Sequence Variation in *Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease Gene*, Low LDL Cholesterol, and Cancer Incidence

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Abstract

Some prospective epidemiologic studies have suggested that a low plasma cholesterol level may be associated with increased risk of cancer. Certain sequence variants in the *proprotein convertase subtilisin/kexin type 9 serine protease gene* (*PCSK9*) are associated with lifelong low total and LDL cholesterol. We therefore analyzed the association of *PCSK9* variation with incidence of cancer between 1987 and 2000 in a prospective study (n = 13,250). The frequency of the *PCSK9* variants studied was 2.4% in blacks and 3.2% in whites. Neither was associated with increased cancer incidence: age- and sex-adjusted hazard ratios were 0.66 [95% confidence interval (95% CI), 0.31-1.39] in blacks and 0.77 (95% CI, 0.54-1.09) in whites. Low baseline total or LDL cholesterol levels in 1987 to 1989 were also not statistically significantly associated with incident cancer: multivariable-adjusted hazard ratios for the lowest compared with the highest quartiles of LDL cholesterol were 1.05 (95% CI, 0.78-1.40) in blacks and 1.16 (95% CI, 0.99-1.36) in whites. These data suggest that a lifelong low cholesterol concentration, as reflected by these *PCSK9* variants, does not increase risk of cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2455–8)

Introduction

By the early 1990s, several prospective epidemiologic studies had reported a modest association between low plasma cholesterol concentrations and increased risk of cancer, particularly colon and lung cancer (1, 2). Reasons for this observation were unclear, but general consensus was that an association between low cholesterol and cancer was likely not a cause and effect relation. Instead, the epidemiologic association was felt to likely suffer from confounding or from ''reverse causality'' (i.e., that subclinical cancer could reduce cholesterol levels; refs. 1, 3).

In that era, before widespread 3-hydroxy-3-methylglutaryl CoA reductase inhibitor (statin) trials, there also was concern from rodent studies and human trials that lowering plasma cholesterol pharmacologically might modestly increase cancer risk (4, 5). However, cholesterol lowering with statins does not seem to increase cancer risk (6-8), so concern about low cholesterol leading to cancer has lessened. The National Cholesterol Education Program's Adult Treatment Panel III in 2001 (9) concluded, ''There is no evidence that currently used cholesterol-lowering drugs promote the development of cancer or induce subtle neurologic diseases. Moreover, clinical experience with these drugs over periods of 30 years for fibrates and bile acid sequestrants and 15 years for statins has uncovered no long-term side effects. Nonetheless, the possibility of long-term side effects, albeit remote, should be one factor to consider when recommending lifetime therapy with a cholesterol-lowering drug.''

One way to further test whether long-term low plasma cholesterol levels may increase cancer risk is to compare cancer risks of people with or without genetic variants that lead to lifelong low cholesterol levels using the approach called ''Mendelian randomization'' (10). Cohen et al. (11) recently showed that plasma LDL cholesterol concentrations are substantially reduced in individuals with certain sequence variations in the *proprotein convertase subtilisin/kexin type 9 serine protease gene* (*PCSK9*). In blacks, two nonsense mutations (142X and 679X) were associated with a 28% reduction in LDL cholesterol and an 88% reduction of coronary heart disease risk in the Atherosclerosis Risk in Communities (ARIC) Study. In whites, a single nucleotide polymorphism sequence variant (46L) was associated with a 15% reduction of LDL cholesterol and a 47% reduction of coronary heart disease risk.

Whether these *PCSK9* variants linked to lifetime low LDL cholesterol concentrations are associated with cancer risk is unknown. We therefore examined this question in the prospective, population-based cohort of the ARIC Study.

Received 6/6/07; revised 7/31/07; accepted 8/31/07.

Grant support: National Cancer Institute grant R03-CA65473 and National Heart, Lung, and Blood Institute contracts N01-HC-55015, 55016, 55018, 55019, 55020, 55021, and 55022.

