. Hypercholesterolemic patients carrying the P1P1 homozygous genotype (with the absence of restriction site) also presented lower reduction in TC, LDL-C, and apo B levels, when compared to other genotypes ($P < 0.05$).

DISCUSSION

Although the efficacy of many lipid-lowering drugs is well documented, it also is recognized that within the same diagnostic class of hyperlipidemia, with both dietary and drug therapies, individual patient responses may vary considerably (22).

Several studies have reported (13–21) such interindividual variation in treatment responses to fluvastatin. It is presumed that some of this variation is due to a genetic predisposition for differential metabolic effects of the lipid-lowering intervention. Considering the crucial role of the LDL receptor (LDLR) in cholesterol homeostasis (33) and the observation that approximately 50% of the interindividual variability in lipid levels may be attributable to genetic influences (22), it is conceivable that common genetic alterations in this gene also may contribute to variation in hypercholesterolemic patients' treatment response to fluvastatin.

In this study, we have investigated (for the first time in a Brazilian population) the effects of *Ava*II (exon 13), *Hinc*II (exon 12), and *Pvu*II (intron 15) polymorphisms at the LDLR gene on treatment response to fluvastatin. As we reported previously (24,25), these polymorphisms were strongly associated with differences on serum lipid levels in Brazilian subjects with high risk for coronary artery disease.

The relative allele frequencies for *Ava*II, *Hinc*II, and *Pvu*II polymorphisms at the LDLR gene found in hypercholesterolemic patients (Table 2) are similar to those previously reported by our group in Brazilian individuals (24,25). However, the A+ allele frequency (0.58) found in the Brazilian hypercholesterolemic (HC) individuals is greater than that observed in hypercholesterolemic patients from London, Italy, Spain, Switzerland, and Germany (34,35). On the other hand, the frequency of the H+ allele (0.56) in HC subjects is similar to that found in Swiss, Germans, and Spanish hypercholesterolemic patients (34,35,36).

The relative allelic frequencies of the *Pvu*II polymorphism found in the Brazilian hypercholesterolemic subjects are similar to those found in other Caucasian individuals from different countries. The relative frequency of the P1 allele (0.78) in the HC group is similar to that observed in hypercholesterolemic patients from Italy, Switzerland, Germany, Israel, Spain, London, The Netherlands, Denmark, and North America (34,37–41).

TABLE 5. Lipid parameters at baseline and percent change after 16 weeks of treatment with fluvastatin, according to the *Pvu*II genotypes[a]

	Baseline levels			Change at week 16 (%)		
Parameters[b]	P1P2 (n = 35)	P1P2/P2P2 (n = 20)	P^c	P1P1	P1P2/P2P2	P^c
TC, mmol/L[d]	8.39 ± 1.32	6.97 ± 0.53	<0.05	–20 ± 9	28 ± 12	0.043
LDL-C, mmol/L	5.87 ± 1.43	5.28 ± 0.64	<0.05	–23 ± 9	–27 ± 7	0.023
Apo B, g/L	2.04 ± 0.29	1.63 ± 0.36	<0.05	–23 ± 9	–27 ± 7	0.023

[a]P1/P2 indicates the presence/absence of restriction site.
[b]Values are mean ± S.D.
[c]*P* values from one-way ANOVA.
[d]TC, total cholesterol; LDL-C, LDL cholesterol; Apo, apolipoprotein.

The strong association between A+A+ (*Ava*II), H+H+ (*Hinc*II), and P1P1 (*Pvu*II) genotypes with higher total cholesterol, LDL-C, and apo B circulating levels found in patients with primary hypercholesterolemia (Fig. 1), support the previous assumption (24,25) that the *Ava*II, *Hinc*II, and *Pvu*II polymorphisms of the LDLR gene are associated with variation on serum lipid levels in the Brazilian population.

In addition, the present study demonstrates that the response to the HMG-CoA reductase inhibitor fluvastatin in Brazilian patients with primary hypercholesterolemia was related, at least in part, to the *Ava*II and *Pvu*II polymorphisms at the LDLR gene. Although all patients have shown a substantial reduction of serum lipid parameters after fluvastatin treatment, patients who have the A+A+ (*Ava*II) genotype demonstrate a statistically significant lower response when compared to other genotypes (Table 3). Lower response to treatment also was observed in individuals with the P1P1 (*Pvu*II) genotype (Table 5).

The mechanism responsible for the varied cellular responses to treatment with an HMG-CoA reductase inhibitor remains unclear. Considering that *Ava*II and *Hinc*II polymorphisms do not involve an amino-acid substitution (42), and *Pvu*II polymorphism is located in an intronic region of the LDLR gene (41), it is conceivable that these polymorphisms have an indirect effect on cholesterol metabolism. This effect may be mediated through a functional mutation in this gene—linkage disequilibrium with these restriction sites—or in a closely linked gene (42).

Leitersdorf et al. (43) demonstrated that the response to fluvastatin was profoundly affected by the type of LDLR mutation in heterozygous familial hypercholesterolemia (FH) patients. The presence of the *Sephardic* mutation, which results in the production of a precursor protein that is not processed to its mature form (class IIa), or of the *Lithuanian* mutation, which creates a class IIb protein (transport-defective slow-processing), was associated to lower response to treatment with 40 mg of fluvastatin.

Recently, several authors (44–46) also have shown the influence of genotype at the LDLR gene locus on the clinical phenotype and cholesterol-lowering response to HMG-CoA reductase inhibitors in patients with heterozygous FH. Moreover, Pedersen and Berg (47) have reported a potential interaction between the *Pvu*II polymorphism at the LDLR gene and variation in the apo E locus (E2, E3, and E4 isoforms) in determining plasma lipid levels. Some studies have demonstrated that the apo E polymorhisms also modulate the response to HMG-CoA reductase inhibitors in hypercholesterolemic individuals (22,48). Therefore, future studies will be necessary to identify the molecular relationship between these polymorphisms and other genetic alterations at the LDLR locus or in the linked gene. However, this will require an increase in interest in the field, and in teaching effort.

In summary, we have demonstrated that normal genetic variations at the LDLR locus contributed significantly to the determination of plasma cholesterol levels and to the variation of treatment response to fluvastatin in patients with primary hypercholesterolemia. Therefore, the identification of the LDLR genetic profile may provide a better prediction of patients' clinical response to fluvastatin.

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