ORIGINAL ARTICLE

Sequence Variations in *PCSK9*, Low LDL, and Protection against Coronary Heart Disease

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ABSTRACT

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BACKGROUND

A low plasma level of low-density lipoprotein (LDL) cholesterol is associated with reduced risk of coronary heart disease (CHD), but the effect of lifelong reductions in plasma LDL cholesterol is not known. We examined the effect of DNA-sequence variations that reduce plasma levels of LDL cholesterol on the incidence of coronary events in a large population.

METHODS

We compared the incidence of CHD (myocardial infarction, fatal CHD, or coronary revascularization) over a 15-year interval in the Atherosclerosis Risk in Communities study according to the presence or absence of sequence variants in the proprotein convertase subtilisin/kexin type 9 serine protease gene (*PCSK9*) that are associated with reduced plasma levels of LDL cholesterol.

RESULTS

Of the 3363 black subjects examined, 2.6 percent had nonsense mutations in *PCSK9*; these mutations were associated with a 28 percent reduction in mean LDL cholesterol and an 88 percent reduction in the risk of CHD (P=0.008 for the reduction; hazard ratio, 0.11; 95 percent confidence interval, 0.02 to 0.81; P=0.03). Of the 9524 white subjects examined, 3.2 percent had a sequence variation in *PCSK9* that was associated with a 15 percent reduction in LDL cholesterol and a 47 percent reduction in the risk of CHD (hazard ratio, 0.50; 95 percent confidence interval, 0.32 to 0.79; P=0.003).

CONCLUSIONS

These data indicate that moderate lifelong reduction in the plasma level of LDL cholesterol is associated with a substantial reduction in the incidence of coronary events, even in populations with a high prevalence of non–lipid-related cardiovascular risk factors.

EXPERIMENTAL, GENETIC, AND EPIDEMI-ologic data support the concept that an elevated plasma level of low-density lipoprotein (LDL) cholesterol is a primary causal factor in the pathogenesis of coronary heart disease (CHD). Population-based studies consistently demonstrate a positive correlation between plasma levels of LDL cholesterol and the prevalence of CHD, and all five single-gene disorders that result in elevated LDL levels are associated with premature coronary atherosclerosis.[1]

The question regarding the reverse situation naturally arises. If elevations in LDL cholesterol cause CHD, do reductions in LDL cholesterol prevent this disease? Reductions in plasma LDL cholesterol levels have been strongly associated with a reduced incidence of coronary events in clinical trials,[2] but the long-term effects of low LDL cholesterol levels on coronary atherosclerosis have been less clearly defined. Data from cross-sectional and cohort studies are consistent with the hypothesis that low levels of LDL cholesterol are protective,[3] but these data are potentially confounded by other factors related to low LDL cholesterol levels that may independently contribute to reductions in cardiovascular events. Studies of genetic disorders that specifically lower the plasma level of LDL cholesterol, such as heterozygous familial hypobetalipoproteinemia, would provide an ideal system in which to assess the consequences of low LDL cholesterol levels independently of other factors that may modify disease progression. These disorders are uncommon and genetically heterogeneous, however, and it has not been possible to determine their effects on CHD.

Recently, we found that approximately 2 percent of black subjects have one of two nonsense mutations (426C→G, encoding Y142X [replacement of the tyrosine at position 142 with a stop codon], and 2037C→A, encoding C679X [replacement of the cysteine at position 679 with a stop codon]) in *PCSK9*, the proprotein convertase subtilisin/kexin type 9 serine protease gene.[4] These two nonsense mutations are associated with a 40 percent reduction in mean LDL cholesterol.[4] Both nonsense mutations are rare among white subjects. We also identified a *PCSK9* sequence variation (137G→T, encoding R46L [replacement of the arginine at position 46 with leucine]) that is more common among white subjects (prevalence, 3.2 percent) than among black subjects (0.6 per-

cent) and that is associated with a 21 percent decrease in plasma LDL cholesterol levels.[5]

The molecular mechanisms by which these sequence variations in *PCSK9* reduce the LDL cholesterol level are not known. PCSK9 is a glycoprotein that is expressed at its highest levels in the liver, intestine, and kidney.[6] Overexpression of PCSK9 or the mouse orthologue in the livers of mice results in a marked reduction in LDL receptors in this organ,[7-10] which is the main pathway for the removal of LDL from the plasma, and a corresponding increase in circulating LDL cholesterol levels. Conversely, mice lacking *Pcsk9* have increased levels of hepatic LDL receptors, and they remove LDL from the plasma at an accelerated rate.[11] Thus, high levels of PCSK9 lead to high plasma levels of LDL cholesterol, whereas low levels of PCSK9 lead to low LDL cholesterol levels.