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doi:10.1158/1055-9965.EPI-07-0502

Table 1. Adjusted HRs (95% CI) of cancer by LDL and total cholesterol quartiles in blacks and whites, ARIC, 1987-2000

	Quartile 4	Quartile 3	Quartile 2	Quartile 1	*P* trend
Blacks					
LDL cholesterol	(*n* = 834)	(*n* = 835)	(*n* = 835)	(*n* = 834)	
Range (mg/dL)	>164	135-164	108-135	<108	
Cases	108	113	105	92	
Model 1*	1.0	1.11 (0.86-1.45)	1.06 (0.81-1.39)	1.01 (0.77-1.34)	0.86
Model 2†	1.0	1.16 (0.89-1.54)	1.06 (0.80-1.40)	1.05 (0.78-1.40)	0.73
Total cholesterol	(*n* = 841)	(*n* = 833)	(*n* = 844)	(*n* = 842)	
Range (mg/dL)	≥242	212-241	184-211	≤183	
Cases	103	111	104	104	
Model 1*	1.0	1.13 (0.87-1.48)	1.10 (0.83-1.44)	1.13 (0.86-1.49)	0.79
Model 2†	1.0	1.16 (0.88-1.54)	1.19 (0.89-1.58)	1.21 (0.91-1.61)	0.56
Whites					
LDL cholesterol	(*n* = 2,400)	(*n* = 2,401)	(*n* = 2,401)	(*n* = 2,400)	
Range (mg/dL)	>161	135-161	112-135	<112	
Cases	333	321	297	314	
Model 1*	1.0	1.01 (0.87-1.18)	0.97 (0.83-1.14)	1.12 (0.96-1.31)	0.30
Model 2†	1.0	1.01 (0.86-1.18)	1.00 (0.85-1.17)	1.16 (0.99-1.36)	0.20
Total cholesterol	(*n* = 2,430)	(*n* = 2,415)	(*n* = 2,459)	(*n* = 2,456)	
Range (mg/dL)	≥239	213-238	188-212	≤187	
Cases	335	316	329	303	
Model 1*	1.0	1.00 (0.86-1.17)	1.06 (0.91-1.23)	1.06 (0.91-1.24)	0.80
Model 2†	1.0	1.03 (0.88-1.20)	1.06 (0.91-1.25)	1.09 (0.93-1.29)	0.74

*Adjusted for age (continuous) and sex.
†Adjusted for age (continuous), body mass index (continuous), triglycerides (continuous), diabetes (yes, no), smoking status (current smoker, nonsmoker), pack-years (continuous), ethanol intake (continuous), sport index (continuous), education (<high school graduate, ≥high school graduate), and sex and current hormone replacement therapy (male and female with no hormone replacement therapy, female with hormone replacement therapy).

Materials and Methods

Population. The ARIC Study is a cohort study of cardiovascular disease in whites and African-Americans in four U.S. communities. Between 1987 and 1989, 7,082 men and 8,710 women ages 45 to 64 years were recruited from Forsyth County, North Carolina; Jackson, Mississippi (African-Americans only); suburban Minneapolis, Minnesota; and Washington County, Maryland. There was a 46% response in the Jackson cohort and a 65% to 67% response in the other three cohorts. The ARIC Study protocol was approved by the institutional review board of each participating university. After written informed consent was obtained, participants underwent a baseline clinical examination (visit 1). Follow-up examinations of the cohort occurred thrice at intervals of ~3 years. The response rates for visits 2 (1990-1992), 3 (1993-1995), and 4 (1996-1998) were 93%, 86%, and 80%, respectively. Participants completed annual telephone interviews between visits and following visit 4.

Risk Factor Measurements. Risk factors examined in these analyses were ascertained at visit 1 as described in detail in the ARIC Study manuals of operation (12). Participants were asked to fast before the clinical examination. Blood was drawn from an antecubital vein of seated participants into vacuum tubes containing EDTA (for measurement of lipids and DNA extraction) or a serum separator gel (glucose). Serum and plasma aliquots were stored at −70°C and shipped to central laboratories for analyses. Total cholesterol and triglycerides were measured by enzymatic methods, and

Table 2. Race-specific and age- and sex-adjusted means and percentages of baseline risk factors by presence of *PCSK9* variants, ARIC, 1987-89

	Blacks			Whites		
	PCSK9^{142X} or *PCSK9*^{679X} (*n* = 85)	Neither variant (*n* = 3,392)	*P* for difference	*PCSK9*^{46L} (*n* = 314)	Neither variant (*n* = 9,459)	*P* for difference
Means						
LDL cholesterol (mg/dL)	98.8	138.9	<0.0001	117.7	138.3	<0.0001
Total cholesterol (mg/dL)	172.2	215.7	<0.0001	195.9	215.4	<0.0001
Triglycerides (mg/dL)	95.8	111.4	0.06	140.0	137.1	0.59
BMI (kg/m^2)	29.3	29.6	0.69	26.9	27.1	0.69
Pack-years of smoking	206.5	240.5	0.41	393.6	341.3	0.03
Ethanol intake (g/wk)	19.0	33.0	0.18	40.2	46.1	0.26
Sports activity index (range, 0-5)	2.08	2.16	0.32	2.47	2.53	0.18
Prevalences						
High school graduate (%)	56.9	59.1	0.67	82.3	82.8	0.83
Diabetes (%)	12.7	16.5	0.35	8.5	8.6	0.96
Current smoking (%)	27.9	29.7	0.72	25.2	24.2	0.68
Current HRT use, women (%)	22.9	18.9	0.45	30.1	23.6	0.05

