Available online at www.sciencedirect.com

SCIENCE DIRECT®

Metabolism Clinical and Experimental 54 (2005) 741–747

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Baseline levels of low-density lipoprotein cholesterol and lipoprotein (a) and the *Ava*II polymorphism of the low-density lipoprotein receptor gene influence the response of low-density lipoprotein cholesterol to pravastatin treatment☆

Carlos Lahoz[a,*], Rocío Peña[a], Jose M. Mostaza[a], Fernando Laguna[a], María F. García-Iglesias[a], Manuel Taboada[b], Xavier Pintó[c] on behalf of the RAP Study Group

[a]*Unidad de Arteriosclerosis, Hospital Carlos III, 28029 Madrid, Spain*
[b]*Centro de Salud de Fuencarral, 28029 Madrid, Spain*
[c]*Unidad de lípidos, Hospital de Bellvitge, 08907 Barcelona, Spain*

Received 18 October 2004; accepted 30 December 2004

Abstract

To investigate some individual and genetic factors that may influence the response of low-density lipoprotein cholesterol (LDL-C) to pravastatin treatment, we recruited 440 subjects with hypercholesterolemia (mean age, 57 years; 43% men) from 21 primary health care centers-outpatient clinics into a prospective, multicentered intervention trial. Pravastatin (20 mg/d) was prescribed for 16 weeks. The main outcome was the percentage variation in LDL-C concentration relative to baseline. Blood analyses and genotyping were performed centrally. The results indicated that LDL-C decreased by 20.5% (range, +21% to −66%) after pravastatin treatment. Baseline concentration of LDL-C (the higher the concentration, the greater the decrease), lipoprotein (a) levels (the lower the concentration, the greater the response), and *Ava*II polymorphism of the LDL-receptor gene significantly influenced the hypolipemic effect ($P < .001$, $P = .014$, and $P = .004$, respectively). These 3 factors combined explained 10.6% of the variation in LDL-C response. Age, sex, smoking habit, alcohol consumption, body mass index, and apolipoprotein E genotype had no significant effect on response. We conclude that baseline levels of LDL-C and lipoprotein (a) together with the *Ava*II polymorphism of the LDL-receptor gene have a significant influence on the LDL-C response to pravastatin treatment in patients monitored in a standard primary health care outpatient clinic setting.

1. Introduction

The prescription of HMG-CoA reductase inhibitors (*statins*) has resulted in a consistent reduction in cardiovascular morbidomortality in primary as well as in secondary prevention trials [1-5]. Since their advent in the decade of the 1990s, the use of the statins has become generalized because of their high efficacy and low secondary effects. The average annual increase in their prescription has been greater than 30% over the past few years in European countries [6]. Their principal action is the reduction of the plasma lipids by inducing a considerable decrease in the concentration of plasma LDL cholesterol while causing a moderate decrease in triglycerides and a modest increase in the concentration of high-density lipoprotein cholesterol (HDL-C) [1-5]. Other possible beneficial effects of the statins not directly related to the decrease in low-density lipoprotein cholesterol (LDL-C) have been described, including anti-inflammatory and antioxidant activities and plaque stabilization; the real importance of these effects remains to be clarified [7].

The statins reduce LDL-C levels between 20% and 60% [8] depending on the dose and type of the drug used. Nevertheless, there appears a wide interindividual variability

☆ The study was supported, in part, by research grants from the Fondo de Investigacion Sanitaria (FIS 98/0201 and FIS 02/0815) and the Fundación para el Fomento y Desarrollo de la Investigación Clínica.

* Corresponding author. Tel.: +34 91 4532530; fax: +34 91 7336614.
E-mail address: clahoz.hciii@salud.madrid.org (C. Lahoz).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.12.020

in response to the same level of dose administered [9,10]. For example, in the WOS study, the percentage response of LDL-C to a fixed dose of 40 mg of pravastatin varied between −60% and +20%. This considerable variability in the hypolipidemic response has been attributable to individual environmental or genetic factors. Among these are sex [11-13], age [14,15], tobacco consumption [14], body mass index (BMI) [14,16], alcohol consumption [15], baseline levels of LDL-C [11,14-16], and the concentration of triglycerides [11,15]. The genetic factors studied to date include several polymorphisms of genes related to lipoprotein metabolism. However, their influence on response appears to be moderate and not universally observed in all of the studies [17]. Furthermore, the data generated from controlled clinical trials, with strict inclusion-exclusion criteria and follow-up, cannot be easily extrapolated to a free-living general population.