The relatively high prevalence of LDL-lowering sequence variations in *PCSK9* provided the opportunity to analyze the effects of specific, lifelong reduction in LDL cholesterol levels on the risk of CHD. Here we report the effects of these sequence variations on the incidence of CHD in the Atherosclerosis Risk in Communities (ARIC) study, a longitudinal, biracial cohort study designed to assess subclinical and clinical atherosclerosis.[12]

METHODS

SUBJECTS

The ARIC study is a prospective study of atherosclerosis initiated in 1987. The study comprises four communities (Jackson, Miss.; Minneapolis; Forsyth County, N.C.; and Washington County, Md.), each of which recruited a randomly selected cohort of approximately 4000 persons 45 to 64 years of age.[12] The subjects participated in four triennial examinations and were interviewed annually by telephone. Records of hospitalizations and deaths were abstracted as previously described.[13] The follow-up data in this study include events up to January 1, 2003. The protocol for the study was approved by the institutional review boards of all centers, and all participants provided written informed consent that included consent for genetic studies.

A total of 15,792 participants underwent an extensive initial examination, which included col-

lection of medical, social, and demographic data. Race or ethnic group was determined by self-identification; participants described themselves as black or white in response to a questionnaire on which the available categories were black, white, Indian, or Asian. The data used in this study included the data from black participants (predominantly, those living in Jackson) and white participants in whom lipoproteins were measured after a fast at baseline and subsequently monitored. The exclusion criteria included use of lipid-lowering drugs (454 persons) and the presence of symptomatic cardiovascular disease (1430 persons) at baseline. Plasma lipids and lipoproteins were assayed in the ARIC central lipid laboratory with commercial reagents, as previously described.[14] The lipid and lipoprotein levels and risk-factor profiles used in this study were obtained at baseline. Hypertension was defined by a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or use of antihypertensive medication. Diabetes mellitus was defined by a fasting glucose level of 126 mg per deciliter (7 mmol per liter) or higher, a nonfasting glucose level of 200 mg per deciliter (11 mmol per liter) or higher, use of hypoglycemic agents, or a history of physician-diagnosed diabetes mellitus. Cigarette smoking was assessed by standardized questionnaires, and current smokers were classified as positive for smoking.

CHD AND CAROTID-ARTERY INTIMA–MEDIA THICKNESS

The incidence of CHD was determined by contacting participants annually, by identifying hospitalizations and deaths during the previous year, and by surveying discharge lists from local hospitals and death certificates from state vital-statistics offices for potential cardiovascular events. All cardiovascular events were adjudicated by independent physician-scientists, as described previously.[15] CHD was defined as a definite or probable myocardial infarction, a silent myocardial infarction detected by electrocardiographic interval changes consistent with an intercurrent ischemic event, death due to CHD, or a coronary-revascularization procedure (coronary bypass graft, coronary angioplasty, or coronary atherectomy). Carotid-artery intima–media thickness, a measure of subclinical atherosclerosis that predicts incident CHD,[13] was determined at baseline with the use of B-mode ultrasonography, as previously described.[16]

GENOTYPING

Fluorogenic 5′-nucleotidase assays for the *PCSK9* alleles encoding Y142X, C679X, and R46L were developed with the use of the TaqMan system (Applied Biosystems). The assays were performed on a 7900HT Fast Real-Time PCR instrument with probes and reagents purchased from Applied Biosystems. Among the 13,761 eligible subjects (10,045 whites and 3716 blacks), there were 419 (255 whites and 164 blacks) from whom genomic DNA was not available, and genotypes were missing because of assay failure for the R46L variant (2.9 percent), the Y142X variant (2.7 percent), and the C679X variant (2.9 percent). The ARIC genotyping laboratory uses a 5 percent blind replicate quality-assurance program for genotype determinations; the agreement for the variants described here was 100 percent.