Abbreviations: BMI, body mass index; HRT, hormone replacement therapy.

high-density lipoprotein cholesterol was measured after dextran-magnesium precipitation. LDL cholesterol was calculated (13). Serum glucose was assayed by a hexokinase/glucose-6-phosphate dehydrogenase method. Prevalent diabetes mellitus was defined as a fasting glucose ≥126 mg/dL (14) or a self-reported history of or treatment for diabetes.

Anthropometrics were assessed with the subject wearing a scrub suit and no shoes. Body mass index was calculated (weight in kilograms/height in meters squared). Questionnaires assessed race (self-identified), education, smoking status, number of cigarettes smoked per day and duration of smoking (pack-years computed), and usual consumption of wine, beer, and hard liquor (grams per day computed). Level of sports physical activity was assessed by the Baecke questionnaire (15).

Genotyping. Using stored DNA from all ARIC participants, fluorogenic 5′-nucleotidase assays for the *PCSK9* alleles encoding Y142X, C679X, and R46L were done with the use of the Taqman system (Applied Biosystems). The assays were carried out on a 7900HT Fast Real-time PCR instrument with probes and reagents purchased from Applied Biosystems. The assay failure rates for the R46L variant, the Y142X variant, and the C679X variant ranged from 2.7% to 2.9%. The ARIC genotyping laboratory uses a 5% blind replicate quality assurance program for genotype determinations; the agreement for the variants described here was 100%.

Cancer Ascertainment. During each clinical examination, participants were asked whether they had ever been diagnosed with cancer. At each annual telephone interview, participants reported all hospitalizations. Among those not reporting cancer at the baseline visit, incident cancers were identified between January 1, 1987 and December 31, 2000 via linkage to state cancer registries and supplemented by the hospital records. This method and the high completeness of ARIC cancer ascertainment were previously described (16, 17). For this analysis, we focused primarily on total cancer (excluding nonmelanoma skin cancer) but conducted subanalyses for common site-specific cancers (i.e., colorectal, lung, female breast, and prostate).

Data Analysis and Statistical Methods. From the original ARIC cohort (n = 15,792), we successively excluded participants who did not want to participate in cancer research (n = 187), who denied permission for DNA testing (n = 79), who were in very small minority groups (n = 96), who did not provide sufficient data to determine baseline cancer status or who had a history of cancer (n = 877), who had missing DNA or *PCSK9* genotypes (n = 795), or who had not fasted 8 h (n = 508). This left 13,250 in the cohort at risk.

Statistical analysis was done by using Statistical Analysis System software (version 9.1; SAS Institute, Inc.). All analyses were conducted race specific because the *PCSK9* variants associated with low LDL cholesterol

Table 3. Crude incidence rate and race-specific and age- and sex-adjusted HRs (95% CI) of cancer by presence of *PCSK9* variants, ARIC, 1987-2000

	No. developing cancer	Person-years	Incidence rate	HR (95% CI)
All cancer				
Blacks				
Neither variant	428	37,445	11.43	
$PCSK9^{142X}$ or $PCSK9^{679X}$	7	937	7.47	0.66 (0.31-1.39)
Whites				
Neither variant	1,253	107,341	11.67	
$PCSK9^{46L}$	33	3,665	9.00	0.77 (0.54-1.09)
Colorectal cancer				
Blacks				
Neither variant	44	38,629	1.14	
$PCSK9^{142X}$ or $PCSK9^{679X}$	2	954	2.10	1.87 (0.45-7.71)
Whites				
Neither variant	129	111,777	1.15	
$PCSK9^{46L}$	5	3,775	1.32	1.16 (0.47-2.83)
Lung cancer				
Blacks				
Neither variant	61	38,720	1.58	
$PCSK9^{142X}$ or $PCSK9^{679X}$	1	959	1.04	0.69 (0.10-4.97)
Whites				
Neither variant	186	112,018	1.66	
$PCSK9^{46L}$	2	3,793	0.53	0.32 (0.08-1.28)
Breast cancer (women)				
Blacks				
Neither variant	81	23,956	3.38	
$PCSK9^{142X}$ or $PCSK9^{679X}$	1	645	1.55	0.45 (0.06-3.26)
Whites				
Neither variant	259	58,027	4.46	
$PCSK9^{46L}$	6	1,996	3.00	0.67 (0.30-1.50)
Prostate cancer				
Blacks				
Neither variant	120	14,090	8.52	
$PCSK9^{142X}$ or $PCSK9^{679X}$	1	315	3.17	0.35 (0.05-2.50)
Whites				
Neither variant	234	51,879	4.51	
$PCSK9^{46L}$	9	1,739	5.18	1.14 (0.59-2.23)

