

# Pharmacogenetic Study of Statin Therapy and Cholesterol Reduction

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**T**HERAPY WITH 3-HYDROXY-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) lowers total and low-density lipoprotein (LDL) cholesterol and has proven to be highly effective for cardiovascular risk reduction. However, there is wide variation in interindividual response to statin therapy, and it has been hypothesized that genetic differences may contribute to this variation. While the implications of this hypothesis are broad in terms of “personalized medicine” and the use of genetic screening to guide selection of lipid-lowering therapy, clinical data addressing pharmacogenetic interactions with statins are limited and have largely focused on the lipid metabolism genes apolipoprotein E (APOE), apolipoprotein B (APOB), cholesteryl ester transfer protein (CETP), and the LDL receptor (LDLR).<sup>1-4</sup>

To explore this issue systematically, we genotyped 148 single-nucleotide polymorphisms (SNPs) across 10 candidate genes known to affect cholesterol synthesis, absorption, and transport and statin metabolism and sought to correlate variation within these genes with the change in total, LDL, and high-density lipoprotein (HDL) cholesterol observed among 1536 individuals

**Context** Polymorphisms in genes involved in cholesterol synthesis, absorption, and transport may affect statin efficacy.

**Objective** To evaluate systematically whether genetic variation influences response to pravastatin therapy.

**Design, Setting, and Population** The DNA of 1536 individuals treated with pravastatin, 40 mg/d, was analyzed for 148 single-nucleotide polymorphisms (SNPs) within 10 candidate genes related to lipid metabolism. Variation within these genes was then examined for associations with changes in lipid levels observed with pravastatin therapy during a 24-week period.

**Main Outcome Measure** Changes in lipid levels in response to pravastatin therapy.

**Results** Two common and tightly linked SNPs (linkage disequilibrium  $r^2=0.90$ ; heterozygote prevalence=6.7% for both) were significantly associated with reduced efficacy of pravastatin therapy. Both of these SNPs were in the gene coding for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the target enzyme that is inhibited by pravastatin. For example, compared with individuals homozygous for the major allele of one of the SNPs, individuals with a single copy of the minor allele had a 22% smaller reduction in total cholesterol (−32.8 vs −42.0 mg/dL [−0.85 vs −1.09 mmol/L];  $P=.001$ ; absolute difference, 9.2 mg/dL [95% confidence interval (CI), 3.8-14.6 mg/dL]) and a 19% smaller reduction in low-density lipoprotein (LDL) cholesterol (−27.7 vs −34.1 mg/dL [−0.72 vs −0.88 mmol/L];  $P=.005$ ; absolute difference, 6.4 mg/dL [95% CI, 2.2-10.6 mg/dL]). The association for total cholesterol reduction persisted even after adjusting for multiple tests on all 33 SNPs evaluated in the HMG-CoA reductase gene as well as for all 148 SNPs evaluated was similar in magnitude and direction among men and women and was present in the ethnically diverse total cohort as well as in the majority subgroup of white participants. No association for either SNP was observed for the change in high-density lipoprotein (HDL) cholesterol ( $P>.80$ ) and neither was associated with baseline lipid levels among those actively treated or among those who did not receive the drug. Among the remaining genes, less robust associations were found for squalene synthase and change in total cholesterol, apolipoprotein E and change in LDL cholesterol, and cholesteryl ester transfer protein and change in HDL cholesterol, although none of these met our conservative criteria for purely pharmacogenetic effects.

**Conclusion** Individuals heterozygous for a genetic variant in the HMG-CoA reductase gene may experience significantly smaller reductions in cholesterol when treated with pravastatin.

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treated with pravastatin during a 24-week period. We evaluated polymorphisms in the genes encoding HMG-CoA reductase (the target for statin

therapy) as well as in the genes encoding squalene synthase (another key enzyme in cholesterol biosynthesis and potential target for cholesterol-lowering

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See also p 2869.