A better understanding of the factors that influence hypolipemic response could help identify patients who would best respond to the treatment. More importantly, perhaps, would be to identify patients who are likely to have a poor hypolipidemic response and to select a more appropriate treatment so as to avoid unnecessary costs and side effects.

The objective of the present study was to evaluate individual environmental and genetic factors that may influence the response of LDL-C to treatment with pravastatin in a population of subjects with hypercholesterolemia followed up in outpatient clinics.

2. Subjects and methods

The design of the study has been described previously [18]. Briefly, the subjects included in the present analysis participated in the RAP study, whose objective was to evaluate the factors that may influence hypolipidemic response to pravastatin. The RAP study is a prospective, multicentered intervention trial in which subjects with hypercholesterolemia who, despite a diet low in saturated fat and cholesterol, required hypolipemic drug therapy based on the National Cholesterol Education Program criteria [19]. Subjects received 20 mg/d of pravastatin over 16 weeks. Participants were recruited from 21 primary health care centers distributed throughout Spain and followed up by their respective physicians at the primary health care center where they provided blood samples before and after the 16 weeks of treatment. Among the exclusion criteria were the following: plasma triglycerides of greater than 4.5 mmol/L, diabetes mellitus, treatment with other hypolipemic agents, acute illness in the previous 3 months, serious or uncontrolled renal, hepatic, digestive, or endocrine disorders, secondary hypercholesterolemias, and those known to be hypersensitive to statins. Over a period of 8 months, there were 656 subjects recruited who, after the dietary phase, fulfilled the criteria for medication. In 442 subjects (76.4%), a complete clinical history was obtained, lipid levels had been measured before and after the treatment, and compliance (assessed by pill counting), was greater than 80%. These patients represent the basis of the present analysis. To all patients with a BMI higher than 25 kg/m^2, a weight-reducing hypocaloric diet was prescribed. Included in the statistical analyses were age, sex, BMI, blood pressure, smoking habit, weight change, and consumption of alcohol. Alcohol intake was self-reported in units per week. The study was approved by the Committee on Ethics and Clinical Investigation of the Hospital Carlos III in Madrid.

3. Laboratory measurements

Venous blood was taken after a 12-hour fast. Cholesterol and triglycerides were measured using enzymatic methods (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein cholesterol was measured after the precipitation of the apolipoprotein B (apoB)–containing particles with phosphotungstic acid. Low-density lipoprotein cholesterol was calculated using the Friedewald formula. Lipoprotein (a) [Lp(a)] was determined using radioimmunoassay kits (Trinity Biotech, Bray, Ireland). All blood analyses were performed in a centralized laboratory.

The *Pvu*II polymorphism of the LDL-receptor gene was determined after amplification of a 800-base pair (bp) fragment using the polymerase chain reaction (PCR) in a thermocycler (PTC-100 MJ Research, Mass). The primers used were 5′-TCC CCT TCA AAA TGC CCT CTT-3′ and 5′-AGC CAC CGA GCC CAG CCT AAG AA-3′. The final volume for the PCR mixture was 25 μL and contained 2 mmol/L of $MgCl_2$, 0.2 mmol/L of dNTP, and 1 U of Taq DNA polymerase. The amplification was for 30 cycles: 95°C for 1 minute, 63°C for 1 minute, and 72°C for 2 minutes 30 seconds. After the digestion of the PCR product with the restriction enzyme *Pvu*II, the fragments were separated by electrophoresis in 1.5% agarose gel and visualized under UV light.

The *Ava*II polymorphism was determined after the PCR amplification of a 228-bp zone in exon 13 of the LDL-receptor gene. The primers were 5′-GTC ATC TTC CTT GCT GCC TGT TTA G-3′ and 5′-GTT TCC ACA AGG AGG TTT CAA GGT T-3′. The 25-μL PCR mixture contained 1.5 mmol/L of $MgCl_2$, 0.2 mmol/L of dNTP, 0.3 μmol/L of each primer, and 1.5 U of Taq DNA polymerase. Amplification of the polymorphic region was performed with 28 cycles: 94°C for 1 minute, 68°C for 2 minutes, and 72°C for 1 minute. The digestion of the amplified product was performed with the restriction enzyme *Ava*II. The fragments were separated by electrophoresis in 2% agarose gels and visualized under UV light.

The apolipoprotein E (apoE) genotype was performed using PCR amplification of a 303-bp fragment. The primers were 5′-CGG GCA CGG CTG TCC AAG GAG-3′ and 5′-CAC GCG GCC CTG TTC CAC GAG-3′. The 25-μL PCR reaction mixture contained 1.6 mmol/L of $MgCL_2$,

0.2 mmol/L of dNTP, 10% of DMSO, and 1 U of Taq DNA polymerase. The amplification was for 30 cycles: 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds. After the digestion of the PCR product with the restriction enzyme *Cfo*-1, the fragments were separated by electrophoresis in 8% polyacrylamide gels. Subsequently, the gel was treated with ethidium bromide and the fragments were visualized under UV light.