STATISTICAL ANALYSIS

Routine comparisons of risk-factor levels between carriers of a *PCSK9* variant and noncarriers were performed with contingency chi-square tests for discrete variables and t-tests for continuous variables. The number of coronary events was not adjusted according to age and sex because these variables did not differ between carriers and noncarriers. Cox proportional-hazards modeling was used to test the null hypothesis that the incidence rate of CHD did not differ between carriers and noncarriers. In this analysis, the dependent variable was the time to an event. The analysis properly accounted for participants who were lost to follow-up or who had not had an event by the end of the study period. Therefore, the analysis included all eligible persons who entered the follow-up period, except for 45 persons who specifically asked that their DNA not be used for research. Hazard ratios based on the regression coefficients from the Cox modeling procedure are reported.

RESULTS

The frequencies of the *PCSK9*[142X] and the *PCSK9*[679X] alleles among black subjects were 0.8 percent and 1.8 percent, respectively (Table 1). Thus, approximately 1 of every 40 black subjects in the ARIC

Table 1. Nonsense Mutations in *PCSK9* and Cardiovascular Risk Factors among 3363 Black Participants in the Study.*

Variable	Noncarriers	Carriers			P Value†
		PCSK9^{142X}	*PCSK9*^{679X}	*PCSK9*^{142X} or *PCSK9*^{679X}	
Mutation status — no. of subjects (%)	3278 (97.4)	26 (0.8)	60 (1.8)	85 (2.6)‡	
Age — yr§	53±6	54±6	53±6	54±6	0.61
Male sex — %	37	42	27	31	0.22
Body-mass index	29.6±6.1	28.7±4.4	29.7±5.5	29.5±5.2	0.88
Total cholesterol — mg/dl	215±44	177±44	172±45	173±44	<0.001
Triglycerides — mg/dl	113±81	97±38	94±39	94±38	0.04
LDL cholesterol — mg/dl	138±42	103±39	100±45	100±43	<0.001
HDL cholesterol — mg/dl	55±17	55±14	54±17	55±16	0.72
Hypertension — %¶	55	42	36	37	0.001
Diabetes — %‖	18	12	13	13	0.26
Smoking — %**	30	38	23	27	0.62
Carotid-artery intima–media thickness — mm	0.73±0.16	0.72±0.17	0.69±0.11	0.70±0.13	0.04
Coronary heart disease — no. of subjects	319	0	1	1	0.008
Stroke — no. of subjects (%)	217 (6.6)	3 (11.5)	3 (5.0)	6 (7.1)	0.87
Death — no. of subjects (%)	580 (17.7)	4 (15.4)	8 (13.3)	12 (14.1)	0.39