**Table 1.** Candidate Genes for Genetic Analysis of Lipid Reduction

Gene Symbol	No. of SNPs	Encoded Protein	OMIM No.	Functional Role
<i>ABCG5</i>	2	ATP-binding cassette, subfamily G, member 5	605459	Cholesterol transport across the plasma membrane
<i>ABCG8</i>	15	ATP-binding cassette, subfamily G, member 8	605460	Cholesterol transport across the plasma membrane
<i>APOB</i>	22	Apolipoprotein B	107730	Major binding protein for LDL cholesterol
<i>APOE</i>	19	Apolipoprotein E	107741	Major binding protein for VLDL/IDL cholesterol
<i>CETP</i>	17	Cholesteryl ester transfer protein	118470	Transfer of cholesteryl esters among lipoprotein particles
<i>CYP3A4</i>	1	Cytochrome P450, subfamily IIIA, polypeptide 4	124010	Statin metabolism
<i>CYP3A5</i>	6	Cytochrome P450, subfamily IIIA, polypeptide 5	606325	Statin metabolism
<i>FDFT1</i>	11	Farnesylidiphosphate farnesyltransferase 1, squalene synthase	184420	Cholesterol synthesis
<i>HMGCR</i>	33	3-Hydroxy-3-methylglutaryl coenzyme A reductase	142910	Cholesterol synthesis
<i>LDLR</i>	22	LDL receptor	606945	Receptor for plasma LDL

Abbreviations: ATP, adenosine triphosphate; IDL, intermediate density lipoprotein; LDL, low-density lipoprotein; OMIM, Online Mendelian Inheritance in Man<sup>13</sup>; SNP, single-nucleotide polymorphism; VLDL, very low-density lipoprotein.

therapy), 2 cholesterol transport adenosine triphosphate-binding cassette proteins, *APOE*, *APOB*, *CETP*, *LDLR*, and the cytochrome P450 system.<sup>5-9</sup> Rare loss-of-function mutations in each of these genes except squalene synthase and the cytochromes have been shown to result in profound effects on lipid levels.<sup>10</sup> On the basis of prior evidence for their central role in controlling lipid levels, these genes were chosen as candidates for examining the hypothesis that their common genetic variants influence the degree of lipid level reduction during pravastatin therapy.

## METHODS

### Study Population

The study population was derived from participants in the Pravastatin Inflammation/CRP Evaluation (PRINCE), a community-based randomized trial and cohort study evaluating the effects of pravastatin, 40 mg/d, or matching placebo on lipid and inflammatory biomarkers during a 24-week period.<sup>11</sup> PRINCE participants were enrolled from 1143 sites representing 49 states and the District of Columbia, with no single site enrolling more than 4 patients. All PRINCE participants were free of statin use in the 6 months prior to enrollment and had no contraindication to statin therapy. For the current pharmacogenetic analysis, we limited our evaluation to PRINCE participants who (1) provided written informed consent for genomic analysis; (2) were randomly allocated to receive pravastatin or placebo and suc-

cessfully completed the full PRINCE study protocol by providing baseline, 12-week, and 24-week blood samples; and (3) underwent successful DNA extraction and genotyping as outlined herein. In total, 1536 PRINCE participants assigned to receive pravastatin fulfilled these criteria and form the basis for these analyses. Of the 1536 study participants, 1362 (88.7%) were self-identified as white, with 100 (6.5%) self-identified as black, 44 (2.9%) as Hispanic, 19 (1.2%) as Asian, and 11 (0.7%) as other. This pharmacogenetic study was approved by Brigham and Women's Hospital's institutional review board.

### SNP Selection and Genotyping

We selected candidate genes based on prior observations that null mutations in all of them, except *FDFT1* and the *CYP3A* genes, grossly alter lipid levels and cause heritable disease. Squalene synthase encoded by the *FDFT1* gene is a target for cholesterol reduction therapy; *CYP3A* genes were included because of their role in statin metabolism, even though they contribute only modestly in the case of pravastatin.<sup>12</sup> Within the 10 selected candidate genes (TABLE 1), we identified the common variation due to SNPs by resequencing in panels of 32 to 96 cell lines from ethnically diverse individuals (roughly one third European, one fourth Asian, one fourth black, and one sixth Hispanic or Native American) and by reviewing the literature. By further exploring linkage disequilibrium, inferred po-