4. Statistical analyses

All the statistical analyses were performed with the SPSS statistical package (SPSS Inc, Chicago, Ill). Quantitative variables are presented as the mean and the SD and the qualitative variables as percentages. To check for normality of distribution, the Kolmogorov-Smirnof test was applied. The triglycerides and Lp(a) values were not normally distributed and needed to be log transformed before applying the statistical analyses. For a clearer presentation, the original nontransformed values are presented in the tables.

To evaluate the trends of the effects of pretreatment variables on LDL response, basal values of LDL-C and Lp(a) were segregated in quartiles. Comparisons between quantitative variables were performed by analysis of variance and between qualitative variables by the χ^2 test. Comparisons between lipid levels before and after the treatment were performed with the Student t test for paired samples. Relationships between LDL-C response and other measured variables were evaluated using linear regression analysis with the dependent variable being the percentage variation of the LDL-C relative to baseline. A multiple linear regression model was used to assess the simultaneous contributions of different variables. Only those variables that had P values less than .10 in the univariate analyses were included in the multivariate analyses. The P values for all tests were 2 tailed, and differences were considered to be statistically significant at the .05 level.

5. Results

Of the 440 participants, 43% were men and the overall mean age was 57.4 years. Table 1 summarizes the characteristics of the study population, including the baseline lipoprotein concentrations. There were no significant differences with respect to baseline characteristics between the subjects who were included and those who were excluded from the study (data not shown). The treatment with 20 mg/d of pravastatin significantly reduced total cholesterol, LDL-C, and triglyceride levels while increasing HDL-C (albeit without reaching statistical significance) and significantly increasing Lp(a) concentration (Table 2). Low-density lipoprotein cholesterol response varied between +21% and −66% (Fig. 1). Also, there was a mean weight loss of 0.360 kg (95%CI, 0.07-0.66; P = .015) during the study.

Table 1
Patients' characteristics at baseline

Characteristic	N = 440
Age [y; mean (SD)]	57.4 (11.6)
Sex (% men)	43.0
BMI [kg/m^2; mean (SD)]	27.2 (3.5)
Hypertensive (%)	38.3
Current smokers (%)	22.3
Alcohol intake [g/wk; mean (SD)]	59.9 (108)
Cardiovascular disease (%)	10.9

Table 2
Percentage variation, relative to baseline, of lipid and lipoprotein concentrations in the overall study population after treatment with pravastatin

	Baseline	Final	% Change	P
Cholesterol (mmol/L)	7.28 (1.10)	6.11 (1.02)	−15.5 (12.2)	<.001
LDL-C (mmol/L)	5.19 (1.06)	4.08 (0.96)	−20.5 (15.8)	<.001
HDL-C (mmol/L)	1.34 (0.96)	1.36 (0.34)	3.7 (19.6)	.097
Triglycerides (mmol/L)	1.60 (0.76)	1.45 (0.70)	−3.7 (35.4)	<.001
Lp(a) (mg/dL)	18.3 (16.0)	19.1 (16.4)	11.8 (67.4)	.015

All values are expressed as the mean (SD).

The *Pvu*II polymorphism of the LDL-receptor gene was determined in 331 subjects. The allelic frequency was 0.765 for the (−) allele and 0.235 for the (+) allele. The *Ava*II polymorphism of the LDL-receptor gene was determined in 335 subjects. The allelic frequency was 0.513 for the (−) allele and of 0.487 for the (+) allele. The apoE polymorphism was determined in 398 subjects. The allelic frequency was 0.016 for the ε2 allele, 0.855 for the ε3 allele, and 0.125 for the ε4 allele. The 3 polymorphisms were distributed according to Hardy-Weinberg equilibrium (χ^2 = 0.432, P = .511; χ^2 = 0.374, P = .543; and χ^2 = 0.97, P = .808, respectively).