* Plus–minus values are means ±SD. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.
† P values are for the comparison between noncarriers and persons carrying either the *PCSK9*^{142X} or *PCSK9*^{679X} variant. P values for sex, hypertension, diabetes, smoking, and coronary events were calculated with use of the chi-square test. P values for age, body-mass index, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and carotid-artery intima–media thickness were calculated with use of the t-test.
‡ One subject had both the *PCSK9*^{142X} and *PCSK9*^{679X} alleles.
§ Age at the inception of the study is shown.
¶ Hypertension was defined by a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or use of antihypertensive medication.
‖ Diabetes mellitus was defined by a fasting serum glucose level of 126 mg per deciliter (7 mmol per liter) or higher, a nonfasting glucose level of 200 mg per deciliter (11 mmol per liter) or higher, use of hypoglycemic agents, or a history of physician-diagnosed diabetes mellitus.
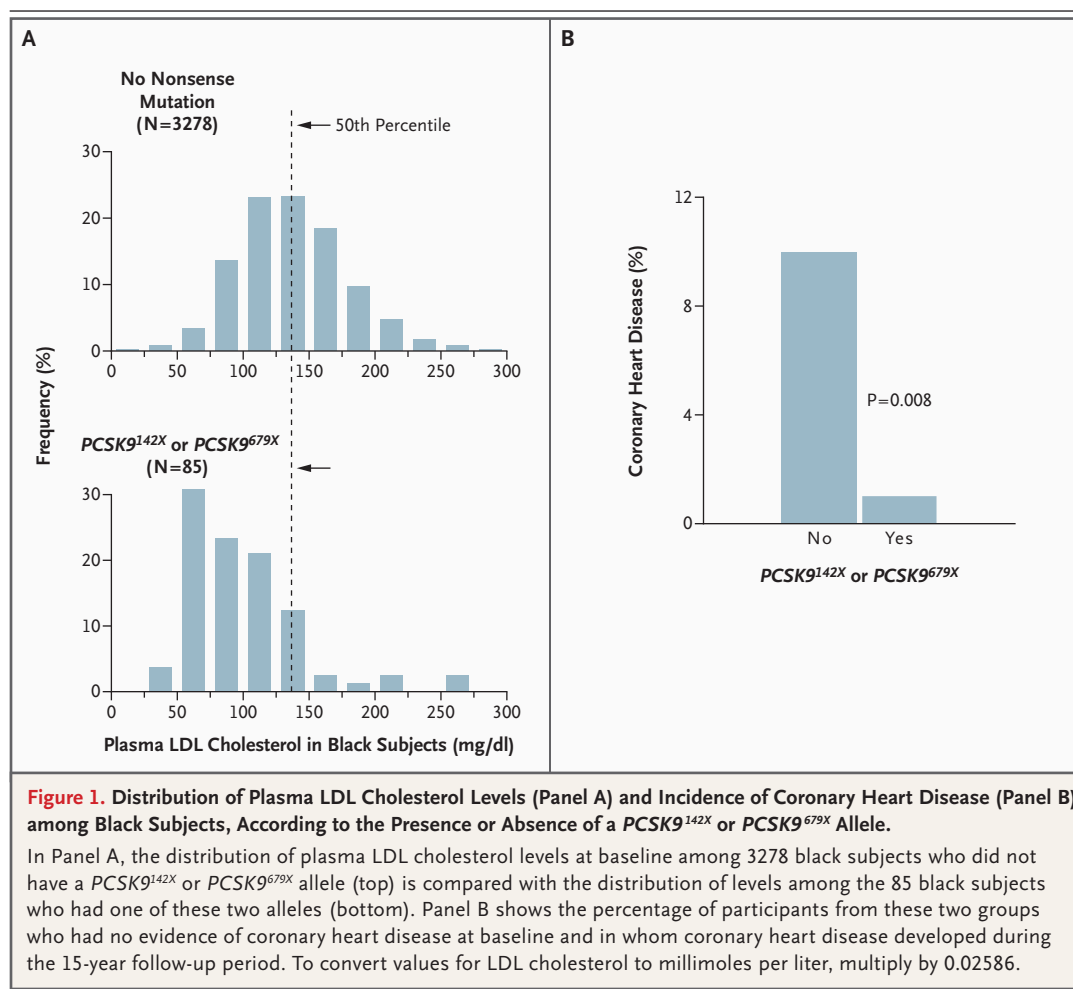** Cigarette smoking was assessed by standardized questionnaires. Smoking status was classified as positive for persons who were current smokers.

study had a nonsense mutation in *PCSK9* — a frequency similar to that observed in two other populations.[4] Both nonsense mutations were rare among white subjects: of 9537 white subjects tested, only 2 had the *PCSK9*^{142X} allele and 4 had the *PCSK9*^{679X} allele. Therefore, analysis of these nonsense mutations was restricted to black subjects. The prevalence of non–lipid-related risk factors was similar in carriers and noncarriers, with the exception of hypertension, which was more common in noncarriers (55 percent vs. 37 percent, P=0.001) (Table 1). Plasma levels of total cholesterol, triglycerides, and LDL cholesterol were significantly lower among subjects with a nonsense mutation in *PCSK9*, but the levels of high-density lipoprotein (HDL) cholesterol were similar in carriers and noncarriers.