tential effects of SNPs on biological function, and inferred haplotypes, we were able to winnow our list of candidate SNPs to a final set of 148 that we believed would capture the important genetic diversity in the 10 selected genes in the study population. Single nucleotide polymorphism 12 of the HMG-CoA reductase gene can be found on chromosome 5 at position 74726928 (Human genome July 2003 UCSC version hg16, based on build 34, National Center for Biotechnology Information) by a match to the sequence AAAAAAAAA-TTTTT[AT]AAATCCTTTATATTA, in which the brackets surround the variable nucleotide. Single nucleotide polymorphism 29 can be found on chromosome 5 at position 74739571 by a match to the sequence TTTTCCAAAC-TCTTT[TG]GTCATATCAGCCTAA. A full listing of the 148 SNPs genotyped in the study is available from the authors.

Genotyping was performed using mass spectrometry-based methods as described elsewhere.<sup>14</sup> Genotypes for HMG CoA reductase SNPs 12 and 29 were successfully determined in 1504 (97.9%) and 1518 (98.8%), respectively, of the 1536 participants in the study sample. A set of reference markers included with the study samples provided no evidence for inaccurate genotype determinations.

### Statistical Analysis and Correction of P Values for Multiple Alleles

To identify potential associations between genotypes in a candidate gene

and response to pravastatin, we first calculated changes in total, LDL, and HDL cholesterol from the values obtained at baseline to the mean values of the 12- and 24-week measurements. These changes followed a normal distribution, allowing us to perform 1-way analysis of variance with F statistics evaluating the difference in the change in lipid level for, at most, 3 genotypes for each SNP. Statistical tests were performed using data only from individuals having mean changes in lipid levels within 3 SDs of the study mean, a constraint that excluded no more than 25 individuals for any of the lipid values measured. Only genotype classes populated by at least 10 individuals were considered.

We determined *P* values by assessing the null distribution of the F statistic for no association of genotype with the difference in lipid levels with 10000 random permutations of genotypes and lipid values.<sup>15</sup> *P* values determined from the analytic F distribution were essentially the same. Because HMG-CoA reductase is the target of statin therapy, squalene synthase is an alternative target for lipid-lowering therapy, and mutations in the other candidate genes are known to have profound effects on lipid levels, we considered each gene to represent a strong, independent prior hypothesis. Accordingly, in addition to the uncorrected *P* values estimated from the permutations, we corrected each *P* value for the multiple SNPs tested by treating SNPs within a shared gene as a set of simultaneously assessed hypotheses. Computationally, we examined the null distribution of the F statistic by rank from permutations involving the set of SNPs from one gene at a time, and applied a step-down procedure to determine corrected *P* values.<sup>15</sup> This permutation approach to correcting *P* values reflects the correlated structure of the multiple hypotheses implicit in the linkage disequilibrium between SNPs belonging to a single locus.

Beyond requiring corrected  $P < .05$  for significance, we sought to limit the potential for false-positive findings by imposing 2 additional qualitative criteria

on any potentially meaningful associations. First, to reduce possible confounding by race, we insisted that associations found in the whole racially mixed cohort had to exist as well in the self-identified white subpopulation, representing 88.7% of the study participants. Second, while we acknowledged the risk of being too conservative, we were interested in identifying the best candidates for genetic influences solely on the change in lipid levels with statin therapy and focused only on SNPs that were not associated with baseline lipid levels.

In addition to the single-SNP analysis and to uncover epistatic effects due to combinations of SNPs within the same gene, we tested for associations between haplotypes and response to pravastatin using an evolutionary approach.<sup>16</sup> Haplotypes were inferred with the program PHASE,<sup>17</sup> but only those predicted with high confidence were used in the analysis. Graphically represented as cladograms, evolutionary trees were derived from the haplotypes through standard assumptions about allele frequency, allele age, and recombination<sup>18</sup> and through the principle of parsimony, which demands minimization of the number of nucleotide changes between adjacent (ie, closely related) haplotypes. Once the trees were constructed, potential associations of the haplotypes and the changes in lipid values were tested using our algorithm that evaluates all of the binary partitions of the tree between pairs of adjacent haplotypes. All genes were examined with the cladistic analysis except *CYP3A4*, which was genotyped at only 1 SNP. Treescan software was used for statistical analyses (version 0.8, University of Vigo, Vigo, Spain).