Among the pretreatment lipid levels, only the baseline concentrations of LDL-C (P < .001) and the Lp(a) (P = .011) significantly influenced the LDL-C response to treatment in the univariate analysis. To better assess this effect, the baseline concentrations of LDL-C and Lp(a) were divided into quartiles (Tables 3 and 4). The change in

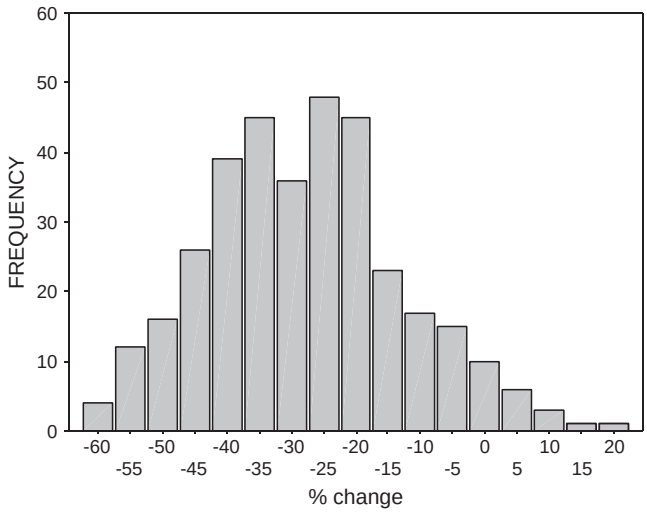

Fig. 1. Percentage change, relative to baseline, in LDL-C after treatment.

Table 3
Percentage and absolute decrease in LDL-C in the overall study population and when subjects were segregated into quartiles of baseline LDL-C concentrations

N = 440	All	Q1	Q2	Q3	Q4	*P*
Mean baseline (mmol/L)	5.19 (1.06)	4.04 (0.36)	4.80 (0.17)	5.40 (0.18)	6.59 (0.95)	–
Absolute decrease (mmol/L)	−1.11 (0.94)	−0.59 (0.69)	−0.93 (0.75)	−1.13 (0.77)	−1.81 (1.06)	.001
Percentage decrease	−20.5 (15.7)	−14.7 (16.8)	−19.4 (15.8)	−21.0 (14.3)	−27.1 (13.5)	.001

Values are expressed as the mean (SD) of each quartile.

LDL-concentration in response to treatment was significant in absolute values ($P < .001$) as well in percentages ($P < .001$) (Table 3). With respect to Lp(a), an elevation in baseline levels was related to a decrease in LDL-C response not only in absolute terms ($P = .022$) but also in percentages ($P = .018$) (Table 4). The mean percentage decrease of LDL-C in the subjects with Lp(a) of greater than 40 mg/dL was 13% and in those with Lp(a) of greater than 50 mg/dL was 9.7%.

To identify factors influencing the LDL-C response, we introduced several individual environmental and genetic factors into a univariate analysis. The results indicated that age, sex, BMI, alcohol consumption, weight change, smoking habit, and the presence of hypertension did not significantly influence the response of the LDL-C to pravastatin treatment.

In the univariate analysis, none of the 3 genotypes studied had a statistically significant effect on LDL-C response to treatment, although there was a significant trend in the *Ava*II polymorphism in that those homozygous for the (−) allele responded less than those heterozygous and these, in turn, responded less than those homozygous for the (+) allele (P of tendency = .032) (Table 5).

In the multivariate analysis, the only 3 factors significantly influencing the LDL-C response were the baseline LDL-C concentration, the baseline Lp(a) concentration, and the *Ava*II polymorphism (Table 6). These 3 variables together accounted for 10.6% of the variance in LDL-C response. In the multivariate analysis, none of the other variables including age, sex, and apoE polymorphism had any statistically significant effect on the LDL-C response to treatment.

6. Discussion

The results of the present study confirm the considerable variability of LDL-C response to a fixed dose of statin, the response varying between −66% and +21%. A daily dose of 20 mg of pravastatin produced a significant decrease in total cholesterol, LDL-C, and triglycerides, together with an increase in the HDL-C. These findings are similar to those described in other studies using the same dose [20-22]. Similarly, the treatment produced a significant increase in the concentration of Lp(a) of slightly less than 1 mg/dL and which is of uncertain clinical relevance. It is not clear what the effects of the statins are in general, or pravastatin in particular, on the concentration of Lp(a). Generally, the concentration of Lp(a) is not modified with statin treatment [23-25] although increments [16,26] as well as decreases [27] have been described, but always in very modest amounts. Also, these increments have been described in studies of short duration [28], whereas in studies of longer duration, the concentrations remain constant [23,24]. The consensus appears to be that there is a transitory increase in Lp(a) concentrations in the first weeks of statin treatment that, subsequently, returns to baseline values.