overlapped little between blacks and whites (11). In analyses of total cancer, person-years at risk were calculated from the time of baseline clinical examination until the date of first cancer diagnosis, death, loss to follow-up, or December 31, 2000, whichever occurred first. In analyses of site-specific cancers, people with multiple cancers of interest were counted more than once. To explore possible confounding factors, means or prevalences of various risk factors were computed by *PCSK9* genotype. Crude cancer incidence rates (per 1,000 person-years) for *PCSK9* genotypes were calculated. Adjusted hazard ratios (HR) for the associations of the *PCSK9* variants and of total and LDL cholesterol with cancer incidence were calculated by using Cox proportional hazards regression. The proportional hazards assumption of the Cox model was found not to be violated by testing an interaction between *PCSK9* variants and time. The median values for quartiles of cholesterol were used for the χ^2 test for trend in HRs.

Because the *PCSK9* variants are uncommon, we estimated the detectable race-specific HRs in association with total cancer in this cohort at $\alpha = 0.05$ and power = 0.8. The detectable HRs for total cancer, assuming the variants increased risk, were 1.51 for blacks and 1.26 for whites.

Results

There were 451 incident cancers in 39,236 person-years of follow-up in blacks and 1,269 cancers in 109,617 person-years in whites. The crude incidence rates per 1,000 person-years were 11.5 in blacks and 11.6 in whites.

A low LDL cholesterol level was not a statistically significant risk factor for cancer in this cohort (Table 1). In whites, the adjusted HR for the lowest versus highest quartile of total cholesterol was 1.09 (95% CI, 0.93-1.29) and for LDL cholesterol was 1.16 (95% CI, 0.99-1.36).

PCSK9 alleles associated with low LDL cholesterol were relatively uncommon. Among blacks, 2.4% had either the 142X or 679X variants (one had both). Among whites, 3.2% had the 46L variant. There was no overlap among genotypes across races. Other than the expected association with total and LDL cholesterol, these *PCSK9* variants were largely unrelated to the other potential risk factors for cancer examined (Table 2).

As shown in Table 3, there was no evidence that cholesterol-lowering variants of *PCSK9* were associated with increased risk of total cancer in blacks (HR, 0.66; 95% CI, 0.31-1.39) or whites (HR, 0.77; 95% CI, 0.54-1.09). This was true in blacks for both 142X and 679X when analyzed separately. There was no appreciable change in the HRs after adjusting for the other risk factors in the footnotes to Table 1 (HR of 0.78 in blacks and HR of 0.77 in whites). Numbers were limited, precluding conclusions about common site-specific cancers (Table 3).

Discussion

In several previous cohorts that were mostly white, a low plasma cholesterol level was reported to be a risk factor for cancer (1, 2). Low total and LDL cholesterol in ARIC was not associated with an increased risk of cancer, although the HR for low LDL cholesterol in whites was slightly elevated (1.16; 95% CI, 0.99-1.36) in comparison with LDL cholesterol in the highest quartile. The novel finding reported here was that the *PCSK9* variants associated with presumably lifelong low plasma cholesterol concentrations were also not related to cancer incidence. This offers further reassurance that any association between plasma total or LDL cholesterol and cancer is unlikely to be causal.

The *PCSK9* variants studied were uncommon, so it would take a very large study to verify that these variants do not increase cancer risk slightly or to study site-specific cancers, which we could not effectively do. However, we had adequate power to detect an important elevation of overall cancer risk if there had been one. HRs of cancer, if anything, tended to be reduced rather than increased in carriers compared with noncarriers of the variants.

In summary, using a Mendelian randomization design, we found no evidence that variants in *PCSK9* associated with low LDL cholesterol levels increase risk of total cancer.

Acknowledgments

We thank the staff and participants in the ARIC Study for their important contributions and Drs. Jonathan Cohen and Helen Hobbs for helpful discussions about *PCSK9*.

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