## RESULTS

The mean (SD) age of the pravastatin-treated patients was 64.0 (12.5) years. A total of 534 (34.8%) were women, 211 (13.7%) were current smokers, and 301 (20%) had diabetes. The mean (SD) baseline levels of total cholesterol, LDL cholesterol, and HDL cholesterol were

218 (39) mg/dL (5.65 [1.01] mmol/L), 132 (3) mg/dL (3.42 [0.08] mmol/L), and 38 (11) mg/dL (0.98 [0.28] mmol/L), respectively. These values are consistent with the PRINCE study population as a whole.

### Polymorphism in the HMG-CoA Reductase Gene

Of the 148 SNPs evaluated across the 10 candidate genes, we found 2 tightly linked SNPs that were significantly associated with a difference in the change in lipid response to pravastatin and that fulfilled our additional qualitative criteria for association. Both of these SNPs were in the HMG-CoA reductase gene (SNP 12 and SNP 29) encoding the target for statin therapy, and the extent of their linkage disequilibrium ( $r^2=0.90$ ;  $P<.001$ ) ensured that the results for the 2 SNPs were essentially equivalent.

In the study population, genotypes for the 2 SNPs were in Hardy-Weinberg equilibrium and were discordant for only 7 individuals. As shown in TABLE 2 for the case of SNP 12, which is represented as a heterozygous genotype in 6.7% of the study individuals, there was a marginally significant difference in age by genotype but no significant differences with regard to sex, traditional risk factors, or pretreatment lipid levels. Only one participant was homozygous for the minor allele of either SNP, which was not a sufficient number for inclusion in the statistical analysis or in the tables.

For individuals with a single copy of SNP 12 in the HMG-CoA reductase gene, the mean change in total cholesterol associated with pravastatin use was  $-32.8$  mg/dL ( $-0.85$  mmol/L) while the mean change for those homozygous for the major allele was  $-42.0$  mg/dL ( $-1.09$  mmol/L), a reduction in overall efficacy of 21.8% (absolute difference, 9.2 mg/dL [95% confidence interval {CI}, 3.8-14.6 mg/dL];  $P=.001$ ). For SNP 29 in the HMG-CoA reductase gene, an almost identical 22.3% smaller effect on total cholesterol reduction was observed (absolute difference, 9.3 mg/dL [95% CI, 3.8-14.7 mg/dL];  $P<.001$ ). Both of these findings remained sig-

nificant after correction for all 33 SNPs evaluated in the HMG-CoA reductase gene (both corrected *P* values < .02) (TABLE 3). These effects were largely due to differences in LDL cholesterol such that individuals heterozygous for the minor allele experienced an approximate 19% smaller LDL reduction after taking pravastatin (both *P* values < .005; absolute difference for SNP 12, 6.4 mg/dL [95% CI, 2.2-10.6 mg/dL] and absolute difference for SNP 29, 6.4 mg/dL [95% CI, 2.2-10.7 mg/dL]). In contrast, there was no significant difference in the change in HDL cholesterol with pravastatin between genotypes.

We addressed the robustness of these findings in several additional analyses. First, in the total cohort, the differences in total cholesterol reduction by genotype persisted even after correction for all 148 SNPs evaluated across all 10 genes (fully corrected *P* = .04 for SNP 12 and *P* = .049 for SNP 29). To address the potential for effect modification by sex, we stratified our analysis for all measured SNPs by sex and, again, found an association for only SNP 12

and SNP 29 in the HMG-CoA reductase gene. Although the trend of lipid change with genotype was the same for both sexes, the association was generally more significant in men.

Second, both SNP 12 and SNP 29 remained significantly correlated with the reduction of total and LDL cholesterol among whites, representing about 88.7% of the total cohort (Table 3). The association with the change in LDL cholesterol became stronger after ethnic stratification; the *P* value for SNP 29 among whites was significant after correction for all 33 SNPs in the HMG-CoA reductase gene.