Several authors have studied the effect of baseline lipid levels on the response to statin treatment [11,14-16,29]. All of them observed a direct association between the baseline concentration of the LDL-C and its decrease with the treatment. In our study, as with the study of Miserez et al [11], we observed that the decrease in LDL-C was greater when the baseline concentration was higher, not only in absolute levels but also in percentages. These findings can be explained on the basis of subjects with the higher concentrations of circulating cholesterol having a higher rate of cholesterol synthesis and a higher activity of HMG-CoA reductase (the rate-limiting enzyme in the intracellular synthesis of cholesterol). Hence, with administration of a statin, the inhibition of the enzyme would be higher and the resultant reduction in LDL-C would be greater.

In contrast to other authors, we did not find that the baseline concentration of triglycerides negatively influenced the response of LDL-C to treatment [11,15,30]. This may be better observed in the presence of hypertriglyceridemia [11] and, probably, would be caused by the LDL particles of these subjects being poorly bound to the LDL receptor because of its lower density and the different distributions of the apoB on the surface of the molecule [31]. In our study population, the mean concentration of the triglycerides was moderate, the selection criterion being subjects with less than 4.5 mmol/L, and only 5% of the subjects having had

Table 4
Percentage and absolute decrease in LDL-C in the overall study population and when subjects were segregated into quartiles of baseline Lp(a) concentrations

n = 434	All	Q1	Q2	Q3	Q4	*P*
Mean baseline (mg/dL)	18.0 (15.8)	1.6 (1.4)	8.8 (2.8)	21.4 (4.1)	39.5 (11.6)	–
Absolute decrease (mg/dL)	−1.12 (0.94)	−1.27 (1.10)	−1.14 (0.89)	−1.13 (0.89)	−0.95 (0.95)	.022
Percentage decrease	−20.5 (15.8)	−22.6 (16.3)	−21.6 (16.0)	−20.9 (14.2)	−17.4 (16.5)	.018

Values are expressed as the means (SD) for each quartile.

levels of greater than 3 mmol/L. Hence, this masking effect is less likely to be observed.

The baseline concentration of Lp(a) was inversely related to the LDL-C response to treatment with pravastatin: the higher the concentration of Lp(a), the lower the decrease in LDL-C. This effect has not been described previously but can be explained quite logically. Lipoprotein (a) is a particle within the LDL and in which the apoB-100 is bound via a single interchain disulfide bridge to a single glycoprotein, apolipoprotein (a) [32]. Its density is somewhat greater than that of LDL such that it overlaps with the fractions corresponding to LDL and HDL_2 when separating lipoproteins by ultracentrifugation. However, when quantifying the amount of cholesterol transported in the LDL molecule using the Friedewald formula, the cholesterol transported by the Lp(a) is included in this subfraction [33] and, given that the concentration of the Lp(a) is hardly modified by the treatment with statins, the higher the Lp(a) concentration, the lower the response of the LDL-C will be. In extreme cases of concentrations of Lp(a) being greater than 50 mg/dL, the decrease in LDL-C was less than half of that reached in the overall study population. Hence, in individuals classified as hyporesponders to statin treatment, one would need to take into account the presence of hyperapoproteinemia (a) as a possible confounding factor.

Among the genetic polymorphisms that could modulate the response to treatment with statins, without doubt the most studied is that of apoE. We [34] as well as others [35-37] did not observe that this polymorphism had any influence on the response of LDL-C, although there are conflicting results in the literature [10,38]. This lack of consistency in the results could be attributable to the several possible effects of the apoE genotype on hypolipidemic response such as that which occurs with its effect on plasma lipids. Factors such as age, sex, BMI, and diet modulate the effect of apoE on baseline lipids and, as such, could also influence the response to statin treatment [39,40].

Given the important role that the LDL receptor plays in the homeostasis of plasma cholesterol, several genetic variations at the LDL-receptor locus have been reported in relation to baseline levels of LDL-C and to response to treatment with statins [41-44]. For example, *Ava*II and *Pvu*II polymorphisms of the LDL-receptor gene have been shown to influence the levels of baseline LDL-C not only in subjects with normocholesterolemia [42] but also in those with hypercholesterolemia [41,45]. As such, these polymorphisms may modulate the response to treatment with statins. Salazar et al [44] investigated 55 subjects with hypercholesterolemia treated with fluvastatin. The results indicated that those who did not carry the restriction site (+/+) of the *Ava*II polymorphism had a lower hypolipemic response than those who did. We, as well, observed that this polymorphism significantly modulates the response to pravastatin, but in the opposite direction to that observed by Salazar et al (ie, the carriers of the (+) allele had a better response in a dose-dependent manner). This disparity in the results can be explained by the treatment schedules used. Salazar et al used 2 different doses of fluvastatin (40 and 80 mg/d) and, when presenting the response with respect to genotypes, they did not discriminate between the 2 doses. Furthermore, the percentage response had not been adjusted for the baseline levels of LDL-C. Their results also indicated that carriers of the allele P2 of the *Pvu*II polymorphism had a better response than those subjects homozygous for the P1 allele [44]. We were not able to confirm this finding. It is difficult to explain the mechanism by which these polymorphisms can have an influence on cholesterol response to statin treatment because the *Pvu*II restriction site is located in an intron and the polymorphism of the *Ava*II does not produce amino acid substitution [42]. As such, any observed effect would need to be mediated by a functional mutation in the gene in linkage disequilibrium with these restriction sites or in a closely linked gene.