The distribution of plasma levels of LDL cholesterol among black carriers of a *PCSK9* variant was shifted toward lower levels (Fig. 1A). The mean plasma LDL cholesterol level was 28 percent lower in the carriers than in the noncarriers (Table 1). Not all subjects with a nonsense mutation had a low plasma level of LDL cholesterol (the levels ranged from 36 to 258 mg per deciliter [0.9 to 6.7 mmol per liter]), but 81 percent had an LDL cholesterol level below the 50th percentile for black subjects.

Among black subjects who did not have a nonsense mutation, 9.7 percent had a coronary event

Figure 1. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Heart Disease (Panel B) among Black Subjects, According to the Presence or Absence of a *PCSK9 142X* or *PCSK9 679X* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 3278 black subjects who did not have a *PCSK9 142X* or *PCSK9 679X* allele (top) is compared with the distribution of levels among the 85 black subjects who had one of these two alleles (bottom). Panel B shows the percentage of participants from these two groups who had no evidence of coronary heart disease at baseline and in whom coronary heart disease developed during the 15-year follow-up period. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

during the 15-year follow-up period (Fig. 1B). In contrast, CHD developed in only 1 of the 85 black participants (1.2 percent) who had a nonsense mutation (P=0.008). According to Cox proportional-hazards modeling, the hazard ratio for CHD among carriers as compared with noncarriers, after adjustment for age and sex, was 0.11 (95 percent confidence interval, 0.02 to 0.81; P=0.03). The incidence of disease remained significantly lower among carriers than among noncarriers (P<0.05) when hypertension and diabetes were added to the model. The only carrier in whom CHD developed was a black man who was obese (body-mass index [the weight in kilograms divided by the square of the height in meters], 34), had hypertension (blood pressure, 186/85 mm Hg), and was a smoker. He also had an Lp(a) lipoprotein level above the 95th percentile for his race and sex as well as a family history of CHD. Despite a very low plasma level of LDL cholesterol

(53 mg per deciliter [1.4 mmol per liter]), he died at the age of 68 years, within 24 hours after his first myocardial infarction.

The frequency of *PCSK9 46L* heterozygosity was 3.2 percent among white subjects (Table 2) and 0.7 percent among black subjects. A total of eight white subjects were homozygous for the *PCSK9 46L* allele. The age, sex distribution, body-mass index, and prevalences of hypertension, diabetes, and smoking were not significantly different between white subjects with the *PCSK9 46L* allele and those without it (Table 2). As was observed for the nonsense mutations, the R46L substitution was associated with a significant reduction in plasma levels of total cholesterol (9 percent) and LDL cholesterol (15 percent). The mean plasma level of LDL cholesterol was slightly lower among the 8 white subjects who were homozygous for the *PCSK9 46L* allele than among the 293 white subjects who were heterozygotes (112±46 mg per deciliter

Table 2. The R46L-Encoding Allele of *PCSK9* and Cardiovascular Risk Factors among 9524 White Subjects in the Study.*

Variable	Noncarriers	Carriers of *PCSK9*[46L]	P Value†
Mutation status — no. of subjects (%)	9223 (96.8)	301 (3.2)	
Age — yr‡	54±6	54±6	0.56
Male sex — %	45	46	0.84
Body-mass index	26.9±4.9	26.8±4.5	0.51
Total cholesterol — mg/dl	214±40	194±37	<0.001
Triglycerides — mg/dl	133±87	135±89	0.79
LDL cholesterol — mg/dl	137±37	116±33	<0.001
HDL cholesterol — mg/dl	51±17	52±17	0.64
Hypertension — %§	25.0	24.6	0.87
Diabetes — %¶	8.0	7.3	0.68
Smoking — %‖	24.6	25.2	0.80
Carotid-artery intima–media thickness — mm	0.73±0.18	0.71±0.16	0.005
Coronary heart disease — no. of subjects	1089	19	0.003
Stroke — no. of subjects (%)	267 (2.9)	9 (3.0)	0.92
Death — no. of subjects (%)	988 (10.7)	25 (8.3)	0.18