Third, neither SNP 12 nor SNP 29 was found to contribute to the variance in baseline levels of total cholesterol or LDL cholesterol, a critical observation to eliminate the potential for confounding on this basis in detecting purely pharmacogenetic effects. Not surprisingly, therefore, the 2 SNPs remain statistically associated with residuals in the change in either total cholesterol or LDL cholesterol after their regression against baseline lipid level, sex, hormone therapy status, age, and

age squared. Moreover, both of the SNPs in the HMG-CoA reductase gene remained significantly associated with the residual change in LDL cholesterol in the subgroup of white participants after correction for the 33 SNPs in the HMG-CoA reductase gene (*P* = .008 and *P* = .02, respectively).

Finally, we repeated all of these analyses among an additional 649 participants in the PRINCE trial who did not receive pravastatin therapy. In this group, we found no evidence of association between the 2 SNPs in the HMG-CoA reductase gene and lipid levels at baseline or the change in lipid levels after placebo treatment. These findings markedly reduce the possibility that our primary observations among those treated represent either regression to the mean or a nonpharmacological effect of study participation.

Evolutionary analysis of haplotypes inferred from the genotype data revealed an association entirely equivalent to the genotype-based association with SNPs 12 and 29. As shown in the FIGURE, haplotype 7 at the tip of one branch in the HMG-CoA reductase cladogram is defined uniquely by the minor alleles of SNPs 12 and 29 and was the sole haplotype significantly associated with lipid response to pravastatin. Indeed, the fact that both SNPs 12 and 29 occur uniquely in the same branch of the cladogram provides a graphical explanation of their high linkage disequilibrium.

**Findings for Other Genes**

In addition to the closely related SNPs in the HMG-CoA reductase gene already described, 3 other SNPs in the remaining 9 genes were associated with a differential effect of pravastatin on lipid reduction but failed to meet at least 1 of our qualitative criteria for association. For example, SNP 4 in the squalene synthase gene (*FDFT1*) was associated with the change in total cholesterol in men and persisted in analyses limited to white men. However, we also found this SNP to be significantly associated with baseline levels of total cholesterol; thus, after regression against baseline levels, the

**Table 2.** Baseline Characteristics of Treated Patients\*

Characteristics	All Treated	HMG-CoA Reductase SNP 12 Genotype†		<i>P</i> Value‡
		AA	AT	
Age, mean (SD), y	64 (12.5)	64 (12.5)	61 (12.9)	.03
Body mass index, mean (SD)§	28.9 (5.4)	28.9 (5.4)	29.2 (5.5)	.59
Smoking				.47
Never	583 (38.0)	530 (37.8)	40 (39.6)	
Past	742 (48.3)	682 (48.6)	48 (47.5)	
Current	211 (13.7)	191 (13.6)	13 (12.9)	
White race/ethnicity	1362 (88.7)	1248 (89.0)	83 (82.2)	.06
Women	534 (34.8)	494 (35.2)	29 (28.7)	.22
Hormone therapy use	238 (15.5)	217 (15.5)	15 (14.9)	.98
Diabetes mellitus	301 (19.6)	272 (19.4)	25 (24.8)	.24
Cholesterol, mean (SD), mg/dL				
Total	218.1 (38.7)	218.1 (38.7)	216.6 (40.1)	.73
LDL	132.2 (29.6)	132.2 (29.6)	130.1 (29.5)	.48
HDL	37.8 (10.8)	37.7 (10.5)	38.5 (13.7)	.57

Abbreviations: HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; SNP, single-nucleotide polymorphism.

SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259.

\*Patients with lipid levels measured at baseline and after both 12 and 24 weeks of treatment. Data are presented as No. (%) unless otherwise indicated.

†As described in the "Methods" section, genotyping for HMG-CoA reductase SNP 12 was successful in 97.9% of the study sample.

‡*P* values assessed statistical differences in each characteristic for the 2 genotypes of HMG-CoA reductase SNP 12 observed in the treated cohort of Pravastatin Inflammation/CRP Evaluation. They were computed by a 2-sided *t* test or by a  $\chi^2$  test for the continuously valued or categorically valued characteristics, respectively.