Table 5
*Pvu*II, *Ava*II, and apoE genotype distributions and baseline and percentage variations in LDL-C concentrations after treatment with pravastatin and segregation with respect to the different genotypes

Polymorphism	Genotypes	Basal LDL-C	% Fall	*P* ANOVA	*P* Trend
*Pvu*II	−/− (n = 192)	5.14 (0.97)	−19.8 (16.6)	.392	.784
	−/+ (n = 123)	5.06 (0.89)	−20.1 (14.4)		
	+/+ (n = 16)	5.49 (0.32)	−14.2 (16.4)		
*Ava*II	−/− (n = 85)	5.34 (1.02)	−17.3 (14.4)	.091	.032
	−/+ (n = 173)	5.10 (1.06)	−20.7 (16.2)		
	+/+ (n = 77)	5.17 (0.83)	−22.5 (14.9)		
ApoE	2/3 (n = 13)	4.75 (0.85)	−17.1 (15.9)	.090	.135
	3/3 (n = 293)	5.24 (1.03)	−21.5 (15.8)		
	3/4 and 4/4 (n = 92)	5.12 (1.03)	−17.6 (15.5)		

ANOVA indicates analysis of variance.

Table 6
Multivariate regression analysis: variables that significantly influence the LDL-C response to treatment

Variable	*B*	95% CI	*P*
Baseline LDL-C	4.210	2.630 to 5.791	<.001
Baseline Lp(a)	−3.833	−6.884 to −0.781	.014
*Ava*II polymorphism	3.407	−5.713 to −1.101	.004

Results from the multivariate analysis indicated that only the baseline levels of the LDL-C and the Lp(a) together with the *Ava*II polymorphism of the LDL-receptor gene significantly influenced the LDL-C response to pravastatin treatment. However, these 3 factors explain only 10.6% of the overall variance in response. As such, their clinical importance is relatively small.

The value of determining genetic polymorphisms to predict the hypolipidemic effects of treatment with statins remains unclear. Their use in routine clinical practice is dubious because no specific polymorphism that has a clinically significant influence on response has been described to date. Furthermore, direct associations between the effect of the polymorphism on the hypolipemic response and clinical outcomes and/or angiographic data are not

always demonstrable [46]. Nor has there been any cost-effectiveness analysis demonstrating any benefit in using these genotypes as markers of statin response.

In conclusion, we identified 3 factors, levels of baseline LDL-C and Lp(a) and the *Ava*II polymorphism, that significantly influence the response of LDL-C to pravastatin treatment administered to patients attending a standard primary health care clinic. However, the amount of variance in response explained by these factors combined was modest.

Acknowledgments

We thank Luisa Elez for her excellent technical assistance.

Appendix A.

The RAP Study Group (*Respuesta Ambulatoria a Pravastatina*)

The RAP study group consists of 195 primary health care clinicians in 17 provinces in Spain and is coordinated by the following principal investigators:

Enric Subirats (Hospital de Puigcerdá, Gerona);
Antonio Robles (Hospital de Requena, Valencia);
Alberto Miján (Hospital General Yagüe, Burgos);
José María Pascual Izuel (Hospital de Sagunto, Valencia);
Fernando Carrasco Miras (Hospital Comarcal La Inmaculada, Huercal Overa, Almería);
Pedro Valdivielso (Hospital Virgen de la Victoria, Málaga);
Alfredo Suarez (Hospital Virgen Blanca, León);
Clotilde Morales (Hospital de Manresa, Barcelona);
Justo Colchero (Hospital Juan Ramón Jiménez, Huelva);
Joima Panisello (Hospital de Igualada, Barcelona);
Isabel Camps (Hospital Arnau de Vilanova, Lérida);
Antonio Espino (Hospital de Osuna, Sevilla);
Francisca Montaner (Hospital de Martorell, Barcelona);
Manuel Zuñiga (Hospital Marqués de Valdecilla, Santander);
José Puzo (Hospital San Jorge, Huesca);
Pedro Saenz de Aranzubia (Hospital de Mérida, Badajoz);
Albert De Luis (Hospital de Calella, Barcelona);
José Ignacio Ruiz Díaz (Hospital General de Baza, Granada);
Jacinto Fernández Pardo (Hospital General, Murcia);
Javier Cazo (Hospital García Orcoyen, Estella, Navarra); and
Angel Brea (Hospital de San Pedro, Logroño, La Rioja).