* Plus–minus values are means ±SD. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.
† P values are for the comparison between noncarriers and persons carrying the *PCSK9*[46L] variant. P values for sex, hypertension, diabetes, and smoking were calculated with use of the chi-square test. P values for age, body-mass index, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and carotid-artery intima–media thickness were calculated with use of the t-test.
‡ Age at the inception of the study is shown.
§ Hypertension was defined by a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or use of antihypertensive medication.
¶ Diabetes mellitus was defined by a fasting serum glucose level of 126 mg per deciliter (7 mmol per liter) or higher, a nonfasting glucose level of 200 mg per deciliter (11 mmol per liter) or higher, use of hypoglycemic agents, or a history of physician-diagnosed diabetes mellitus.
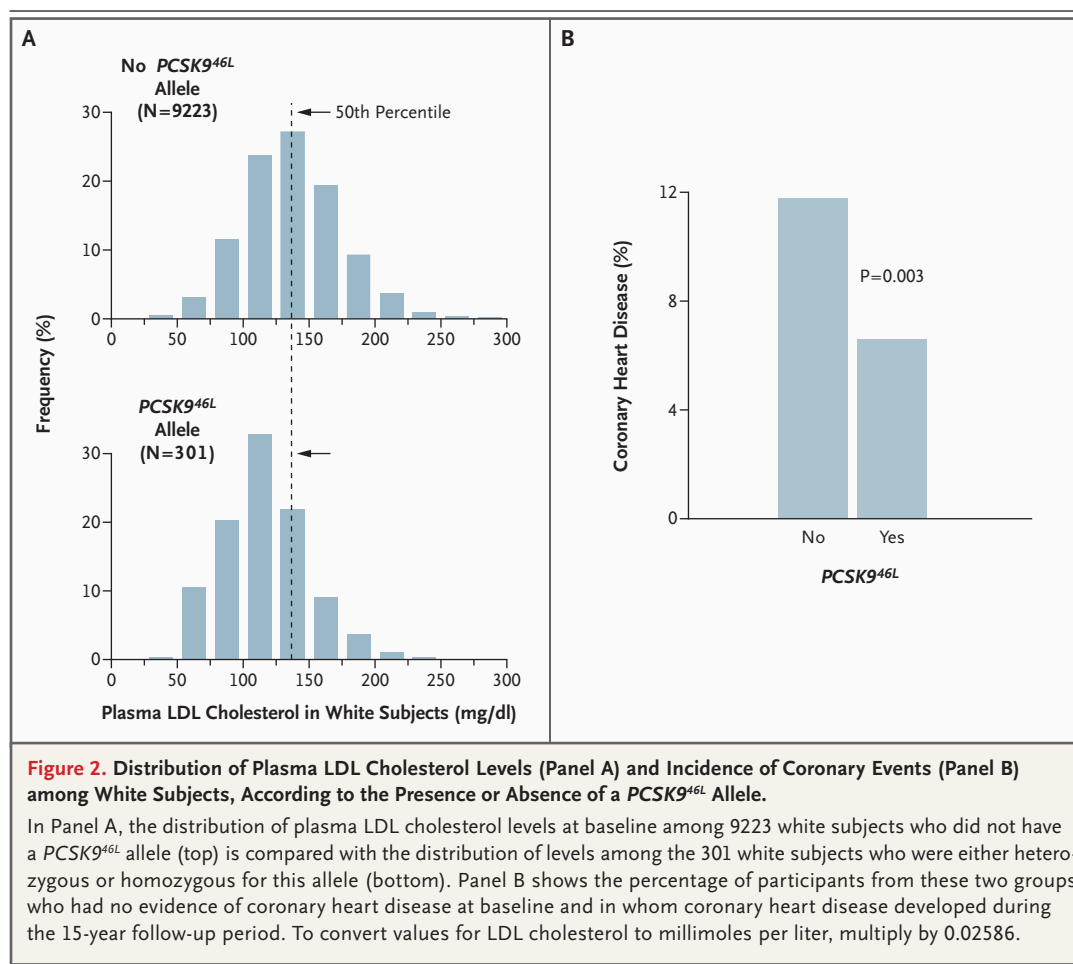‖ Cigarette smoking was assessed by standardized questionnaires. Smoking status was classified as positive for persons who were current smokers.