§Body mass index was calculated as weight in kilograms divided by the square of height in meters.

association with residuals was no longer significant.

Similarly, while SNP 17 in the *APOE* gene was associated with a greater reduction in LDL cholesterol among individuals heterozygous or homozygous for the minor allele ( $P=.001$ ; corrected  $P=.047$ ), it too was associated with baseline LDL levels (corrected  $P<.001$ ). Moreover, its effect was attenuated and no longer fully significant in our analysis limited to white participants ( $P=.02$ ; corrected  $P=.22$ ). The minor allele of SNP 15 in the cholesteryl ester transfer protein (*CETP*) was associated with a smaller increase in HDL cholesterol among men ( $P=.007$ ; corrected  $P=.02$ ) but, again, it was also associated with baseline HDL cholesterol level (corrected  $P=.003$ ) and the effect was not present among women ( $P=.59$ ; corrected  $P=.99$ ), among white men ( $P=.04$ ; corrected  $P=.17$ ), or in the sex-combined data ( $P=.046$ ; corrected  $P=.26$ ). Finally, in the cladistic evolutionary analysis, none of the haplotypes in genes other than HMG-CoA reductase genes was both significantly associated with the change in lipid levels in response to pravastatin and not associated with baseline lipid levels.

**COMMENT**

In this analysis of 148 SNPs across 10 genes known to be involved in cholesterol synthesis and statin metabolism, we found 2 common and closely linked polymorphisms in the HMG-CoA reductase gene that were significantly associated with a 22% smaller reduction in total cholesterol and a 19% smaller reduction in LDL cholesterol following 24 weeks of pravastatin therapy. For total cholesterol, these effects remained significant after adjustment for all SNPs evaluated and were consistent in magnitude and direction among men and women and among whites as well as the total cohort.

It is of particular interest that the 2 SNPs with the most robust association with lipid level reduction after pravastatin therapy both lie within the HMG-CoA reductase gene, encoding the target for statin therapy. Whether the

genetic effect can be explained by altered expression, activity, or drug binding is uncertain, but we considered several possible molecular interpretations. The HMG-CoA reductase gene spans about 24 200 base pairs on chromosome 5q13.3 (July 2003 human genome assembly, National Center for Biotechnology Information build 34) with an inferred mature transcript of at least 4471 bases spliced from about 19 exons (RefSeq: NM\_000859).<sup>19</sup> Single nucleotide polymorphisms 12 and 29 are separated by about 12 650 base pairs and reside in introns 5 and 15, respectively, both distant enough from recognized splicing borders that they are unlikely to interfere with the expected function of known splicing signals.

In contrast with exons and parts of some introns in the HMG-CoA reduc-

tase gene, sequences surrounding the 2 SNPs are not conserved with sequences from the mouse genome, suggesting that they may not be directly involved in a conserved biological process across species. Moreover, neither SNP is part of a CpG dinucleotide sequence, a potential target for methylation and effects on transcription. It is thus possible that the SNPs we have identified are linked to other genetic changes within functional parts of the HMG-CoA reductase gene. For example, in our multiethnic SNP discovery panel, both SNPs are tightly linked to a third SNP in a 3' untranslated exon of the HMG-CoA reductase gene, which is retained in the mature RNA message. This SNP was not genotyped in the current study, but it may influence the stability of HMG-CoA reduc-