References

[1] Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. N Engl J Med 1995; 333:1301-7.

[2] Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: Results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA 1998;279: 1615-22.

[3] Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383-9.

[4] Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 1996;335:1001-9.

[5] Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. N Engl J Med 1998;339: 1349-57.

[6] Walley T, Folino-Gallo P, Schwabe U, van Ganse E. Variations and increase in use of statins across Europe: Data from administrative databases. BMJ 2004;328:385-6.

[7] Davignon J, Laaksonen R. Low-density lipoprotein–independent effects of statins. Curr Opin Lipidol 1999;10:543-59.

[8] Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol 1998;81:582-7.

[9] Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation 1998;97:1440-5.

[10] Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. Atherosclerosis 2001;158:183-93.

[11] Miserez AR, Rossi FA, Keller U. Prediction of the therapeutic response to simvastatin by pretreatment lipid concentrations in 2082 subjects. Eur J Clin Pharmacol 1994;46:107-14.

[12] Leitersdorf E. Gender-related response to fluvastatin in patients with heterozygous familial hypercholesterolaemia. Drugs 1994; 47(Suppl 2):54-8.

[13] Nakajima K. Sex-related differences in response of plasma lipids to simvastatin: The Saitama Postmenopausal Lipid Intervention Study. S-POLIS Group. Clin Ther 1999;21:2047-57.

[14] Shear CL, Franklin FA, Stinnett S, et al. Expanded Clinical Evaluation of Lovastatin (EXCEL) study results. Effect of patient characteristics on lovastatin-induced changes in plasma concentrations of lipids and lipoproteins. Circulation 1992;85:1293-303.

[15] Streja L, Packard CJ, Shepherd J, Cobbe S, Ford I. Factors affecting low-density lipoprotein and high-density lipoprotein cholesterol response to pravastatin in the West Of Scotland Coronary Prevention Study (WOSCOPS). Am J Cardiol 2002;90:731-6.

[16] Leitersdorf E, Eisenberg S, Eliav O, et al. Genetic determinants of responsiveness to the HMG-CoA reductase inhibitor fluvastatin in patients with molecularly defined heterozygous familial hypercholesterolemia. Circulation 1993;87:35-44.

[17] Dornbrook-Lavender KA, Pieper JA. Genetic polymorphisms in emerging cardiovascular risk factors and response to statin therapy. Cardiovasc Drugs Ther 2003;17:75-82.

[18] Lahoz C, Pena R, Mostaza JM, et al. Apo A-I promoter polymorphism influences basal HDL-cholesterol and its response to pravastatin therapy. Atherosclerosis 2003;168: 289-95.

[19] Summary of the second report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel II). JAMA 1993;269:3015-23.

[20] Hunninghake DB, Knopp RH, Schonfeld G, et al. Efficacy and safety of pravastatin in patients with primary hypercholesterolemia: I. A dose-response study. Atherosclerosis 1990;85:81-9.

[21] McPherson R, Bedard J, Connelly P, et al. Comparison of the short-term efficacy and tolerability of lovastatin and pravastatin in the management of primary hypercholesterolemia. Clin Ther 1992;14: 276-91.

[22] Lambrecht LJ, Malini PL. Efficacy and tolerability of simvastatin 20 mg vs pravastatin 20 mg in patients with primary hypercholesterolemia. European Study Group. Acta Cardiol 1993;48:541-54.

[23] Fieseler HG, Armstrong VW, Wieland E, et al. Serum Lp(a) concentrations are unaffected by treatment with the HMG-CoA reductase inhibitor pravastatin: Results of a 2-year investigation. Clin Chim Acta 1991;204:291-300.

[24] Hunninghake DB, Stein EA, Mellies MJ. Effects of one year of treatment with pravastatin, an HMG-CoA reductase inhibitor, on lipoprotein a. J Clin Pharmacol 1993;33:574-780.

[25] Comparison of the efficacy, safety and tolerability of simvastatin and pravastatin for hypercholesterolemia. The Simvastatin Pravastatin Study Group. Am J Cardiol 1993;71:1408-14.

[26] McKenney JM, McCormick LS, Weiss S, Koren M, Kafonek S, Black DM. A randomized trial of the effects of atorvastatin and niacin in patients with combined hyperlipidemia or isolated hypertriglyceridemia. Collaborative Atorvastatin Study Group. Am J Med 1998; 104:137-43.