[2.9±1.2 mmol per liter] vs. 116±33 mg per deciliter [3.0±0.8 mmol per liter]); therefore, the two groups were pooled in subsequent analyses. The distribution of plasma LDL cholesterol levels among persons heterozygous or homozygous for the R46L-encoding allele was shifted toward lower levels (Fig. 2A), although the magnitude of the shift was smaller than that observed with the nonsense mutations (Fig. 1A).

Despite its more moderate LDL-lowering effect, the *PCSK9*[46L] allele was associated with a significant reduction in the incidence of CHD (Fig. 2B). Persons who were heterozygous or homozygous for *PCSK9*[46L] had a 47 percent reduction in the rate of coronary events (6.3 percent vs. 11.8 percent). The hazard ratio for CHD among *PCSK9*[46L] carriers relative to noncarriers, after adjustment for age and sex, was 0.5 (95 percent confidence interval, 0.32 to 0.79; P=0.003). CHD developed in only 1 of 25

blacks who were heterozygous for the *PCSK9*[46L] allele. This woman was an obese (body-mass index, 35), nondiabetic, nonhypertensive smoker who had an LDL cholesterol level of 111 mg per deciliter (2.9 mmol per liter) and an HDL cholesterol level of 27 mg per deciliter (0.7 mmol per liter). She reported no family history of heart disease and had a myocardial infarction at the age of 53 years.

To determine whether the three *PCSK9* alleles associated with lower plasma levels of LDL cholesterol were also associated with a reduced risk of carotid atherosclerosis, we compared carriers and noncarriers with respect to carotid-artery intima–media thickness, a surrogate measure of coronary atherosclerosis. The mean intima–media thickness was slightly but significantly lower among carriers than among noncarriers, both in the group of black subjects (Table 1) and in the group of white subjects (Table 2).

Figure 2. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a *PCSK9^46L* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 9223 white subjects who did not have a *PCSK9^46L* allele (top) is compared with the distribution of levels among the 301 white subjects who were either heterozygous or homozygous for this allele (bottom). Panel B shows the percentage of participants from these two groups who had no evidence of coronary heart disease at baseline and in whom coronary heart disease developed during the 15-year follow-up period. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

DISCUSSION

The principal finding of this study is that sequence variations in *PCSK9* associated with lower plasma levels of LDL cholesterol conferred protection against CHD. The reduced incidence of CHD associated with LDL-lowering *PCSK9* alleles was observed in two different populations. A graded association between reduced LDL cholesterol levels and a decreased risk of coronary events was found: the nonsense mutations that lowered plasma levels of LDL cholesterol by about 40 mg per deciliter (1.0 mmol per liter) were associated with an 88 percent reduction in the incidence of CHD, whereas the *PCSK9^46L* allele, which lowered LDL cholesterol levels by about 20 mg per deciliter (0.5 mmol per liter), was associated with a 50 percent reduction in CHD. The reductions in CHD associated with these *PCSK9* sequence variations were larger than those predicted from LDL-lowering trials,[2] presumably reflecting the beneficial effects of lifelong reductions in plasma LDL cholesterol. These data suggest that relatively moderate reductions in LDL cholesterol level (20 to 40 mg per deciliter) would markedly reduce the incidence of CHD in the population if sustained over a lifetime.

In several observational studies in large populations, reduced plasma levels of LDL cholesterol have been associated with reduced rates of CHD.[17,18] What has not been clear from these studies is the extent to which the risk reduction is directly attributable to lower plasma levels of LDL cholesterol and the extent to which it is due to confounding effects from factors associated with LDL cholesterol levels, such as body weight, diet, medical therapy, or hormonal status. The identification of LDL-lowering alleles of *PCSK9* that were sufficiently common allowed us to stratify subjects according to genotype and to assess the association between these alleles and coronary events.