**Table 3.** Association of HMG-CoA Reductase Genotype With Lipid Changes\*

Group	Mean Change (% Change) in Lipid Level by Genotype, mg/dL†		Mean Change (% Change)‡	P Value§	Corrected P Value
	AA or TT	AT or TG			
<b>Total Cholesterol</b>					
SNP 12					
Entire study	-42.0 (-18.8)	-32.8 (-14.4)	-9.2 (-21.8)	.001	.01
Whites	-42.2 (-18.9)	-33.1 (-14.3)	-9.1 (-21.6)	.003	.04
SNP 29					
Entire study	-41.8 (-18.7)	-32.5 (-14.6)	-9.3 (-22.3)	<.001	.01
Whites	-42.0 (-18.9)	-32.6 (-14.4)	-9.4 (-22.5)	.002	.03
<b>LDL Cholesterol</b>					
SNP 12					
Entire study	-34.1 (-25.2)	-27.7 (-20.1)	-6.4 (-18.7)	.005	.07
Whites	-34.3 (-25.4)	-27.4 (-19.5)	-6.9 (-20.0)	.003	.05
SNP 29					
Entire study	-34.0 (-25.1)	-27.6 (-20.3)	-6.4 (-18.9)	.003	.06
Whites	-34.3 (-25.3)	-27.2 (-19.6)	-7.0 (-20.4)	.002	.03
<b>HDL Cholesterol</b>					
SNP 12					
Entire study	2.2 (6.8)	2.3 (6.9)	-0.1 (4.6)	.83	>.99
Whites	2.1 (6.7)	2.7 (8.2)	-0.6 (28.1)	.25	.97
SNP 29					
Entire study	2.2 (6.7)	2.1 (6.2)	0.1 (-2.4)	.91	>.99
Whites	2.1 (6.6)	2.6 (7.6)	-0.5 (22.4)	.37	>.99

Abbreviations: HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; SNP, single-nucleotide polymorphism.  
 SI conversions: To convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259.  
 \*To exclude possible confounding due to population stratification by ethnicity, the statistical analysis was also applied to the subgroup of participants who were self-identified as white.  
 †Mean change in total, LDL, and HDL cholesterol levels, respectively, for the average of measurements after 12 and 24 weeks of pravastatin treatment. Genotypes AA and TT are homozygous for the major allele of the HMG-CoA reductase gene SNPs 12 and 29, respectively. Genotypes AT and TG are heterozygous genotypes for SNPs 12 and 29, respectively.  
 ‡Difference in mean changes in lipids for individuals in the 2 genetic classes and percentage mean changes from the homozygous genetic class.  
 §P values from permutation test for the analysis of variance F statistic.  
 ||P values corrected for all 33 SNPs in the HMG-CoA reductase gene.

tase RNA in the cell. We can likely exclude any involvement for SNP 25 encoding the amino acid substitution I638V in HMG-CoA reductase because this SNP was neither associated with the change in lipid nor linked to SNPs 12 or 29. Determining if 1 or more of the HMG-CoA reductase candidate SNPs provides a molecular explanation of the data observed will thus require further study.

Other than the 2 SNPs in the HMG-CoA reductase gene, we observed no other SNPs associated with lipid changes following pravastatin therapy using our conservative analysis plan. The alternative associations that met

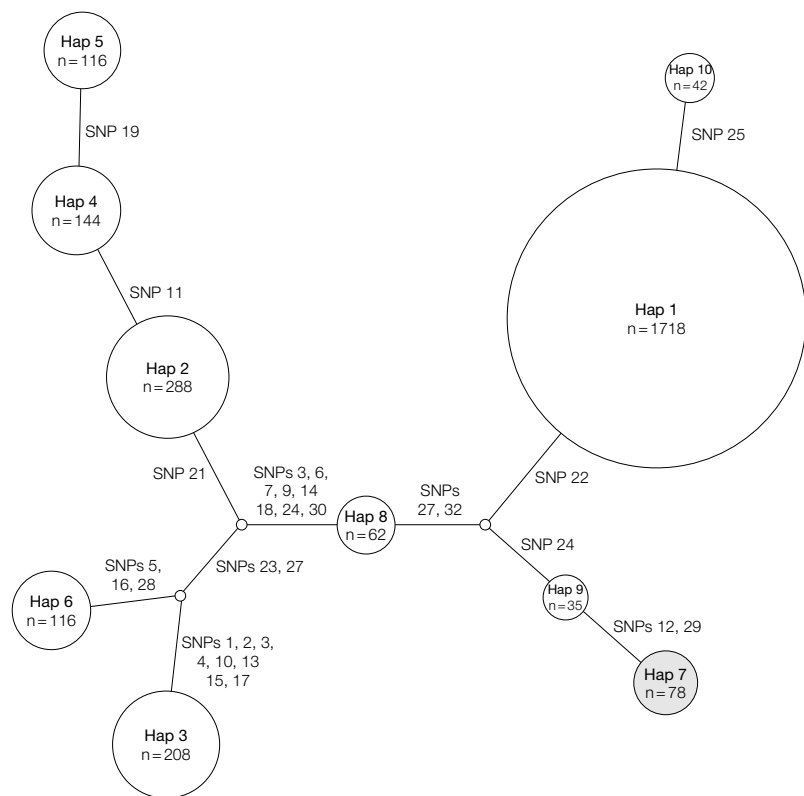
some but not all of our qualitative criteria and any others that may exist in our data may be described adequately only by less conservative statistical constraints or more complex genetic analysis. For example, we recognize that while our study had sufficient power to detect large effects due to relatively common alleles, the power for smaller effects due to rare alleles was limited. Estimated from the observed variance in the change in total cholesterol among the 1536 participants, the statistical power of our study was sufficient to detect a change in total cholesterol as small as 9 mg/dL (0.23 mmol/L) for alleles of 3% or greater frequency. For al-