[27] Gonbert S, Malinsky S, Sposito AC, et al. Atorvastatin lowers lipoprotein(a) but not apolipoprotein(a) fragment levels in hypercholesterolemic subjects at high cardiovascular risk. Atherosclerosis 2002;164:305-11.

[28] Klausen IC, Gerdes LU, Meinertz H, Hansen FA, Faergeman O. Apolipoprotein(a) polymorphism predicts the increase of Lp(a) by pravastatin in patients with familial hypercholesterolaemia treated with bile acid sequestration. Eur J Clin Invest 1993;23:240-5.

[29] Narita Y, Kitazoe Y, Kurihara Y, et al. Increase or decrease of HDL-cholesterol concentrations during pravastatin treatment depending on the pre-treatment HDL cholesterol levels. Eur J Clin Pharmacol 1997;52:461-3.

[30] Drmanac S, Heilbron DC, Pullinger CR, et al. Elevated baseline triglyceride levels modulate effects of HMGCoA reductase inhibitors on plasma lipoproteins. J Cardiovasc Pharmacol Ther 2001;6: 47-56.

[31] Packard CJ, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler Thromb Vasc Biol 1997;17:3542-56.

[32] Scanu AM, Lawn RM, Berg K. Lipoprotein(a) and atherosclerosis. Ann Intern Med 1991;115:209-18.

[33] Scanu AM. Lipoprotein(a), Friedewald formula, and NCEP guidelines. National Cholesterol Education Program. Am J Cardiol 2001;87:608-609, A9.

[34] Pena R, Lahoz C, Mostaza JM, et al. Effect of apoE genotype on the hypolipidaemic response to pravastatin in an outpatient setting. J Intern Med 2002;251:518-25.

[35] Ojala JP, Helve E, Ehnholm C, Aalto-Setala K, Kontula KK, Tikkanen MJ. Effect of apolipoprotein E polymorphism and *Xba*I polymorphism of apolipoprotein B on response to lovastatin treatment in familial and non-familial hypercholesterolaemia. J Intern Med 1991;230:397-405.

[36] Sanllehy C, Casals E, Rodriguez-Villar C, et al. Lack of interaction of apolipoprotein E phenotype with the lipoprotein response to lovastatin or gemfibrozil in patients with primary hypercholesterolemia. Metabolism 1998;47:560-5.

[37] Gerdes LU, Gerdes C, Kervinen K, et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction: A substudy of the Scandinavian simvastatin survival study. Circulation 2000;101:1366-71.

[38] Ordovas JM, Lopez-Miranda J, Perez-Jimenez F, et al. Effect of apolipoprotein E and A-IV phenotypes on the low density lipoprotein response to HMG CoA reductase inhibitor therapy. Atherosclerosis 1995;113:157-66.

[39] de Knijff P, Havekes LM. Apolipoprotein E as a risk factor for coronary heart disease: A genetic and molecular biology approach. Curr Opin Lipidol 1996;7:59-63.

[40] Corella D, Guillen M, Saiz C, et al. Environmental factors modulate the effect of the APOE genetic polymorphism on plasma lipid concentrations: Ecogenetic studies in a Mediterranean Spanish population. Metabolism 2001;50:936-44.

[41] Salazar LA, Hirata MH, Giannini SD, et al. Effects of *Ava*II and *Hinc*II polymorphisms at the LDL receptor gene on serum lipid levels of Brazilian individuals with high risk for coronary heart disease. J Clin Lab Anal 1999;13:251-8.

[42] Ahn YI, Kamboh MI, Aston CE, Ferrell RE, Hamman RF. Role of common genetic polymorphisms in the LDL receptor gene in affecting plasma cholesterol levels in the general population. Arterioscler Thromb 1994;14:663-70.

[43] Salazar LA, Hirata MH, Forti N, et al. *Pvu*II intron 15 polymorphism at the LDL receptor gene is associated with differences in serum lipid concentrations in subjects with low and high risk for coronary artery disease from Brazil. Clin Chim Acta 2000;293:75-88.

[44] Salazar LA, Hirata MH, Quintao EC, Hirata RD. 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. J Clin Lab Anal 2000;14:125-31.

[45] Salazar LA, Hirata MH, Giannini SD, et al. Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. Clin Chim Acta 2000;300:139-49.

[46] Maitl-van der Zee AH, Klungel OH, Stricker BH, et al. Genetic polymorphisms: Importance for response to HMG-CoA reductase inhibitors. Atherosclerosis 2002;163:213-22.