The possibility exists that the nonsense mutations and R46L-encoding allele of *PCSK9* reduce CHD by a mechanism unrelated to the LDL-lowering effect. We found no evidence that the observed association between the *PCSK9* nonsense mutations or R46L-encoding variant and the reduction in CHD was due to a difference in non–lipid-related risk factors. Nonsense mutations in *PCSK9* were not associated with cardiovascular risk factors (other than LDL cholesterol level) in a prior study.[4] Among black subjects in the current study, hypertension was the only risk factor that was significantly less prevalent in carriers of a *PCSK9* variant than in noncarriers. Among white subjects, the prevalences of all the major cardiovascular risk factors, including hypertension, were similar in carriers and noncarriers. These data are consistent with the notion that reductions in the incidence of CHD associated with sequence variations in *PCSK9* are related to LDL-lowering effects. Nonetheless, PCSK9 may also have direct atherogenic effects that are independent of plasma levels of LDL cholesterol.

Anecdotal observations of atherosclerotic disease in persons with heterozygous familial hypobetalipoproteinemia have raised the possibility that low plasma levels of LDL cholesterol may not prevent cardiovascular disease in the presence of other cardiovascular risk factors.[19] In the current study, nonsense mutations in *PCSK9* were associated with an 88 percent reduction in incident CHD among black subjects, despite the very high prevalence of non–lipid-related cardiovascular risk factors in this population. More than one half of the black participants in the ARIC study had hypertension, almost one third smoked, and nearly 20 percent had diabetes. The significant reduction in the incidence of CHD among black subjects with the nonsense mutations suggests that a lifelong history of reduced LDL cholesterol levels significantly lowers the risk of CHD, even in the presence of multiple risk factors.

Several studies have estimated the potential effect of cholesterol-lowering interventions on the burden of CHD in the population. A recent meta-analysis of 58 trials indicated that an LDL cholesterol reduction of 38.7 mg per deciliter (1.0 mmol per liter) reduces the risk of coronary events by 36 percent after five years.[2] Similar estimates were obtained from an analysis of the 10 largest cohort studies of plasma cholesterol and CHD.[2] The decrease in LDL cholesterol levels in those studies is similar to the reduction associated with the two nonsense mutations in *PCSK9* (37 mg per deciliter [1.0 mmol per liter]), but in the current study the corresponding reduction in CHD was substantially larger (88 percent). Even the more moderate reduction in LDL cholesterol levels associated with the *PCSK9*[46L] allele (about 20 mg per deciliter) was associated with a reduction in CHD (47 percent) that is similar to that achieved in statin trials (36 percent).[2] Adjustment according to the plasma LDL cholesterol level did not substantially alter the hazard ratio for CHD among persons with the nonsense mutations (from 0.11 to 0.15) or with the R46L-encoding sequence variation (from 0.50 to 0.60). This result suggests that a single measurement of plasma LDL cholesterol does not capture the effect of a lifetime of reduced plasma levels. These data suggest that lifelong reduction of LDL levels confers greater benefit than does a similar reduction instituted later in life. This finding is consistent with the observation that coronary atherosclerosis develops early in life[20-23] and suggests that earlier introduction of an intervention that lowers lipid levels even moderately may confer increased protection from CHD.[24]

The participants described in the current report were 45 to 64 years old at the inception of the study and were followed for an average of 15 years. The study does not address whether the cardioprotective effects of the LDL-lowering *PCSK9* sequence variations persist in older age groups. The difference between carriers and noncarriers in the incidence of heart disease may decline with aging, as the absolute rates of disease increase. Moreover, it is not known whether the beneficial effect of decreased LDL cholesterol levels on cardiovascular disease results in an overall reduction in mortality rates. The number of deaths observed during the follow-up period was slightly lower among carriers than among noncarriers both in black subjects and in white subjects, but the difference did not reach statistical significance. Further investigation in the ARIC study populations and in cohorts of elderly persons will be required to answer these questions.

Statins are the cornerstone of cholesterol-lowering therapy for the prevention of CHD. Recent clinical trials have shown that the reduction in the rate of coronary events is directly related to the magnitude of the reduction in LDL cholesterol levels.[2] Ironically, statin treatment

increases the expression of both the LDL-receptor gene (*LDLR*) and *PCSK9*.[25] The increased expression of *PCSK9* may attenuate the LDL-lowering effect of statins. Observations in genetically modified mice suggest that inhibition of PCSK9 activity would enhance the LDL-lowering effects of statins.[11] These findings, together with the results of the current study, make PCSK9 an attractive new target for LDL-lowering therapy.

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