leles with a frequency as low as 1%, we had adequate power to detect a change in total cholesterol of 15 mg/dL (0.39 mmol/L) or greater. Thus, we think it is unlikely that we have missed other major pharmacogenetic effects.

Our study has limitations that require consideration. First, although the critical variants observed were in the HMG-CoA reductase gene itself, we evaluated only pravastatin; thus, care must be taken before generalizing these data to other statins. Second, the design of our study did not allow evaluation of dose-response effects. However, the dose of pravastatin used is the highest dose routinely given for this agent and is the only dose that has been tested in clinical end-point trials. Third, despite our study's large sample size and our conservative statistical analysis, these data require independent confirmation, as would be true for any genetic study. Nonetheless, it seems unlikely that our study population, which was derived from 49 states and the District of Columbia, is subject to any major selection bias. Finally, despite the statistical robustness of our data, the proportion of the variance that can be explained by HMG-CoA reductase SNPs 12 and 29 is small in comparison with the expected influence of clinical determinants such as compliance and diet.

We recognize that these data have considerable pathophysiological interest and provide strong clinical evidence that there may be promise in the concept of "personalized medicine" and the use of genetic screening to target certain therapies. The absolute difference in total cholesterol reduction associated with the HMG-CoA reductase genotype in our data was 9 mg/dL (0.23 mmol/L), an effect large enough to affect health on a population basis. Future studies must determine whether this difference can be offset by dose adjustment or the choice of an alternative nonstatin lipid-lowering therapy. In the meantime, clinical reminders to take treatment daily and to titrate dose as necessary to achieve National Cholesterol Education Program goals remain critical issues for practice.<sup>20</sup>

**Figure.** Evolutionary Analysis of the Association of Mean Total Cholesterol Change With Haplotypes of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase



Each node in the tree corresponds to a different haplotype (Hap) as labeled, with node area proportional to the number of observations of the corresponding haplotype among participants meeting the study criteria. Lines connect haplotypes inferred to have arisen close to each other during the evolutionary history of the 3-hydroxy-3-methylglutaryl coenzyme A reductase locus. Small, unlabeled nodes correspond to haplotypes inferred to have existed during evolution but not found in the study population. Only haplotype 7 (gray), which is uniquely defined by single-nucleotide polymorphisms (SNPs) 12 and 29, was associated with an altered response to pravastatin therapy.

**Author Contributions:** Drs Chasman and Ridker had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Stanton, Ridker

**Acquisition of data:** Chasman, Stanton, Ridker.

**Analysis and interpretation of the data:** Chasman, Posada, Subrahmanyam, Cook, Stanton, Ridker.

**Drafting of the manuscript:** Chasman, Ridker.

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Humanity needs practical men, who get the most out of their work, and, without forgetting the general good, safeguard their own interests. But humanity also needs dreamers, for whom the disinterested development of an enterprise is so captivating that it becomes impossible for them to devote their care to their own material profit.

—Marie Curie (1867-1934)